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# Assessing the Safety of Fish Retail Shops and Associated Environment for Antimicrobial Resistant Non–Typhoidal *Salmonella*

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## Abstract

Non-typhoidal salmonellosis stands among the major foodborne illnesses. Outbreaks from contaminated fishes have been witnessed worldwide. A cross-sectional study was performed. A total of 368 samples (fish and associated environmental samples) were collected to assess the presence of non–typhoidal *Salmonella* in fishes and associated environment of 22 retail fish meat shops. The overall occurrence of the organisms was found to be 4.35% (16/368) with highest in fish rinsing water (18.18%, 4/22) followed by knife swabs (11.11%, 3/27), chopping board swabs (8.33%, 2/24), meat swabs (6.52%, 3/46), floor swabs (4.54%, 1/22), gill and intestine swabs (4.34%, 1/23), fish holding water (2.43%, 1/41). *S*.Typhimurium (87.5%, 14/16) was found as the prevalent serovar. Major virulence genes harboured were *sipA* gene (87.5%) followed by *stn* (75%), *sop*B (68.75%), *sop*E1 (56.25%) *mgt*C (43.75%) and *spv*C and *gip*A (12.5%) genes each. The isolates were highly resistant against Tetracycline and Ampicillin (93.75%, 15/16) followed by Nalidixic acid (50%), Ciprofloxacin (37.5%) Ofloxacin, Cefotaxime and Sulfisoxazole (25%) each, Chloramphenicol (12.5%) and Streptomycin (6.25%). Ten *Salmonella* (62.5%) isolates were multi drug resistant (MDR). The most commonly occurring resistance genes were *gyr*A (92.30%, 12/13), $bla_{\text{TEM}}$ (53.33%, 8/15), *aad*A1 and *str*A (50%, 2/4), *sul1* (30.76%, 4/13) while *tetA* was not found in any of the isolates. Resistance to critically important fluoroquinolones and highly important Cephalosporin and Tetracycline antibiotics detected in *Salmonella* isolates is a serious threat to public health.

**Keywords:** Non-typhoidal *Salmonella*, fish, multidrug resistant, public health

## Introduction

Fisheries, an important food production sector, helps in providing the country with nutritional stability along with the other agricultural produce including varied animal diets. Besides the nutritional aspect and the increasing demand for fish and fish products forming a part of the human diet, the risk of food illnesses through contaminated fish/fish products seems highly significant, and among the contaminants, bacteria remain the main cause of foodborne illness constituting around 66% of the problems (Abebe et al., 2020). Of all the bacterial foodborne illnesses, non-typhoidal *Salmonella* stands as the second leading cause of foodborne disease worldwide (Wong & Chen, 2013). The disease, salmonellosis results from the consumption of contaminated food products (Zhao et al., 2008 and Pires et al., 2014). The disease, salmonellosis it accounts for 93.8 million cases worldwide per year, resulting in 1,55,000 deaths, of which, 80.3 million are foodborne cases (Majowiez et al., 2010) and 3.4 million cases are of invasive illness (Ao et al., 2015).

Fishes generally do not usually harbour *Salmonella* (Heinitz et al., 2000) but if infected, acts as a passive carrier of *Salmonella* without apparent symptoms and serious disorder. Fish and shellfish may accumulate *Salmonella* from polluted waters (Newaj-Fyzul et al., 2008), contaminated soil (Setti, 2009 and Hoffmann et al., 2016), supply chain and handling process (Olsvik et al., 2013), storage and processing (Panisello et al., 2000) and under environmental

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stress conditions such as high temperature (Zheng et al., 2004).

The human consequences of exposure to non - typhoidal *Salmonella* may differ from having no effect, asymptomatic form, symptomatic with acute gastroenteritis. Mild symptoms include diarrhea, nausea, mild fever, abdominal cramping, headache and dehydration. However, serious complications such as septicemia can occur in people with a weakened immune system and require treatment (Coburn et al., 2007). Occasionally chronic conditions in the form of appendicitis, meningitis and arthritis can occur as a result of non-typhoidal *Salmonella* infection (WHO/FAO, 2002 and FDA, 2012). Typhoidal serovars, on the other hand are few in number (Typhi, Paratyphi and Sendai), totally human restricted and cause invasive, systemic disease in immunocompetent individuals with comparatively longer incubation period (Gal-Mor et al., 2014).

Moreover, massive and indiscriminate usage of antibiotics in food animals including aquaculturehas facilitated the selection and emergence of antimicrobial resistance (AMR) (Davies & Davies, 2010) in the organisms. Considering AMR as a major global crisis (Asín-Prieto et al*.,* 2015; Holmes et al*.,* 2016 and Boucher et al*.,* 2017), World Health Organization has identified the spread of multidrug-resistant non-typhoidal *Salmonella* strains as a significant human and animal health issue (WHO, 2018).

Thus, the present study investigates the occurrence of non-typhoidal *Salmonella* in fishes sold at retail shops along with their associated environment. The antibiotic resistance profile to explore and understand the present and future public health threat was also undertaken.

## Materials and Methods

A total of 368 fish (farmed fresh water fishes sold at retail outlets) samples (gills (n=23), intestines (n=23), fish meat swabs (n=46), rinsing water (n=22), fish skin swabs (n=25), knife swabs (n=27), chopping boards swabs (n=24), hand swabs (n=44), floor swabs (n=22), fish holding water (water container in which fishes were kept before being slaughtered) (n=41), container swabs (n=47) and utensils swabs (n=24) were collected from 22 retail fish meat shops representing, Haldwani (n=10), Lalkuan (n=3), Pantnagar (n=6) and Kiccha (n=6) areas of Uttarakhand state, India during November 2019-March 2020. Briefly, sterile 100 ml sterile sample containers (Abdos, India) were used for the collection of water/ rinse water samples (10 ml). The swab samples were collected using sterile buffered peptone water (BPW, BD Difco, USA) swabs (10 ml) prepared in sterile polypropylene tubes (Hi-Media, India). The samples were collected aseptically and brought immediately to the laboratory under cold chain for processing.

