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Prevalence, Antimicrobial resistance and Virulence profile of *Salmonella* from Aquaculture farms of central Kerala, India

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Abstract

Salmonella belonging to Enterobacteriaceae is considered as the leading cause of food borne illness all over the world and products from aquaculture often act as vehicles of their transmission. In this study, a total of 150 samples including mud, water, fish/shrimp and feed from 38 aquaculture farms from three districts of central Kerala *viz*., Thrissur, Ernakulam and Kottayam were screened for the presence of *Salmonella.* The overall prevalence of *Salmonella* in aquaculture farms was 7.9%. By serotyping, 100% of *Salmonella* isolates (n=8) from aquaculture were identified as *Salmonella* enterica subspecies enterica serovar Typhimurium with antigenic formulae 4,5,12: i: 1,2. None of the shrimp farms selected in this study harboured *Salmonella*. Antibiotic sensitivity testing revealed that 100% of S. Typhimurium were sensitive to the 17 antibiotics tested. Screening of the S. Typhimurium isolates for 11 virulence genes; *inv*A, *fim*A, *stn*, *spv*C, *sop*B, *mgt*C, *bcf*C, *csg*D, *avr*A, *hil*A and *pho*P/*pho*Q belonging to *Salmonella* Pathogenicity Islands (SPI) 1 to 5 by PCR revealed that the isolates carried all the virulence genes except *spv*C gene, indicating its pathogenic potential. This study suggests that finfish farms are probable reservoirs of *Salmonella* with high virulence potential and can pose a potential threat to public health safety. Proper policies and regulations are to be adopted to control the occurrence of *Salmonella* in finfish culture systems.

Keywords: *Salmonella*, aquaculture, *S*. Typhimurium, antibiotic sensitivity, virulence

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Introduction

The global aquaculture production is increasing at an average growth rate of 5.3% per year from 2000-2018 and India stands second after China (FAO, 2020) with six and half fold growth in aquaculture production for the past two decades (Jayasankar et al., 2018). Though fish and fish products are accepted worldwide as source of healthy nutrition, employment and foreign exchange, they often carry risk of having various foodborne pathogens. Among these pathogens, *Salmonella* of Enterobacteriaceae ranks first (15 outbreaks) in causing fish associated food borne illness with single bacterial etiology across the world (Sheng & Wang, 2021). Within the 2579 *Salmonella* serotypes, other than S. Typhi and S. Paratyphi, all are typically referred to as non-typhoidal *Salmonella* (NTS). During the past few years, food borne transmitted invasive NTS infections emerged as the leading cause of food borne illness with 153 million cases and 57,000 deaths every year and pose a substantial challenge to public health worldwide (CDC, 2021).

Generally, *Salmonella* is not an autochthonous bacterial flora of fish; but can get contaminated either from aquaculture environment or through processing chain. *Salmonella* may enter the aquaculture environment through domestic stock and integrated farming animals, wild animals, poor sanitation, inappropriate disposal of human and animal waste, contaminated feed, fertilization of pond with poultry litter or cow dung or through water run-off from contaminated area (Ampofo & Clerk, 2010). *Salmonella* has high survival and persistence in aquatic environments of tropical countries (Fernandes et al., 2018). *Salmonella* is capable of producing biofilms over food and food contact surfaces such as plastics, stainless steel working spaces, equipment *etc*; that may result in

cross contamination in processing areas of aquatic products (Wang et al., 2015). Therefore, aquatic products often act as vehicles of *Salmonella* cross contamination and transmission across the countries during export and import (Fernandes et al., 2018). Since *Salmonella* is a zero tolerant microbe with respect to food safety, their mere presence in raw aquaculture products remains as a major cause of detention and rejection of shipments in international markets (FAO, 2010).

