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Prevalence and antimicrobial resistance of *Salmonella* spp. in raw retail frozen imported freshwater fish to Eastern Province of Saudi Arabia

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PEER REVIEW

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Comments

This is a good research work in which author determined the proportion of imported frozen fish contaminated with *Salmonella* spp. among retail food stores and supermarkets in the Eastern Province of Saudi Arabia. The results add valuable informations to the formulation of food safety and food hygiene measures.

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ABSTRACT

Objective: To determine the proportion of imported frozen fish contaminated with *Salmonella* among retail food stores and supermarkets in the Eastern Province of Saudi Arabia.

Methods: A total of 223 frozen freshwater fish purchased from different supermarkets and grocery stores were analyzed for the presence of foodborne pathogen *Salmonella*. The isolation of *Salmonella* was determined and confirmed by using the methods of US Food and Drug Administration's Bacteriological Analytical Manual, CHROMagar *Salmonella* plus, biochemical tests and API 20E strips. Antimicrobial susceptibilities of *Salmonella* isolates were determined by the disk diffusion method on Muller–Hinton agar, as described by Kirby–Bauer, in accordance with the guidelines of the Clinical and Laboratory Standards Institute.

Results: Out of the total 223 fish samples (20 of catfish, 18 of carfu, 20 of mirgal, 25 of milkfish, 35 of mackerel, 75 of tilapia, and 30 of rohu), 89 (39.9%) were tested positive for *Salmonella*. The prevalence of positive samples were reported for the freshwater fish of pangas (60.0%, $n=12$), carfu (27.7%, $n=5$), mirgal (35.0%, $n=7$), milkfish (52.0%, $n=13$), mackerel (31.4%, $n=11$), tilapia imported from Thailand (64.0%, $n=16$), tilapia imported from India (28.0%, $n=14$), rohu imported from Thailand (26.6%, $n=4$) and rohu imported from Myanmar (46.6%, $n=7$). A total of 140 isolates of *Salmonella* spp. were yielded from at least seven different types of frozen freshwater fish imported from 5 different countries and were tested for their susceptibility to 16 selected antimicrobial agents. The highest antibiotic resistance was observed to tetracycline (90.71%) followed by ampicillin (70%) and amoxicillin–clavulanic acid (45%).

Conclusions: The obtained results of this study shows that these raw retail imported frozen freshwater fish are contaminated with potentially pathogenic *Salmonella* spp. And the study recommend and suggest that there is a need for adequate consumer measures.

KEYWORDS

Salmonella, Antibiogram, Frozen fish, Food safety

1. Introduction

Capture fisheries and aquaculture supplied the world with about 148 million tonnes of fish in 2010 (with a total value of USD 217.5 billion, of which about 128 million tonnes was utilized as food for people, and preliminary data for 2011 indicate increased production of 154 million tonnes, of which 131 million tonnes was destined as food[1].

There is a global trade in aquaculture products which has considerably increased in recent decades, and the expansion of aquaculture production, particularly from Asia, has the potential to meet most of the growing global demand for fish and fishery products[2]. According to Food and Agriculture Organization (FAO), aquaculture supplies about 50% of the global demand for fish and fishery products with about 90% of the aquaculture products

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coming from Asian region^[3]. Fishery products have been recognized as a major carrier of food-borne pathogens^[4,5]. Pathogenic bacteria associated with fish and fishery product can be categorized into three general groups: (1) indigenous bacteria that belong to the natural microflora of fish (*Clostridium botulinum*, pathogenic *Vibrio* spp., *Aeromonas hydrophila*); (2) enteric bacteria (nonindigenous bacteria) that are present due to fecal contamination (*Salmonella* spp., *Shigella* spp., pathogenic *Escherichia coli*, *Staphylococcus aureus*); and (3) bacterial contamination during processing, storage or preparation for consumption (*Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Clostridium perfringens*, *Salmonella* spp.)^[6].

Salmonella is a member of Enterobacteriaceae, Gram-negative, facultative anaerobe, motile, with peritrichous flagella, non-spore forming rods that responsible of causing salmonellosis. In humans, these pathogenic bacteria caused enteric fever (typhi or paratyphi) and acute gastroenteritis^[7].