The isolation and identification of non–typhoidal *Salmonella* was performed as described by Keelara et al. (2013) using standard protocol. Briefly, the samples were first pre-enriched in buffered peptone water (BPW) (Difco, USA), i.e., one part of the sample was added to 9 parts of BPW, mixed thoroughly, and incubated at 37ºC for 24 h. Thereafter, 0.1 ml of the pre-enriched sample was transferred to 9.9 ml of Rappaport Vassiliadis enrichment broth (RV-10) (Difco, USA) and incubated at 42ºC for 24 h. A loop full of the enriched sample from RV-10 was then plated onto Xylose Lysine Tergitol-4 (XLT-4) agar (Hi-Media) and incubated at 37ºC for 24 h. A total of 3-4 individual black coloured colonies were picked from each plate and inoculated onto triple sugar iron (TSI) (Difco, USA) and the urea slants (HiMedia, India) for biochemical confirmation. All presumptive *Salmonella* isolates tested positive on TSI and negative on urea slants were subjected to DNA isolation (Pui et al., 2011).

The DNA of the organisms was extracted by using thermal lysis method (Reischl et al*.,* 2000). A duplex PCR was used for the confirmation of *Salmonella* genus and *Salmonella* Typhimurium isolates targeting *ompC* (204bp) and *typh* (401bp) genes, respectively (Alvarez et al., 2004). Primers used for the genus identification were *ompC*-F (5′-ATCGCTGACTTATGCAATCG-3′), *ompC*-R (5′CGGGTTGCCTTATAGGTCTG-3′) and for the serovarTyphimurium, *typh*-F (5′-TTGTTCACTTTTTACCCCTGAA-3′) and *typh*-R (5′-CCCTGACAGCCGTTAGATATT-3′). The PCR cycling conditions were as mentioned by Alvarez et al. (2004).

Antimicrobial resistance (AMR) profile of all *Salmonella* isolates was prepared by using standard Kirby-Bauer disc diffusion method (Bauer et al., 1966). A panel of nine antimicrobials comprising of Ampicillin (AMP, 10µg), Streptomycin (S, 10µg), Nalidixic Acid (NA, 30µg), Ciprofloxacin (CIP, 5µg), Cefotaxime (CTX, 30µg), Tetracycline (TE, 30µg),

Ofloxacin (OF, 5μg), Chloramphenicol (C, 30μg) and Sulfisoxazole (SF, 300μg) representing seven different antimicrobial classes was used. The results were interpreted as per Clinical and Laboratory Standards Institute for disk-diffusion assay (CLSI, 2019), using ATCC25922 *Escherichia coli* culture as a reference strain. The isolates resistant to three or more classes of antimicrobials were classified as Multidrug Resistant (MDR) (Magiorakos et al., 2012).

The *Salmonella* isolates exhibiting resistance to a class of antimicrobial were subjected to MIC determination using E-strips (Himedia, India) as per Cetinkaya et al. (2008). Clinical and Laboratory Standard Institute standards recommendations were used to interpret the MIC breakpoint (CLSI, 2019).

The phenotypic resistant isolate of the class were screened for commonly prevalent genes for resistance. The gene $bla_{TEM}$ (Randall et al., 2004) for Beta-lactam group, for Streptomycin resistance, genes *aad A1 and strA* (Madsen et al., 2000), for Sulfonamide resistant isolates, gene *sul1* (Randall et al., 2004) while for Tetracycline resistant isolates gene coding *tet(A)* (Ng et al., 1999) were targeted for the study. Quinolones resistant isolates was screened for *gyrA* gene (Chau et al., 2007). The primer sequences and respective amplicon sizes are given in Table 2 and the cycling conditions were as followed in respective references.

## Results and Discussion

Of the total 368 samples screened, 16 isolates belonged to genus *Salmonella* through PCR thus revealing an overall presence of 4.35%. The occurrence is less than studies reporting a range between 5.8% (Suwanrangsi et al., 1998) to 31.74% (David et al., 2009). Nambiar & Iyer (1991), Hatha & Lakshmanaperumalsamy (1997), Sanath et al. (2003), Kumar et al. (2008) and Yagoub (2009) observed higher prevalence rate of 8.66%, 14.25%, 30%, 28.80% and 56% respectively from fish samples. Prabhakar et al. (2020) reported an overall prevalence of 20.7% (17/82) in seafood with 19.3% (11/57) in landing centres and 24% (6/25) in retail markets in Mumbai, India. The comparably low prevalence obtained in our study could be due to difference in the locations coupled with prevailing hygienic environmental as well as managemental conditions practiced by respective retail shop owners. It was observed that the shop owners washed their hands in between and changed the holding water apart from keeping more than one container for holding live fishes.