Consumption of undercooked or raw aquatic products contaminated with *Salmonella* often causes food borne outbreaks and is very rare with aqua cultured products when compared to seafood (Hamilton et al., 2018). Pathogenicity of *Salmonella* is determined by the presence of various virulence genes located either in chromosomes as *Salmonella* Pathogenicity Islands (SPI's) or in plasmids as (*Salmonella* plasmid virulence) *spv* R, A, B, C and D. Such virulence genes influence invasiveness, attachment, toxin production, growth and survival of *Salmonella* within host and results in Salmonellosis (Beshiru et al., 2019). Emergence of antibiotic resistance in *Salmonella* strains from aquaculture due to misuse of antibiotics in farming practices exerts more pressure on health care systems with high treatment cost and failures.

The presence of *Salmonella* in aquaculture farms poses risk for public health and increases the economic burden due to export rejections (Hamilton et al., 2018). Even though microbiological safety of aquaculture products from *Salmonella* remains as a serious concern for consumers, industrialists and regulatory policy makers all over the world, limited data is available in this regard (FAO, 2010; Zhang et al., 2015; Sing et al., 2016; Li et al., 2017). Therefore, the aim of the study is to assess the prevalence, antimicrobial resistance and virulence genes of *Salmonella* from aquaculture farms of central Kerala.

Materials and Methods

A total of 38 aquafarms (shrimp farms; n1=14 and fish farms; n2=24) were selected for screening of *Salmonella* from three districts of central Kerala *viz*., Thrissur, Ernakulam and Kottayam. From the 38 farms, a total of 150 samples that includes mud (n=38), water (n=38), fish/shrimp (n=43), feed (n=31) were collected aseptically and processed within 3h of collection.

All the samples were screened for the presence of *Salmonella* according to BAM, USFDA (Andrews and Hammack, 2007). For pre-enrichment, 25 g (25 ml in case of water) of samples were homogenised with Lactose broth (BD & Difco, USA) in a stomacher blender (Seward, UK) and incubated at 37°C for 24 h. For further selective enrichment of *Salmonella,* 1ml and 0.1 ml aliquots of pre-enriched lactose broth were transferred to 10 ml tetrathionate broth (TTB) (Oxoid, USA) and 10 mL Rappaport-Vassiliadis (RV) (BD and Difco, USA) medium respectively. Incubation for TTB was done at 37°C for 24 h, while RV medium was incubated at 42°C for 24 h. From each selective enrichment broth, a loopful of culture was streaked onto hektoen enteric agar (HEA) (BD and Difco, USA), bismuth sulphite agar (BSA) (BD and Difco, USA), and xylose lysine deoxycholate agar (XLD) (BD and Difco, USA). All selective plates were incubated at 37°C for 24 h and typical colonies of *Salmonella* were taken and sub cultured onto trypticase soy (TS) agar (BD and Difco, USA) slants for further identification.

For tentative identification, all presumptive *Salmonella* isolates were subjected to standard biochemical tests according to BAM, USFDA (Andrews & Hammack, 2007). Isolates with typical biochemical reactions were further confirmed using PCR (Rahn et al., 1992). Crude lysate was prepared from overnight grown cultures of *Salmonella* in Luria Bertani (LB) (BD & Difco, USA) and used as template for PCR targeting *invA* gene of 284 bp amplicon size. A 25 µl of PCR mixture contained 0.4 pM concentration of primer, 200 µM of dNTP (Thermo fisher scientific), 1X reaction buffer (20 mM Tris HCl; pH 8.0, 50 mMKCI, 1.5 mM $MgCl_2$), 1U of Taq polymerase (Thermo fisher scientific) and 2 ul of template DNA. PCR reactions were carried out with the following conditions: initial denaturation at 95°C for 2 min, followed by 35 cycles of 95°C for 30 sec, 64°C for 30 sec, and 72°C for 30 sec and a final extension of 5 min at 72°C in Veriti™ 96-Well Thermal Cycler (Thermo Fisher Scientific, United States). For visualization of PCR products, 1.8% agarose gel (Sigma Aldrich, USA) was used. Reference strain *Salmonella* enterica subsp. enterica serovar Paratyphi A (ATCC® 9150™) was used as positive control.