In Saudi Arabia, data regarding the presence of medically significant *Salmonella* spp. in imported frozen freshwater fish are limited or not available. Thus, present study was conducted to determine the prevalence and antibiotic resistance of *Salmonella* spp. in imported frozen freshwater fish imp in Eastern Province of Saudi Arabia.

2. Materials and methods

2.1. Sample collection

Starting from December 2012 till March 2013, a total of 223 frozen freshwater fishes (Catfish, Carfu, Mirgal, Milkfish, Mackerel, Tilapia and Rohu) were purchased from different supermarkets and grocery stores. The samples originated from five countries, namely, Thailand, India, Bahrain, Myanmar and Vietnam, respectively. All fish samples during collection were placed in sterile polypropylene bag, placed in polystyrene box containing crushed ice and the temperatures was between 4 °C and 8 °C during transportation. The samples were transported to the laboratory and examined on the same day for the presence of *Salmonella* spp.

2.2. Isolation and biochemical identification

The isolation of *Salmonella* was determined by using the methods described in Bacteriological Analytical Manual (BAM, 2011)^[8]. The fish samples were gently removed from coolers and processed in aseptic condition. The gills, intestines parts and skin parts were removed by using sterile knives and forceps and placed on sterile tray and chopped thoroughly with sterile knife. About 25

g of samples (fish gills, intestines parts and skin) were placed into a stomacher bag containing 225 mL of buffered peptone water and homogenized using a stomacher (Seward Stomacher 400 circulator, UK) for 2 min and incubated for 18–24 h at 37 °C. From this nonselective pre-enrichment, 0.1 mL and 1.0 mL were, respectively, transferred into 10 mL of Rappaport–Vassiliadis broth and 10 mL of selenite cystine broth, and incubated for 18–24 h at 42 °C (Rappaport–Vassiliadis) and at 37 °C (selenite cystine). A drop from each selective enrichment broth was streaked onto selective Hektoen agar, Rambach agar and CHROMagar *Salmonella* agar (CHROMagar, Paris, France) plates and incubated for 24–48 h at 37 °C. Suspected colonies on selective agar plates were purified and bio-typed by using biochemical tests and API 20E strips (BioMerieux, Marcy, France). *Salmonella enterica* subsp. *enterica* serovar *enteritidis* American Type Culture Collection 13076 was used as a reference strain for CHROMagar, biochemical tests and API 20E strips.

2.3. Antibiotic susceptibility testing

Antimicrobial susceptibility was determined by the disk diffusion method on Muller–Hinton agar, as described by Kirby–Bauer, in accordance with the guidelines of the Clinical and Laboratory Standards Institute. The isolates were tested against 18 antibiotics which included: amikacin 30 µg, amoxicillin–clavulanic acid 30 µg, ampicillin 10 µg, cefotaxime 30 µg, ceftazidime 30 µg, ceftriaxone 30 µg, chloramphenicol 30 µg, ciprofloxacin 5 µg, kanamycin 30 µg, nalidixic acid 30 µg, nitrofurantoin 300 µg, norfloxacin 10 µg, piperacillin 100 µg, polymyxin B 300 units, tetracycline 30 µg, ticarcillin 75 µg, tobramycin 10 µg, trimethoprim/sulfamethoxazole (1.25 µg/23.75 µg). The antibiotics discs were obtained from Oxoid (Baringstoke, Hampshire, United Kingdom). Cultures were grown overnight in tryptic soy broth and incubated at 37 °C. The overnight cultures were diluted to a turbidity of 0.5 on McFarland scale. The cultures were streaked on Mueller Hinton Agar (Oxoid, Baringstoke, Hampshire, United Kingdom) plates using a cotton swab. After 30 min, 3–4 antibiotic discs were placed on the plates and were incubated at 37 °C for 18–24 h. After the incubation period, the diameter of inhibition zones was measured and compared with interpretive chart proposed by the ‘Performance Standards for Antimicrobial Disk Susceptibility Tests’ and which were classified as resistant (CLSI, 2010)^[9]. *Escherichia coli* American Type Culture Collection 25922 was used as a reference strain for antimicrobial disk control.

2.4. Statistical analysis

Overall prevalence rates for specific fish types were

calculated using the Kruskal–Wallis test. The distribution of antibiotic resistance was compared for the three groups of resistance, intermediate and susceptible. The distribution of antibiotic resistance for each fish type was used to calculate two measures of diversity, dominance and entropy, widely used in ecology. The dominance measure ranges from 0 in which all antibiotics (in this case) are equally present, to 1, in which one antibiotic dominates. The entropy measure ranges from 0 in which only one antibiotic is present (no diversity) to high values, showing that different antibiotics are present. The calculations were done using PAST software version 2.04[10].