The highest presence of *Salmonella* was observed in rinsing water (18.18%, 4/22) samples followed by knife swabs (11.11%, 3/27), chopping board swabs (8.33%, 2/24), meat swabs (6.52%, 3/46), floor swab (4.54%, 1/22), gill swab and intestine swab (4.34%, 1/23) each and fish water (2.43%, 1/41). None of the hand, fish skin, container, and utensil swab samples tested positive for *Salmonella.* The high presence of *Salmonella* observed in rinsing water (18.18%, 4/22) samples draws attention to the fact that lack of adequate provision of good quality rinsing water in retail meat shops can act as a source of *Salmonella* contamination (Faleke et al., 2017) (Fig. 1). It is seen that the rinsing water may at times and places be collected from a nearby available sources such as contaminated water supplies or ponds/rivers. Also, the water may get contaminated with fish faeces which can further carry the contamination to subsequent steps of processing (Faleke et al., 2017). They reported an overall prevalence of 28.8% *Salmonella* with 73.33% contamination in processing water in Sokoto market in Nigeria.

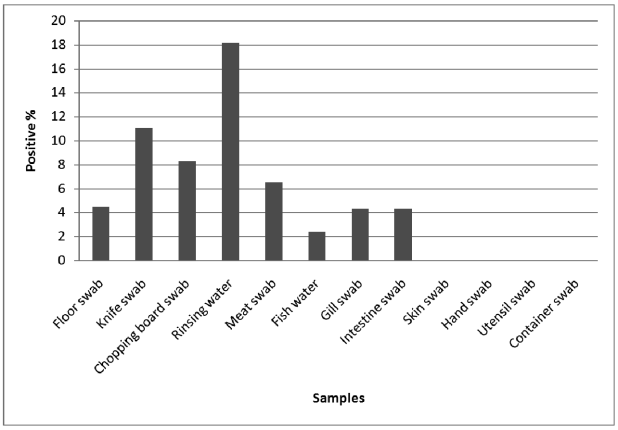


Fig. 1. Distribution of *Salmonella* spp. across different samples

Contamination of water may take place due to improper hygienic and sanitation conditions during storage of processing water in contaminated vessels or either from the source it is procured. Presence of *Salmonella* in knife (11.11%, 3/27) and chopping board swabs (8.33%, 2/24) indicates its repetitive use without being properly washed or sanitized after each slaughter process. Floor contamination indicates unhygienic conditions indicating a potential source for *Salmonella* contamination (Saini, 2019).

Gills and intestine contamination was also reported by Hatha & Lakshmanaperumalsamy (1997) in their study (body surface 32%, gill, 26.7% and intestine 41.3%) conducted in Coimbatore, South India. A study on environmental samples collected from an abattoir in Ethiopia, Teklu & Negussie (2011) reported an overall prevalence of 8.9% in animal samples and 7.2% in the abattoir environment. The authors reported *Salmonella* presence in 10 (5%) skin swabs, 18 (8.9%) hand swabs, 15 (7.4%) knife swabs and 2 (7%) water samples. However, in our study, none of the hand swab and skin swab was found positive for *Salmonella.* This may be due to washing of hands in between and lesser soil contaminated fishes arriving at the shop at the day/time of sampling.

Of the sixteen *Salmonella* isolates, fourteen were identified as *Salmonella* Typhimurium as they exhibited 401 bp amplicon while two were identified as 'others' (non *S*. Typhimurium) on failure of detection of *typh* gene. *S*. Typhimurium is among the highly prevalent zoonotic serovar and thus was targeted for the study. Other serotypes that may predominate are Enteritidis, Stanley and Weltevreden in Asia (Fernandes et al., 2018). A 10.4% prevalence of *Salmonella* in fresh fish was found in Iran, where five different serotypes were detected, namely *S*. Typhimurium, *S.* Enteritidis, *S*. Typhi, *S*. Paratyphi B and *S*. Newport (Rahimi et al., 2013).

Out of sixteen *Salmonella* isolates, ten were found to be multidrug-resistant (MDR). The highest resistance was observed against Tetracycline and Ampicillin (93.75%) followed by Nalidixic acid (50%), Ciprofloxacin (37.5%), Ofloxacin, Cefotaxime and Sulfisoxazole (25%) each, Chloramphenicol (12.5%) and Streptomycin (6.25%). Of the 14 *S.* Typhimurium isolates, maximum resistance was exhibited against Tetracycline and Ampicillin (92.85%) followed by Nalidixic acid (50%), Ciprofloxacin (28.57%), Ofloxacin, Sulfisoxazole and Cefotaxime (21.42%) each, Chloramphenicol (14.28%), Streptomycin (7.14%) (Table 1).

In the year 2017, WHO specifically ranked fluoroquinolone-resistant *Salmonella* as a high priority pathogen for the research and development of new antibiotics (Tacconelli et al., 2018). Hence the emergence of resistance for fluoroquinolones in non-typhoidal *Salmonella* is of a global concern. Other than quinolones, high resistance was also seen towards Ampicillin (87.5%). The high-level antibiotic resistance of *Salmonella* in the present study may be related to the uncontrolled use of these antimicrobials for animal therapy, prophylaxis, or as

Table 1. Detail of multidrug resistance pattern of non-typhoidal *Salmonella* isolates from retails fish markets of Uttarakhand

| Sample No. | Sample type | Serotype | Resistant phenotype | No. of antimicrobials | Class of antimicrobials | Location |
|---|---|---|---|---|---|---|
| 1 | Floor swab | *S.*Typhimurium | S, TE, OF, NA, CIP | 5 | 3 | Lalkuan |
| 2 | Knife swab | *S.*Typhimurium | CTX, TE, C, AMP, NA, CIP | 6 | 4 | Lalkuan |
| 3 | Knife swab | Other | CTX, TE, OF, AMP, NA, CIP, SF | 7 | 4 | Haldwani |
| 4 | Chopping board swab | *S.*Typhimurium | CTX, TE, AMP, NA | 4 | 3 | Haldwani |
| 5 | Rinsing water | *S.*Typhimurium | C, AMP, NA | 3 | 3 | Haldwani |
| 6 | Rinsing water | *S.*Typhimurium | TE, AMP, SF | 3 | 3 | Haldwani |
| 7 | Rinsing water | Other | TE, AMP, CIP | 3 | 3 | Pantnagar |
| 8 | Meat swab | *S.*Typhimurium | CTX, TE, OF, AMP, NA, CIP, SF | 7 | 4 | Haldwani |
| 9 | Fish water | *S.*Typhimurium | TE, OF, AMP, NA, CIP, SF | 6 | 4 | Kiccha |
| 10 | Gill swab | *S.*Typhimurium | TE, AMP, NA | 3 | 3 | Haldwani |