All PCR confirmed (*invA* positive) *Salmonella* isolates were sent to National *Salmonella* Centre (Veterinary), ICAR-Indian Veterinary Research Institute (IVRI), Bareilly, Uttar-Pradesh for serotyping.

Antimicrobial susceptibility test was performed for *Salmonella* on Mueller Hinton (MH) agar (BD and Difco, USA) by Kirby-Bauer disc diffusion assay. Overnight grown young cultures of *Salmonella* in Trypticase Soy Broth (TSB) (BD and Difco, USA) was evenly spread over MH agar after adjusting optical density to 0.5 MacFarland standard. Then antibiotics paper discs impregnated with antibiotics (HiMedia, India) were aseptically placed over the culture and kept for incubation at 37°C for 24 h. The antibiotics tested were co-trimoxazole (COT, 25 mcg), ampicillin (AMP, 10 mcg), chloramphenicol (C, 30 mcg), pefloxacin (PF, 5 mcg), amoxiclav (AMC, 30 mcg), cefotaxime (CTX, 30 mcg), ceftriaxone (CTR, 30 mcg), cefoxitin (CX, 30 mcg), ceftazidime (CAZ, 30 mcg), cefpodoxime (CPD, 10 mcg), aztreonam (AT, 30 mcg), imipenem (IPM, 10 mcg), gentamicin (GEN, 10 mcg), azithromycin (AZM, 15 mcg), tetracycline (TE, 30 mcg), ciprofloxacin (CIP, 5 mcg) and nitrofurantoin (NIT, 300 mcg) (HiMedia, India). *Escherichia coli* ATCC 25922 was used as a quality control organism in this assay and all the results

Table 1. Details of *Salmonella* virulence genes selected for this study

Sl. No.	Genes	Function	References
1	*fim A* [Fimbrial A]	Codes for type 1 fimbrial protein A chain and helps in colonisation on host epithelial cells	Kumar et al., 2009, Deguenon et al., 2019
2	*stn* [Salmonella enterotoxin]	Codes for enterotoxin production	Deguenon et al., 2019
3	*spv C* [Salmonella plasmid virulence C]	Plasmid virulence marker	Borges et al., 2013
4	*sop B* [Salmonella outer protein B]	Have role in renal administration of pathogenic routes (Belongs to SPI-5)	Sanchez et al., 2010,
5	*mgtC* [Magnesium transporter C]	Promotes intramacrophage survival of *Salmonella* within the host (Belongs to SPI-3) and factor required for growth in low Mg^{2+} medium. Also involved in regulating membrane potential by activating Na^{+}/K^{+}-ATPase.	Elkenany et al., 2019
6	*csgD* [Curli subunit G]	Promotes the biofilm formation of *Salmonella*	Elkenany et al., 2019
7	*bcfC* [Chaperone-usher fimbriae C]	Promotes the biofilm formation of *Salmonella*	Elkenany et al., 2019
8	*avrA*	Codes for TTSS (Type Three Secretory System) complex for *Salmonella* (Belongs to SPI-1) and mainly involved in the enteritis pathway	Borges et al., 2013
9	*hilA* [Hyper invasive locus A]	Helps *Salmonella* to invade epithelial cells and also induce apoptosis of host macrophages (Belongs to SPI 1)	Cardona-Castro et al., 2002
10	*phoP/phoQ*	Part of pleiotropic two component regulatory system which act as global transcriptional regulator by promoting phosphate intake and also helps to survive low acidic conditions	Soto et al., 2006
11	*invA* [Invasive A]	Promotes the epithelial invasion	Rahn et al., 1992

underwent standardization and evaluation according to guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2020).

All salmonella strains isolated in this study from aquaculture environment were screened for the presence of virulence genes such as *fim*A, *stn, spv*C, *sop*B, *mgt*C, *bcf*C, *csg*D, *avr*A, *hil*A and *pho*P/*pho*Q by PCR (Table 1).

