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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 knifes 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

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