growth promoters (Ibrahim et al., 2020). In the marine environment antimicrobials are typically used during the fatten phase delivered via feed medication, bioencapsulation, immersion baths, dip, flush, or in exceptional cases, intramuscularly or intraperitoneally (Smith et al., 2008). The contribution of resistant microbes from various sources seems to be the major base of resistance in the environment (Ibrahim et al., 2020). This phenomenon can limit the therapeutic options for clinical cases that require antimicrobial treatment.

Multi drug resistance has been reported by many authors and is a cause of concern. Zhang et al. (2015) reported 43.3% multidrug resistant *Salmonella* isolates. In our study, Haldwani observed higher number of MDR isolates as Haldwani, a big town catering to the larger population daily may obtains fishes from highly commercialized ponds where antibiotic usage may be high. Capuano et al. (2013) detected 85.71% multidrug resistance in *S.* Typhimurium isolates. In the year 2011, about 5% of *Salmonella* spp. tested by CDC was resistant to five or more type of antimicrobial drugs (CDC, 2015). Increasing resistance against newer antimicrobial drugs (fluoroquinolones and broad spectrum cephalosporins), recommended to treat invasive salmonellosis might lead to treatment failure.

*Salmonella* isolates showed very high MIC values for Nalidixic acid i.e.> 256 μg $mL^{-1}$. MICs for Ciprofloxacin resistance in *Salmonella* isolates ranged from 0.25-8μg $mL^{-1}$ (Table 2).

For Cefotaxime, MICs was 0.38μl/ml for two *S.*Typhimurium isolates. One *S.*Typhimurium and one 'other' *Salmonella* serovar were found Cefotaxime resistant as per the CLSI breakpoint (>4 μg /ml) and showed MIC value >16 μg /ml. An increase in MIC is a serious threat because treatment failures have been reported for isolates with MIC value for Ciprofloxacin as low as 0.125 μg /ml (Sjölund-Karlsson et al., 2014). Detection of resistance toward Ciprofloxacin and Cefotaxime indicates indiscriminate use of these antimicrobials (Saini, 2019). Ciprofloxacin and Cefotaxime are classified as the highest priority- critically important antimicrobial by the WHO (2018).

Out of 15 Beta lactam resistant and intermediate isolates, 8 isolates carried the $bla_{TEM}$ gene. Out of 4 Streptomycin resistant and intermediate isolates, 2 isolates (1 resistant and 1 intermediate) carried *aad*1 gene, and 2 intermediate resistant isolates harboured *str*A gene. Tetracycline resistance was detected by the presence of the *tet*A gene. None of the Tetracycline resistance isolate harboured the *tet*A gene. Two Sulfisoxazole resistant and 2 intermediate resistant isolates carried the *sul1* gene (317bp). Resistance towards quinolones (Nalidixic acid, Ciprofloxacin, and Ofloxacin) was detected by the presence of *gyr*A genes. Out of 13 phenotypically resistant to quinolones, 12 *Salmonella* isolates harboured the *gyr*A gene (Table 3).

The gene *blaTEM* has been also reported by Djeffal et al. (2017) and Trongjit et al. (2017) in *Salmonella* isolates from humans and livestock. The beta-lactam

Table 2. Distribution of MIC values of *Salmonella* isolates

| S. No. | Sample | Location | Serovar | NA μg $mL^{-1}$ | CIP μg $mL^{-1}$ | CTX μg $mL^{-1}$ |
|---|---|---|---|---|---|---|
| 1 | Floor swab | Lalkuan | *S.*Typhimurium | >256 | 4 | NT* |
| 2 | Knife swab | Lalkuan | *S.*Typhimurium | >256 | 6 | >16 |
| 3 | Fish water | Kiccha | *S.*Typhimurium | >256 | 8 | NT |
| 4 | Meat swab | Haldwani | *S.*Typhimurium | >256 | 8 | .38 |
| 5 | Knife swab | Haldwani | Other | >256 | 0.25 | >16 |
| 6 | Gill swab | Haldwani | *S.*Typhimurium | >256 | NT | NT |
| 7 | Rinsing water | Haldwani | *S.*Typhimurium | >256 | NT | NT |
| 8 | Chopping board | Haldwani | *S.*Typhimurium | >256 | NT | .38 |
| 9 | Rinsing water | Pantnagar | Other | NT | 4 | NT |