Results and Discussion

In this study, a total of 38 aquaculture farms from three districts of central Kerala, India were screened for the presence of *Salmonella* and the overall prevalence of *Salmonella* in aquaculture farms was 7.9% (n=3). Among the 150 samples tested from 38 farms including fish/shrimp, water, mud and feed, *Salmonella* was isolated from finfishes; mullet, tilapia and pangasius. None of the water, mud and feed samples tested harboured *Salmonella.* Out of 43 presumptive colonies in HEA and XLD, only 8 isolates were confirmed as *Salmonella* with biochemical tests and PCR targeting *invA* gene. By serotyping, all the 8 *Salmonella* isolates were identified as *Salmonella* enterica subspecies enterica serovar Typhimurium with antigenic formulae 4,5,12: i: 1,2. None of the shrimp farms selected in this study harboured *Salmonella*.

Salmonella is considered as a contaminant of raw aquaculture products. Some studies conclude that *Salmonella* is inherently present in shrimp aquaculture environment and can be treated as part of natural flora (Bhaskar et al., 1995). But in contrast, Dalsgaard et al. (1995) reported that *Salmonella* is not a natural flora in tropical brackish water environments. Koonse et al. (2005) reports that raw aqua cultured products are more likely to be contaminated with *Salmonella* when compared to raw wild caught aqua products. This might be due to the fact that, *Salmonella* may enter aquafarming systems through various inputs such as contaminated feed (Antunes et al., 2018), untreated, animal manure excreta of farm associated animals and birds (Ogg et al., 1989), inflow water (Antunes et al., 2018) *etc*. In addition, the workers associated with aquafarming practices could also be a potential source of *Salmonella* contamination (Hamilton et al., 2018). The warm water together with high microbial load and other ambient conditions prevailing in tropical aquatic environment makes suitable habitat for *Salmonella* and promotes the growth, multiplication and long-term survival of *Salmonella* serovars (FAO, 2010). Interestingly, the absence of *Salmonella i*n shrimp farms of Kerala selected for this study might be due to proper adoption and implementation of good management practices (GMP) including strict biosecurity measures in shrimp farms compared to fish farms in this region. From Asian countries, S. Weltevreden was reported as the most common *Salmonella* serotype from aquatic environment as well as seafood (Koonse et al., 2005; Kumar et al., 2009; Noor Uddin et al., 2015). *But this study identifies S.* Typhimurium as the only *Salmonella* serotype from aquaculture systems of Kerala. S. Typhimurium was reported from aquaculture farms of Malaysia (Sing et al., 2016) and China (Zhang et al., 2015; Li et al., 2017).

In this study, all the S. Typhimurium isolates (n=8) were sensitive to all the 17 antibiotics tested. The results highlight the responsible usage of antibiotics in finfish farms of Kerala selected for this study. Generally, antibiotics are being used excessively in aquaculture farms as therapeutics, prophylactics and as growth promoters (Zhang et al., 2015). Such misuse of antibiotics in sublethal doses in aquafarms contributes significantly in emergence of antibiotic resistance among the aquatic microflora. The genes responsible for resistance against antibiotics may get easily transmitted between the host, pathogen and environment and makes the situation more complex.

In this study, all S. Typhimurium isolates from aquaculture were screened for the presence of 11 virulence genes which belongs to SPI 1 to 5 such as *inv*A, *fim*A, *stn, spv*C, *sop*B, *mgt*C, *bcf*C, *csg*D, *avr*A, *hil*A and *pho*P/*pho*Q by PCR. The results revealed that 100% of the S. Typhimurium were found to be positive for all the genes screened except *spv*C gene which codes for plasmid virulence. As reported earlier, all *Salmonella* strains harbours *inv*A gene and it was targeted widely for PCR detection of various *Salmonella* serotypes (Rahn et al., 1992). The fimbrial operon *fim*A gene, chromosomal encoded toxin production *stn* gene and hyperinvasive locus A (*hil*A) gene were also considered as conserved genes among *Salmonella* serotypes (Thung et al., 2018). The *mgt*C gene upregulates and promotes magnesium as well as phosphate intake of *Salmonella* which is very much needed for intramacrophage survival within the host (Elkenany et al., 2019). In addition, the high-level constitutive expression of *mgt*C gene in S. Typhimurium confers to increased thermotolerance (Gall et al., 2018) which may act as a critical factor