3. Results

The prevalence and contamination rates of imported frozen fish with *Salmonella* are presented in Table 1. Out of 223 samples examined, 140 *Salmonella* spp. isolates were isolated. *Salmonella* spp. were detected in 89 fish samples with prevalence rate of 39.9%. The prevalence of positive samples were reported for the freshwater fish of catfish (60.0%, $n=12$), carfu (27.7%, $n=5$), mirgal (35.0%, $n=7$), milkfish (52.0%, $n=13$), mackerel (31.4%, $n=11$), tilapia imported from Thailand (64.0%, $n=16$), tilapia imported from India (28.0%, $n=14$), rohu imported from Thailand (26.6%, $n=4$) and rohu imported from Myanmar (46.6%, $n=7$) as shown in Table 1.

Table 1

Prevalence of *Salmonella* spp. in raw retail frozen imported fish to Eastern Province of Saudi Arabia.

Fish type	Country	No. of samples	No. of positive samples	Prevalence (%)	No. of isolates
Catfish	Thailand	20	12	60.0	28
Carfu	Thailand	18	5	27.7	9
Mirgal	Thailand	20	7	35.0	12
Milkfish	Vietnam	25	13	52.0	15
Mackerel	Bahrain	35	11	31.4	13
Tilapia	Thailand	25	16	64.0	20
Tilapia	India	50	14	28.0	22
Rohu	Thailand	15	4	26.6	8
Rohu	Myanmar	15	7	46.6	13
Total		223	89	39.9	140

All isolates (140) of *Salmonella* spp. isolated from at least seven different types of frozen freshwater fish imported from five different countries were tested for their susceptibility to 18 selected antimicrobial agents. Most of *Salmonella* isolates showed resistance to tetracycline (90.71%, $n=127$) followed by ampicillin (70%, $n=98$) and amoxicillin–clavulanic acid (45%, $n=63$) as presented in Table 2. The highest antibiograms of *Salmonella* exhibited resistance to tetracycline, ampicillin and amoxicillin–clavulanic acid were isolated from catfish, tilapia (India), tilapia (Thailand), rohu, milkfish and mirgal, respectively. The lowest antibiograms of *Salmonella* exhibited resistance to tetracycline, ampicillin and

amoxicillin–clavulanic acid were isolated from mackerel and carfu imported Bahrain and Thailand, respectively. As Table 2 shows, the highest value of dominance is for number of resistance isolates, for those ones, tetracycline predominates.

Table 2

Antibiogram of *Salmonella* spp. isolated from raw retail frozen imported fish to Eastern Province of Saudi Arabia.

Antibiotic	No. of resistant isolates (%)	No. of intermediate isolates (%)	No. of susceptible isolates (%)
Amikacin	0	0	0
Amoxicillin–clavulanic acid	63 (45)	0	77 (55)
Ampicillin	98 (70)	0	42 (30)
Cefotaxime	0	0	0
Ceftazidime	0	0	0
Ceftriaxone	0	0	0
Chloramphenicol	0	28 (20)	112 (80)
Ciprofloxacin	0	0	0
Kanamycin	0	36 (25.71)	104 (74.28)
Nalidixic acid	0	25 (17.85)	115 (82.14)
Nitrofurantoin	0	0	0
Norfloxacin	0	0	0
Piperacillin	0	0	0
Polymyxin B	0	0	0
Tetracycline	127 (90.71)	0	13 (9.28)
Ticarcillin	0	48 (34.28)	92 (65.71)
Tobramycin	0	0	0
Trimethoprim/sulfamethoxazole	0	0	0
Measures of diversity based on antimicrobial of <i>Salmonella</i> spp.			
Dominance D	0.3581	0.2669	0.1718
Shannon H	1.06	1.354	1.818

$n=140$.

The highest value of antimicrobial diversity is for number of susceptibility, for those ones, none of antibiotics predominates. Partly because of the absence of resistance in many of the antibiotics, comparison of the three groups (resistant, intermediate and susceptible) using the Kruskal–Wallis test did not yield any statistical significance between them ($H=2.898$, $P=0.3929$).