*Not tested

Table 3. Distribution of antimicrobial resistant genes (ARGs) profile in *Salmonella* isolates

| S. No. | Sample type | Serotype | Resistance Pattern | Resistance genes |
|---|---|---|---|---|
| 1 | Floor swab | *S.*Typhimurium | S,TE,OF,NA,CIP | *gyrA, aadA1, sul1* |
| 2 | Knife swab | *S.*Typhimurium | CTX,TE,C,AMP,NA,CIP | *gyrA* |
| 3 | Chopping board swab | *S.*Typhimurium | CTX,TE,AMP,NA | *gyrA,* $bla_{TEM}$ |
| 4 | Intestine swab | *S.*Typhimurium | TE,AMP | *gyrA* |
| 5 | Rinsing water | *S.*Typhimurium | C,AMP,NA | *gyrA,* $bla_{TEM}$ |
| 6 | Meat swab | *S.*Typhimurium | CTX,TE,OF,AMP,NA,CIP,SF | *gyrA,* $bla_{TEM}$, *sul1, strA* |
| 7 | Knife swab | Other | CTX,TE,OF,AMP,NA,CIP,SF | *gyrA,* $bla_{TEM}$ |
| 8 | Knife swab | *S.*Typhimurium | TE,AMP | $bla_{TEM}$ |
| 9 | Chopping board swab | *S.*Typhimurium | TE,AMP | $bla_{TEM}$ |
| 10 | Rinsing water | Other | TE,AMP,CIP | *gyrA,* $bla_{TEM}$ |
| 11 | Rinsing water | *S.*Typhimurium | TE,AMP,SF | *gyrA, strA,* |
| 12 | Fish water | *S.*Typhimurium | TE,OF,AMP,NA,CIP,SF | *gyrA, sul1, aadA1* |
| 13 | Gill swab | *S.*Typhimurium | TE,AMP,NA | *gyrA* |
| 14 | Rinsing water | *S.*Typhimurium | TE,AMP | *gyrA* |
| 15 | Meat swab | *S.*Typhimurium | TE,AMP | $bla_{TEM}$ |
| 16 | Meat swab | *S.*Typhimurium | TE, AMP | *sul1* |

resistance in *Enterobacteriaceae* is usually attributed to the production of enzymes such as extended spectrum beta-lactamases (Ali et al., 2018). These enzymes have been recognized in many species of the *Enterobacteriaceae* family including different serotypes of *Salmonella enterica* (Pokharel et al., 2006). Acquisition of such resistance gene encoding ESBLs, the attainment of resistance to beta-lactams is becoming a serious health concern. The presence of *str*A gene has been described from plants animals and humans (Carattoli et al., 2002). This gene conferring resistance to streptomycin along with *aad1* in our isolates is of concern owing to the horizontal transfer of the genes. The high prevalence of *aad*A1 gene among all Streptomycin resistant *Salmonella* isolates was also reported by Chen et al. (2004).

Randall et al. (2004) recognized the *sul*1 gene as the most common gene associated with Sulfonamide resistant *S.* Typhimurium. Ahmed et al. (2016) reported a *sul*1 gene in 96.7% Sulfonamide resistant *S.* Typhimurium in Egypt. Sharma et al. (2019) also found predominant presence of the *sul*1 gene in 82.35% of screened *Salmonella* isolates from North India. In contrast, Odoch et al. (2018) could not detect *sul*1 gene among the Sulfonamide resistant *Salmonella* isolates.

The absence of the *tet*A gene in the resistant isolates of our study may be either due to the limitations of the Kirby disc diffusion method or the presence of genes other than *tetA* in those isolates (Kerrn et al., 2002). A zone of inhibition in Kirby disc diffusion method depends upon drug solubility, rate of drug diffusion through agar, the thickness of the agar medium, and the drug concentration impregnated into the disc (Kerrn et al., 2002).

Twelve quinolone resistant and intermediately resistant *Salmonella* isolates harboured the *gyr*Agene. The chief mechanisms responsible for resistance against fluoroquinolones include mutations in the quinolones resistance determining region (QRDR) of DNA gyrase and topoisomerase (Seminati et al., 2005) and the presence of plasmid-mediated Quinolone resistance gene (PMQR) (Ferrari et al., 2013). Fluoroquinolones are also the drug of choice

to treat invasive salmonellosis in adults. Further investigation in assessing the mutation in a gene can be carried out through sequencing to confirm their role in exhibiting resistance to fluoroquinolones.

Fishes are generally looked for contamination of chemical contaminants. However, the presence of food pathogens cannot be overlooked. The overall presence of 4.35% *Salmonella* in the fish and environmental samples of fish retail shops warrants public health threat. The study reflects that implementation of strict quality assurance in production, handling and sale of fish is necessary. Initial contamination in the food production chain can hamper the safety of fish products.

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## References

Abebe, E., Gugsa, G. and Ahmed, M. (2020) Review on Major Food-Borne Zoonotic Bacterial Pathogens. J. Trop. Med.

Ahmed, H.A., El-Hofy, F.I., Shafik, S.M., Abdelrahman, M.A. and Elsaid, G.A. (2016) Characterization of virulence-associated genes, antimicrobial resistance genes, and class 1 integrons in *Salmonella enterica* serovar Typhimurium isolates from chicken meat and humans in Egypt. Foodborne Pathog. Dis. 13(6): 281-288

Ali, T., Ali, I., Khan, N.A., Han, B. and Gao, J. (2018) The growing genetic and functional diversity of extended spectrum beta-lactamases. Bio.Med. research international. 14: 665

Alvarez, J., Sota, M.,Vivanco, A., Perales, I., Cisterna, R., Rementeria, A. and Garaizar, J. (2004) Development of a multiplex PCR technique for detection and epidemiological typing of *Salmonella* in human clinical samples. J. Clin. Microbiol. 42: 1734-1738

Ao, T. T., Feasey, N.A., Gordon, M.A., Keddy, K.H., Angulo, F.J. and Crump, J.A. (2015) Global Burden of Invasive Non-Typhoidal Salmonella Disease. Emerg. Infect. Dis. 21(6): 941-949