for long term persistence of Salmonella in adverse conditions. The biofilm forming genes of *Salmonella* such as *csg*D and *bcf*C have important role in *Salmonella* persistence as well as in virulence (Elkenany et al., 2019). The *avr*A gene codes for Avr protein which belongs to acetyltransferases family and is translocated into intestinal epithelial cells during the initial stages of *Salmonella* invasion. They have potential role in immunosuppression of host upon *Salmonella* infection (Wu et al., 2012). The role of plasmid virulence factors in pathogenicity of *Salmonella* serotypes are still unclear, but it is presumed that they affect the intramacrophage survival of *Salmonella* within the host (Singh et al., 2018).

Among the various non-typhoidal Salmonella serotypes, S. Typhimurium is identified as a common serotype with cosmopolitan profile and wide host range (Ferrari et al, 2019) among food animals. The presence of various chromosomal virulence genes which belongs to SPI 1-5 among the S. Typhimurium strains isolated from aquaculture farms notifies them as potential human pathogens. They can cause considerable food borne infection and their presence in aquaculture farms pose a serious risk towards public health safety and security. Many previous studies reported that S. Typhimurium alone account for approximately 50% of all *Salmonella* isolates globally reported from human clinical cases (Tennant et al., 2016) after *S.* Enteritidis.

In conclusion, aquaculture farms especially fish farms selected for this study was contaminated with *Salmonella* and pose a potential risk to public health. The absence of resistance towards antibiotics can be considered as a positive sign towards consumer safety. But, the presence of potentially virulent S. Typhimurium in finfish farms of Kerala emphasis the need for integrated *Salmonella* surveillance from different sources such as animals, human beings and environment to find out the possible source of origin. Adequate training should also be envisaged for aquafarmers to understand the link between the faecal bacteria contamination and the likelihood of *Salmonella* occurrence in farming systems. Aquafarmers should also be trained to identify potential *Salmonella* sources which are within their control among various aquaculture inputs. Additionally, government authorities should strictly monitor the adoption and implementation of the GMP/HACCP protocols together with proper biosecurity measures in aquafarms. Such interventions strategies are inevitable to control and eliminate the *Salmonella* from aquaculture so as to ensure public health and safety.

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References

Ampofo, J.A. and Clerk, G.C. (2010) Diversity of Bacteria Contaminants in Tissues of Fish Cultured in Organic Waste-Fertilized Ponds Health Implications. Open J. Fish. Res. 3: 142-146

Andrews, W.H., Jacobson, A. and Hammack, T. (2007) BAM Chapter 5: Salmonella. Bacteriological Analytical Manual. Available at online at https://www.fda.gov/food/laboratory-methods-food/bam-chapter-5-salmonella

Antunes, P., Campos, J., Mourão, J., Pereira, J., Novais, C. and Peixe, L. (2018) Inflow water is a major source of trout farming contamination with Salmonella and multidrug resistant bacteria. Sci. Total Environ. 642: 1163-1171

Beshiru, A., Igbinosa, I.H. and Igbinosa, E.O. (2019) Prevalence of antimicrobial resistance and virulence gene elements of Salmonella serovars from ready-to-eat (RTE) shrimps. Front. Microbiol. 10: 1613

Bhaskar, N., Setty, T.R., Reddy, G.V.S., Manoj, Y.B., Anantha, C.S., Raghunath, B.S. and Antony, J.M. (1995) Incidence of Salmonella in cultured shrimp Penaeus monodon. Aquac. Res. 138(1-4): 257-266

Borges, K.A., Furian, T.Q., Borsoi, A., Moraes, H.L., Salle, C.T. and Nascimento, V.P. (2013) Detection of virulence-associated genes in Salmonella Enteritidis isolates from chicken in South of Brazil. Pesqui. Vet. Bras. 33(12): 1416-1422