4. Discussion

In this study, *Salmonella* spp. were isolated from at least seven different types of frozen fish (pangas, carfu, mirgal, milkfish, mackerel, tilapia and rohu) imported from five different countries (Thailand, India, Bahrain, Vietnam and Myanmar) to Eastern Province of Saudi Arabia. The overall prevalence of *Salmonella* spp. contamination of frozen imported fish (39.9%) observed in this study is significantly very high. *Salmonella* contaminates in fish and fishery products have also been reported from other countries and areas such as India, Mexico, Thailand, Hong Kong, Spain and Turkey[11–14]. The highest *Salmonella* incidence in fishery products was determined in Central Pacific and African countries while it was lower in Europe and including

Russia and North America^[15]. In this study, the highest numbers of *Salmonella* strains were isolated from catfish (imported from Thailand), tilapia (imported from India), tilapia (imported from Thailand), milkfish (imported from Vietnam), rohu (imported from Myanmar), mackerel (imported from Bahrain) and mirgal (imported from Thailand), while the lowest number of *Salmonella* strains were isolated from carfu and rohu (imported from Thailand).

In the Asia-Pacific region, cultured fishes are fed by both commercial and homemade feeds (fresh feed material or farm feed material). According to FAO, homemade feeds are used to reduce cost of production^[16]. Homemade feed is usually made from chicken viscera, kitchen refuse, chicken bone and other food waste materials^[17]. Such feeds can be a source of pathogenic bacteria such as *Salmonella* spp. which can be transmitted to catfish and tilapia and ultimately to consumers^[18]. In a recent study in Malaysia, Budiati *et al.* reported that feeds, such as chicken offals and spoiled eggs, can pose a potential source of *Salmonella* spp. and the high risks associated with the dissemination of antibiotic resistance genes among bacteria associated with catfish, tilapia and environment of aquaculture systems^[19]. In this study, number of *Salmonella* isolates resistant to tetracycline (90.71%) followed by ampicillin (70%) and amoxicillin-clavulanic acid (45%), and this could indicate and reflect the wide spread use of excessive antibiotics in fish farming. The increase and spread of antimicrobial-resistant *Salmonella* has been associated with extensive use of antimicrobial agents, not only in human and veterinary medicine, but also in livestock production for disease prevention or as growth promoters in the animal husbandry and aquaculture which lead to development of resistance among environmental species^[20–22]. This increase of antimicrobial-resistant *Salmonella* phenomenon could limit the therapeutic options for clinical cases that require antimicrobial treatment^[23].

The main finding in the study is that there were high rates of *Salmonella* contamination of a wide variety of imported freshwater frozen fish to Eastern Province of Saudi Arabia, and this highlights a lack of applications of adequate hygiene for *Salmonella* control in the fisheries production in some countries. Thus, to ensure the safety of imported fish and fisheries products for human consumption, the study recommends the surveillance of foodborne *Salmonella* through all imported frozen fish and fisheries products should be adopted and considered seriously by food control authorities in the study region.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

This study was conducted in order to determine the proportion of imported frozen fish contaminated with *Salmonella* among retail food stores and supermarkets in the Eastern Province of Saudi Arabia.

Research frontiers

Salmonella is a member of Enterobacteriaceae, Gram-negative, facultative anaerobe, motile, with peritrichous flagella, non-spore forming rods that responsible of causing salmonellosis. In humans, these pathogenic bacteria caused enteric fever (typhi or paratyphi) and acute gastroenteritis.

Related reports

There is a global trade in aquaculture products which has considerably increased in recent decades and the expansion of aquaculture production, particularly from Asia, has the potential to meet most of the growing global demand for fish and fishery products. According to FAO, aquaculture supplies about 50% of the global demand for fish and fishery products with about 90% of the aquaculture products coming from the Asian region.

Innovations and breakthroughs

The obtained results of this study shows that these raw retail imported frozen freshwater fish are contaminated with potentially pathogenic *Salmonella* spp., and the study recommends and suggests that there is a need for adequate consumer measures.

Applications

In Saudi Arabia, data regarding the presence of medically significant *Salmonella* spp. in imported frozen freshwater fish are limited or not available. Thus, present study was conducted to determine the prevalence and antibiotic resistance of *Salmonella* spp. in imported frozen freshwater fish imported in Eastern Province of Saudi Arabia.

Peer review

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