Asín-Prieto, E., Rodríguez-Gascón, A. and Isla, A. (2015) Applications of the pharmacokinetic/pharmacodynamic (PK/PD) analysis of antimicrobial agents. J. Infect. Chemother. 21(5): 319-329

Bauer, A.W., Kirby, W.M.M., Sherris, J. C. and Turck, M. (1966) Antibiotic Susceptibility Testing by a Standardized Single Disk Method. Am. J. Clin. Pathol. 45: 493-496

Boucher, H.W., Ambrose, P.G., Chambers, H.F., Ebright, R.H., Jezek, A., Murray, B.E., Newland, J.G., Ostrowsky, B., Rex, J.H. and Infectious Diseases Society of America (2017) White paper: developing antimicrobial drugs for resistant pathogens, narrow-spectrum indications, and unmet needs. J. Infect. Dis. 216(2): 228-236

Capuano, F., Mancusi, A., Capparelli, R., Esposito, S. and Proroga, Y.T. (2013) Characterization of drug resistance and virulotypes of *Salmonella* strains isolated from food and humans. Foodborne. Pathog. Dis. 10(11): 963-968

Carattoli, A., Tosini, F., Giles, W.P., Rupp, M.E., Hinrichs, S.H., Angulo, F.J., Barrett, T.J. and Fey, P.D. (2002) Characterization of plasmids carrying CMY-2 from expanded-spectrum Cephalosporin resistant *Salmonella* strains isolated in the United States between 1996 and 1998. Antimicrob. Agents Chemother. 46: 1269-1272

Centers Disease Control and Prevention (CDC) (2015) *Salmonella*- reports of active Salmonella outbreak investigations.https://www.cdc.gov/salmonella/ outbreaks-active.html

Cetinkaya, F., Cibik, R., Soyutemiz, G.E., Ozakin, C., Kayali, R. and Levent, B. (2008) *Shigella* and *Salmonella* contamination in various foodstuffs in Turkey. Food Control. 19(11): 1059-1063

Chau, T.T., Campbell, J.I., Galindo, C.M., Hoang, N.V.M., Diep, T.S., Nga, T.T.T., Chau, N.V.V., Tuan, P.Q., Page, A.L., Ochiai, R.L. and Schultsz, C. (2007) Antimicrobial drug resistance of *Salmonella* entericaserovar Typhi in Asia and molecular mechanism of reduced susceptibility to the fluoroquinolones. Antimicrob. Agents. Chemother. 51(12): 4315-4323

Chen, S., Zhao, S., White, D.G., Schroeder, C.M., Lu, R., Yang, H., McDermott, P.F., Ayers, S. and Meng, J. (2004) Characterization of multiple-antimicrobial-resistant *Salmonella* serovars isolated from retail meats. Appl. Environ. Microbiol. 70(1): 1-7

CLSI (Clinical and Laboratory Standards Institute) (2019)Performance Standards for Antimicrobial Susceptibility Testing. Twenty-Ninth Edition. CLSI document M100. Wayne PA

Coburn, B., Grassl, G.A. and Finlay, B.B. (2007) *Salmonella*, the host and disease: a brief review. Immunol. Cell Biol. 85(2): 112-118

David, O.M., Wandili, S., Kakai, R. and Waindi, E.N. (2009) Isolation of *Salmonella* and *Shigella* from fish harvested from the Winam Gulf of Lake Victoria, Kenya. J. Infect. Developing. Countries. 3(2): 99-104

Davies, J. and Davies, D. (2010) Origins and evolution of antibiotic resistance. Microbiol. Mol. Biol. Rev. 74(3): 417-433

Djeffal, S., Bakour, S., Mamache, B. Elgroud,R., Agabou, A. Chabou, S., Hireche, S., Bouaziz, O., Rahal, K. and Rolain, J.M. (2017) Prevalence and clonal relationship of ESBL-producing *Salmonella* strains from humans and poultry in northeastern Algeria. BMC Vet Res. 13: 132

Faleke, O.O., Jolayemi, K.O., Igoh, Y.O., Jibril, A.H. and Ayedun, J.O. (2017) *Salmonella* species on meat contact surfaces and processing water in Sokoto main market and abattoir. Nigeria. Maced. Vet. Rev. 40(1): 59-65

FDA (Food and Drug Administration) (2012) Bad bug book: Foodborne pathogenic microorganisms and natural toxins handbook, 2nd edn. US Food and Drug Administration, Silver Spring. 12-16

Fernandes, D.V.G.S., Vinicius Silva Castro, V.S., Neto, A.C. and Figueiredo, E.E.S. (2018) *Salmonella* spp. in the fish production chain: a review. Cienc. Rural: 48

Ferrari, R., Galiana, A., Cremades, R., Rodríguez, J.C., Magnani, M., Tognim, M.C.B., Oliveira, T. C. and Royo, G. (2013) Plasmid- mediated quinolone resistance (PMQR) and mutations in the topoisomerase genes of *Salmonella* enterica strains from Brazil. Braz. J. Microbiol. 44(2): 657-662

Gal-Mor, O., Boyle, E.C. and Grassl, G.A. (2014) Same species, different diseases: how and why typhoidal and non-typhoidal *Salmonella* entericas erovars differ. Front Microbiol. 5: 391

Hatha, A.M. and Lakshmanaperumalsamy, P. (1997) Prevalence of *Salmonella* in fish and crustaceans from markets in Coimbatore, South India. Food. Microbiol. 14(2): 111-116

Heinitz, M.L., Ruble, R.D., Wagner, D. E. and Tatini , S. R. (2000) Incidence of *Salmonella* in fish and seafood. J. Food Protec. 63(5): 579-592