Cardona-Castro, N., Restrepo-Pineda, E. and Correa-Ochoa, M. (2002) Detection of hilA gene sequences in serovars of Salmonella enterica subspecies enterica. Mem. Inst. Oswaldo Cruz. 97: 1153-1156

CDC (2021) Centers for disease control and prevention. Salmonella home page, Retrieved from https://www.cdc.gov/salmonella/index.html

CLSI. Performance standards for antimicrobial susceptibility testing. 30th Ed. CLSI supplement M100. Wayne. P. A, Clinical and laboratory Standards Institute, 2020

Dalsgaard A, Huss HH, H-Kittikun A, Larsen J. (1995) The prevalence of *Vibrio cholerae* and *Salmonella* in a

major shrimp production area in Thailand. Int J Food Microbiol. 28(1): 101-113

Deguenon, E., Dougnon, V., Lozes, E., Maman, N., Agbankpe, J., Abdel-Massih, R.M., Djegui, F., Baba-Moussa, L. and Dougnon, J. (2019) Resistance and virulence determinants of faecal Salmonella spp. isolated from slaughter animals in Benin. BMC Res. Notes. 12(1): 1-7

Elkenany, R., Elsayed, M.M., Zakaria, A.I., El-Sayed, S.A.E.S. and Rizk, M.A. (2019) Antimicrobial resistance profiles and virulence genotyping of Salmonella enterica serovars recovered from broiler chickens and chicken carcasses in Egypt. BMC Vet. Res. 15(1): 1-9

FAO (2020) The State of World Fisheries and Aquaculture (SOFIA) Available online on https://www.fao.org/publications/sofia/2020/en/

FAO (2010) Expert Workshop on the Application of Biosecurity Measures to Control Salmonella Contamination in Sustainable Aquaculture. Mangalore, India, 19–21 January. FAO Fisheries and Aquaculture Report No 937. Rome, FAO. 39

Fernandes, D.V.G.S., Castro, V.S., Cunha, A.D. and Figueiredo, E.E.D.S. (2018) Salmonella spp. in the fish production chain: a review. Ciência Rural, 48. Available online on https://doi.org/10.1590/0103-8478cr20180141

Ferrari, R.G., Rosario, D.K., Cunha-Neto, A., Mano, S.B., Figueiredo, E.E. and Conte-Junior, C.A. (2019) Worldwide epidemiology of Salmonella serovars in animal-based foods: a meta-analysis. Appl. Environ. Microbiol. 85(14): 00591-19

Gall, A.R., Hegarty, A.E., Datsenko, K.A., Westerman, R.P., SanMiguel, P. and Csonka, L.N. (2018) High level, constitutive expression of the mgtC gene confers increased thermotolerance on Salmonella enterica serovar Typhimurium. Mol. Microbiol. 109(3): 327-344

Hamilton, K.A., Chen, A., Johnson, E.D.G., Gitter, A., Kozak, S., Niquice, C., Zimmer-Faust, A.G., Weir, M.H., Mitchell, J. and Gurian, P.L. (2018) Salmonella risks due to consumption of aquaculture-produced shrimp. Microb. Risk Anal. 9: 22-32

Jayasankar, P. (2018) Present status of freshwater aquaculture in India-A review. Indian J. Fish. 65(4):157-65

Koonse B, Burkhardt W, III, Chirtel S, Hoskin GP. (2005) Salmonella and the sanitary quality of aquacultured shrimp. J. Food Prot. 68(12): 2527–2532

Kumar, R., Surendran, P.K. and Thampuran, N. (2009) Distribution and genotypic characterization of Salmonella serovars isolated from tropical seafood of Cochin, India. J. Appl. Microbiol. 106(2): 515-524

Li, K., Petersen, G., Barco, L., Hvidtfeldt, K., Liu, L. and Dalsgaard, A. (2017) Salmonella Weltevreden in integrated and non-integrated tilapia aquaculture systems in Guangdong, China. Food Microbiol. 65: 19-24

Makino, S.I., Kurazono, H., Chongsanguam, M., Hayashi, H., Cheun, H.I., Suzuki, S. and Shirahata, T. (1999) Establishment of the PCR system specific to Salmonella spp. and its application for the inspection of food and fecal samples. J. Vet. Med. Sci. 61(11): 1245-1247

Noor Uddin, G.M., Larsen, M.H., Barco, L., Minh Phu, T. and Dalsgaard, A. (2015) Clonal occurrence of Salmonella Weltevreden in cultured shrimp in the Mekong Delta, Vietnam. PLoS One. 0(7): 0134252

Ogg, J.E., Ryder, R.A. and Smith Jr, H.L. (1989) Isolation of Vibrio cholerae from aquatic birds in Colorado and Utah. Appl. Environ. Microbiol. 55(1): 95-99

Rahn, K., De Grandis, S.A., Clarke, R.C., McEwen, S.A., Galan, J.E., Ginocchio, C., Curtiss Iii, R. and Gyles, C.L. (1992) Amplification of an invA gene sequence of Salmonella typhimurium by polymerase chain reaction as a specific method of detection of Salmonella. Mol. Cell. Probes. 6(4): 271-279

Sánchez, S., Sánchez, S., Herrera-León, S., Porrero, C., Blanco, J., Dahbi, G., Blanco, J.E., Mora, A., Mateo, R., Hanning, I. and Vidal, D. (2013) Prevalence of Shiga toxin-producing Escherichia coli, Salmonella spp. and Campylobacter spp. in large game animals intended for consumption: relationship with management practices and livestock influence. Vet. Microbiol. 163(3-4): 274-281

Sheng, L. and Wang, L. (2021) The microbial safety of fish and fish products: Recent advances in understanding its significance, contamination sources, and control strategies. Compr. Rev. Food Sci. Food Saf. 20(1): 738-786

Sing, C.K., Khan, M.Z.I., Daud, H.H.M. and Aziz, A.R. (2016) Prevalence of Salmonella sp in African Catfish (Clarias gariepinus) obtained from farms and wet markets in Kelantan, Malaysia and their antibiotic resistance. Sains Malays. 45(11): 1597-1602

Singh, Y., Saxena, A., Kumar, R. and Kumar Saxena, M. (2018) Virulence system of Salmonella with special reference to Salmonella enterica. *Intech*. 41-53

Soto, S.M., Rodríguez, I., Rodicio, M.R., Vila, J. and Mendoza, M.C. (2006) Detection of virulence determinants in clinical strains of Salmonella enterica serovar Enteritidis and mapping on macro restriction profiles. J. Med. Microbiol. 55(4): 365-373

Tennant, S. M., MacLennan, C. A., Simon, R., Martin, L. B., Khan, M. I. (2016) Non-typhoidal *Salmonella* disease: current status of vaccine research and development. Vaccine. 34: 2907-10

Thung, T.Y., Radu, S., Mahyudin, N.A., Rukayadi, Y., Zakaria, Z., Mazlan, N., Tan, B.H., Lee, E., Yeoh, S.L., Chin, Y. Z. and Tan, C.W. (2018) Prevalence, virulence

genes and antimicrobial resistance profiles of Salmonella serovars from retail beef in Selangor, Malaysia. Front Microbiol. 8: 2697

Wang, C., Chang, T., Yang, H. and Cui, M. (2015) Antibacterial mechanism of lactic acid on physiological and morphological properties of Salmonella Enteritidis, Escherichia coli and Listeria monocytogenes. Food Control. 47: 231-236

Wu, H., Jones, R.M. and Neish, A.S. (2012) The Salmonella effector AvrA mediates bacterial intracellular survival during infection in vivo. Cell Microbiol. 14(1): 28-39

Zhang, J., Yang, X., Kuang, D., Shi, X., Xiao, W., Zhang, J., Gu, Z., Xu, X. and Meng, J. (2015) Prevalence of antimicrobial resistance of non-typhoidal Salmonella serovars in retail aquaculture products. Int. J. Food Microbiol. 210: 47-52