Hoffmann, M., Luo, Y., Monday, S.R., Gonzalez-Escalona, N., Ottesen, A.R., Muruvanda, T., Wang, C., Kastanis, G., Keys, C., Janies, D. and Senturk, I.F. (2016) Tracing origins of the *Salmonella* Bareilly strain causing a food-borne outbreak in the United States. J. Infect. Dis. 213(4): 502-508

Holmes, A.H., Moore, L.S., Sundsfjord, A., Steinbakk, M., Regmi, S., Karkey, A., Guerin, P. J. and Piddock, L. J. (2016) Understanding the mechanisms and drivers of antimicrobial resistance. The Lancet. 387: 176-187

Ibrahim, M., Ahmad, F., Yaqub, B., Ramzan, A., Imran, A., Afzaal, M., Mirza, S. A., Mazhar, I., Younus, M., Akram, Q., Ali Taseer, M. S., Ahmad, A., and Ahmed, S. (2020) Current trends of antimicrobials used in food animals and aquaculture. Antibiotics and Antimicrobial Resistance Genes in the Environment. 39-69

Keelara, S., Scott, H. M., Morrow, W. M., Gebreyes, W.A., Correa, M., Nayak, R., Stefanova, R. and Thakur, S. (2013) Longitudinal study of the distribution of similar antimicrobial resistant Salmonella serovars in pigs and their environment in two distinct swine production system. Appl. Environ. Microbiol. 79(17): 5167-5178

Kerrn, M.B., Klemmensen, T., Frimodt-Møller, N. and Espersen, F. (2002) Susceptibility of Danish *Escherichia coli* strains isolated from urinary tract infections and bacteraemia, and distribution of sul genes conferring sulphonamide resistance. J. Antimicrob. Chemother. 50(4): 513-516

Kumar, K., Saklaini, A.C., Singh, S. and Singh, V.P. (2008) Evaluation of specificity for invA gene PCR for detection of *Salmonella* spp. In Proceeding of VIIthAnnual Conference of Indian Association of Veterinary Public Health Specialists (IAVPHS)

Madsen, L., Aarestrup, F. M. and Olsen, J. E. (2000) Characterization of streptomycin resistance determinants in Danish isolates of *Salmonella* Typhimurium. Vet. Microbiol. 75: 73-78

Magiorakos, A.P., Srinivasan, A., Carey, R.B., Carmeli, Y., Falagas, M.E., Giske, C.G., Harbarth, S., Hindler, J.F., Kahlmeter, G., Olsson-Liljequist, B., Paterson, D.L., Rice, L.B., Stelling, J., Struelens, M.J., Vatopoulos, A., Weber, J.T. and Monnet, D.L. (2012) Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin. Microbiol. Infect. 18(3): 268-81

Majowiez, S.E., Musto, J., Scallan, E., Angulo, F.J., Kirk, M., O'Brien, S.J., Jones, T.F., Fazil, A. and Hoekstra, R. M. (2010) The global burden of Non –Typhoidal *Salmonella* gastroenteritis. Clin. Infect. Dis. 50(6): 882-889

Nambiar, V. and MahadevaIyer, K. (1991) Distribution of *Salmonella* serotypes in fish in retail trade in Kochi. Fish. Technol. 28(1): 33-37

Newaj Fyzul, A., A. Mutani, A. Ramsubhag, and Adesiyun, A. (2008) Prevalence of Bacterial Pathogens and their Anti microbial Resistance in Tilapia and their Pond Water in Trinidad. Zoo. Publ. Health. 55(4): 206-213

Ng, L.K., Mulvey, M.R., Martin, I., Peters, G.A. and Johnson, W. (1999) Genetic characterization of antimicrobial resistance in Canadian isolates of *Salmonella* serovar Typhimurium DT104. Antimicrob. Agents Chemother. 43: 3018-3021

Odoch, T., Sekse, C., L'Abee-Lund, T.M., Høgberg Hansen, H.C., Kankya, C. and Wasteson, Y. (2018) Diversity and antimicrobial resistance genotypes in Non-Typhoidal *Salmonella* isolates from poultry farms in Uganda. Int. J. Environ. Res. Public Health 15(2): 324

Olsvik, P.A., Vikeså, V., Lie, K.K. and Hevrøy, E.M. (2013) Transcriptional responses to temperature and low oxygen stress in Atlantic salmon studied with next-generation sequencing technology. BMC genomics. 14(1): 817

Panisello, P. J., Rooney, R., Quantick, P. C. and Stanwell-Smith, R. (2000) Application of foodborne disease outbreak data in the development and maintenance of HACCP systems. Int. J. Food Microbiol. 59: 221-234

Pires, S.M., Vigre, H., Makela, P. and Hald, T. (2014) Using outbreak data for source attribution of human salmonellosis and campylobacteriosis in Europe. Foodborne. Pathog. Dis. 7(11): 1351-1361

Pokharel, B.M., Koirala, J., Dahal, R.K., Mishra, S.K., Khadga, P.K. and Tuladhar, N.R. (2006) Multidrug-resistant and extended-spectrum beta-lactamase (ESBL)-producing *Salmonella* enterica (serotypes Typhi and Paratyphi A) from blood isolates in Nepal: surveillance of resistance and a search for newer alternatives. Int. J. Infect. Dis. 10(6): 434-438

Prabhakar, P., Lekshmi, M., Ammini, P., Nayak, B. B. and Kumar, S. (2020) *Salmonella* contamination of seafood in landing centers and retail markets of Mumbai, India. J. AOAC. Intern. 103(5): 1361-1365

Pui, C. F., Wong, W. C., Chai, L. C., Nillian, E., Ghazali, F. M., Cheah, Y. K., Nakaguchi, Y., Nishibuchi, M. and Radu, S. (2011) Simultaneous detection of *Salmonella* spp., *Salmonella* Typhi and *Salmonella* Typhimurium in sliced fruits using multiplex PCR. Food Control. 22: 337-342

Rahimi, E., Shakerian, A. and Falavarjani, A.G. (2013) Prevalence and antimicrobial resistance of *Salmonella* isolated from fish, shrimp, lobster, and crab in Iran. Comp. Clin. Pathol. 22: 59-62

Randall, L.P., Cooles, S.W., Osborn, M.K., Piddock, L. J. V. and Woodward, M. J. (2004) Antibiotic resistance genes, integrons and multiple antibiotic resistance in thirty-five serotypes of *Salmonella* enterica isolated from humans and animals in the UK. J. Antimicrob. Chemother. 53(2): 208-216

Reischl, U., Linde, H.J., Metz, M., Leppmeier, B. and Lehn, N. (2000) Rapid identification of methicillin-resistant *Staphylococcus aureus* and simultaneous species confirmation using real-time fluorescence PCR. J. Clin. Microbiol. 38(6): 2429-2433

Saini. S, (2019) Molecular characterization of Non-Typhoidal *Salmonella* serovars isolated from commercial broiler farms and retail chicken meat shops with reference to virulence and antimicrobial resistance. (M.V.Sc. thesis. Govind Ballabh Pant University of Agriculture and Technology.Pantnagar)

Sanath, K. H., Sunil, R., Venugopal, M.N., Karunasagar, Indrani and Karunasagar, I. (2003) Detection of Salmonella spp. in tropical seafood by polymerase chain reaction. Int. J. Food Microbiol. 88(1): 91-95

Seminati, C., Mejia, W., Mateu, E. and Martin, M. (2005) Mutations in the quinolone- resistance determining region (QRDR) of *Salmonella* strains isolated from pigs in Spain. Vet. Microbiol. 106(3-4): 297-301

Setti, I.A. (2009) Characteristics and dynamics of *Salmonella* contamination along the coast of Agadir, Morocco. Appl. Environ. Microbiol. 75: 7700-7709

Sharma, J., Kumar, D., Hussain, S., Pathak, A., Shukla, M., Kumar, V.P., Anisha, P.N., Rautela, R., Upadhyay, A.K. and Singh, S. P. (2019) Prevalence, antimicrobial resistance and virulence genes characterization of Non-Typhoidal *Salmonella* isolated from retail chicken meat shops in Northern India. Food Control. 102: 104-111

Sjölund-Karlsson, M., Howie, R.L., Crump, J.A. and Whichard, J.M. (2014) Fluoroquinolone susceptibility testing of *Salmonella* enterica: detection of acquired resistance and selection of zone diameter breakpoints for levofloxacin and ofloxacin. J. Clin. Microbiol. 52(3): 877-884

Smith P.R., Le Breton A., Horsberg T. E. and Corsin F. (2008) Guidelines for Antimicrobial Use in Aquaculture. Guide to Antimicrobial Use in Animals. 207-218

Suwanrangsi, S., Srimanobhas, K. and Keerativiriyaporn, S. (1998) Incidence of *Salmonella* in fishery products. RAP Publ. (FAO). 24: 254-262

Tacconelli, E., Carrara, E., Savoldi, A., Harbarth, S., Mendelson, M., Monnet, D.L., Pulcini, C., Kahlmeter, G., Kluytmans, J., Carmeli, Y. and Ouellette, M. (2018) Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Inf. Dis. 18(3): 318-327

Teklu, A. and Negussie, H. (2011) Assessment of risk factors and prevalence of *Salmonella* in slaughtered small ruminants and environment in an export abattoir, Modjo, Ethiopia. Am. Eurasian. J. Agric. Environ. Sci. 10: 992-999

Trongjit, S., Angkititrakul, S., Tuttle, R.E., Poungseree, J., Padungtod, P. and Chuanchuen, R. (2017) Prevalence and antimicrobial resistance in *Salmonella* enterica isolated from broiler chickens, pigs and meat products in Thailand–Cambodia border provinces. Microbiol. Immunol. 61(1): 23-33

WHO/FAO (2002) Risk assessments of *Salmonella* in eggs and broiler chickens. World Health Organization and Food and Agriculture Organization of the United Nations, Geneva

Wong, M.H.Y. and Chen, S. (2013) First detection of oqxAB in *Salmonella* spp. isolated from food. Antimicrob. Agen. Chemother. 57(1): 658-660

WHO (2018) Global antimicrobial resistance surveillance system (GLASS) report: early implementation. 2017-2018

Yagoub, S.O. (2009) Isolation of Enterobacteriaceae and Pseudomonas spp. from raw fish sold in fish market in Khartoum state. Afr. J. Microbiol. Res.1(7): 085-088

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

Zhao, S., White, D.G., Friedman, S.L., Glenn, A., Blickenstaff, K., Ayers, S.L., Abbott, J.W., Hall-Robinson, E. and McDermott, P. F. (2008) Antimicrobial resistance in *Salmonella* enterica serovar Heidelberg isolates from retail meats, including poultry, from 2002 to 2006. Appl. Environ. Microbiol. 74(21): 6656-6662

Zheng, D., Mai, K., Liu, S., Cao, L., Liufu, Z., Xu, W., Tan, B. and Zhang, W. (2004) Effect of temperature and salinity on virulence of *Edwardsiellatarda* to Japanese flounder, Paralichthysolivaceus. Aqua. Res. 35(5): 494-500