



Prevalence of antimicrobial resistance of non-typhoidal *Salmonella* serovars in retail aquaculture products



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ABSTRACT

Aquaculture products can become sources of *Salmonella* by exposure to contaminated water or through processing practices, thus representing a public health hazard. A study was conducted on *Salmonella* contamination in aquaculture products sampled from marketplaces and retailers in Shanghai, China. A total of 730 samples (including fish, shellfish, bullfrog, clam, shrimp and others) were obtained from 2006 to 2011. Among them, 217 (29.7%) were positive for *Salmonella*. Thirty-eight serovars were identified in the 217 *Salmonella* isolates. The most prevalent were *Salmonella* Aberdeen (18.4%), *S. Wandsworth* (12.0%), *S. Thompson* (9.2%), *S. Singapore* (5.5%), *S. Stanley* (4.6%), *S. Schwarzengrund* (4.6%), *S. Hvittingfoss* (4.1%) and *S. Typhimurium* (4.1%). Many resistant isolates were detected, with 69.6% resistant to at least one antimicrobial drug. We observed high resistance to sulfonamides (56.5%), tetracycline (34.1%), streptomycin (28.6%), ampicillin (23.5%) and nalidixic acid (21.2%). Lower levels of resistance were found for gentamicin (3.2%), ciprofloxacin (2.3%), ceftiofur (1.3%), cefotaxime (0.9%), ceftazidime (0.5%) and cefepime (0.5%). A total of 43.3% of the *Salmonella* isolates were multidrug-resistant and 44 different resistance patterns were found. This study provided data on the prevalence, serovars and antimicrobial resistance of *Salmonella* from retail aquaculture products in Shanghai, and indicated the need for monitoring programs for microbiologic safety in such projects and for more prudent drug use in aquaculture production in order to reduce the risk of development and spread of antimicrobial resistance.

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1. Introduction

Non-typhoidal *Salmonella* serovars are important foodborne pathogens that cause millions of illnesses in humans and significant economic losses worldwide (Majowicz et al., 2010). Foods that have been implicated in salmonellosis include beef, pork, dairy products, dairy, vegetables and seafood (Brands et al., 2005; Heinritz et al., 2000; Zhao et al., 2008). *Salmonella* in aquaculture products, due to contaminated water during production and cross-contamination during handling, is a major public health concern. The survival rate of *Salmonella* in aquatic environments is very high; it outlives even *Vibrio cholerae* in highly eutrophic river water (Spector, 1998).

Salmonella is also an important causative agent for the food-borne diseases in human who consumed aquaculture products. The incidence of salmonellosis caused by the consumption of contaminated seafood, such as raw shellfish, is a primary concern of public health agencies in many countries (Feldhusen, 2000). In the United States, Seafood and shellfish accounted for 7.42% of all food poisoning related deaths from *Salmonella* infections between 1990 and 1998 (Heinritz et al., 2000). In China, a major producer and consumer of food of animal origin, the per capita consumption of meat and aquaculture products has increased significantly in recent decades (Chen et al., 2008; Liu et al., 2008). With the increase in consumption of aquaculture products, particularly raw products, potential risks of exposure to foodborne pathogens have also increased.

In recent years, antimicrobial resistance in *Salmonella*, particularly multidrug-resistant (MDR) strains, has become a major public health issue. Most infections with MDR *Salmonella* are acquired by eating contaminated foods of animal origin (White et al., 2001). Excessive use of antibiotics in aquaculture can be a significant contributor to the increase in the prevalence of antibiotic-resistant bacteria in seafood (Cabello,

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2006). Several studies have shown the presence of antibiotic resistance in bacteria from seafood produced in or exported to developed countries (Zhao et al., 2003; Sapkota et al., 2008). However, few studies have shown bacterial antibiotic resistance in fish available for local consumption in developing countries where the use of antibiotics is reported to be high (Radu et al., 2003). This is especially important in East and Southeast Asia, where the vast majority of aquaculture production and consumption occurs (FAO, 2014).

Historically, most studies on the prevalence and characterization of antimicrobial resistance in *Salmonella* have focused on isolates from clinical and veterinary sources, and other food products (meat and poultry) (Khan et al., 2009; Randall et al., 2004). Information on the potential role of aquaculture food products in the dissemination of multidrug-resistant *Salmonella* in China is very limited. In this study, we characterized *Salmonella* isolates recovered from aquaculture products from Shanghai, China between 2006 and 2011.

2. Materials and methods

2.1. Sample collection

Salmonella isolates were obtained through the World Health Organization Global Foodborne Infections Network Surveillance System. A total of 730 samples of aquaculture products including fish, bullfrog, spiral shell, clam, *Solen strictus* (Gould's razor shell), shrimp and other shellfish were randomly collected in supermarkets and retailers in Shanghai, China, from 2006 to 2011. Samples were placed in sterile plastic sample bags and chilled on ice during transport to the laboratory at Shanghai Center for Disease Control. All samples were analyzed on the day of arrival.

2.2. *Salmonella* isolation

Isolation was performed according to the U.S. FDA Bacteriological Analytical Manual (FDA, 1992). Twenty-five grams of each sample was aseptically prepared, transferred to a sterile plastic bag containing 225 mL of lactose broth and incubated for 24 h at 37 °C. A 1 mL portion of the pre-enrichment was added to 10 mL Rappaport–Vassiliadis (RV) broth and incubated for 24 h at 42 °C. Samples from RV enrichment broth were plated on Xylose-lysine-desoxycholate agar, *Salmonella*–*Shigella* agar, Brilliant Green agar, Bismuth Sulfite agar and MacConkey agar plates. These were incubated at 37 °C for 24 h. Bacterial colonies showing morphological characteristics of *Salmonella* were then confirmed as *Salmonella* using API 20E test strips (bioMérieux, France).

2.3. *Salmonella* serotyping

Salmonella isolates were serotyped on slides using a microtiter agglutination test for O and H antigens according to the manufacturer's instructions (SSI, Copenhagen, Denmark). Serovar was assigned according to the Kauffmann–White scheme (Grimont and Weill, 2007).

2.4. Antimicrobial susceptibility testing

Antimicrobial susceptibility was conducted for *Salmonella* isolates using the Kirby–Bauer disk diffusion method (CLSI, 2009). Agar diffusion assays were performed on Muller–Hinton agar with disks containing 16 different antibiotic agents (Oxoid, UK): ampicillin 10 µg (AMP), cefotaxime 30 µg (CTX), nalidixic acid 30 µg (NAL), ciprofloxacin 5 µg (CIP), ceftazidime 30 µg (CAZ), gentamicin 10 µg (GEN), tetracycline 30 µg (TET), chloramphenicol 30 µg (CHL), ceftiofur 30 µg (EFT), amoxicillin/clavulanic acid 30 µg (AMC), trimethoprim/sulfamethoxazole 1.25/23.75 µg (SXT), ofloxacin 5 µg (OFX), cefepime 5 µg (FEP), trimethoprim 5 µg (W), sulfonamides 300 µg (S3), and streptomycin 10 µg (STR). *Escherichia coli* ATCC 25922 and ATCC 35218 were used as quality control organisms. Results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2010).

2.5. Statistical analysis

Chi square or Fisher's exact test was used for data analysis with SAS 9.2 (SAS Institute, Cary, N.C.). A P-value of <0.05 was considered statistically significant.

3. Results

3.1. Isolation and identification of *Salmonella*

Among the 730 samples analyzed, 29.7% (n = 217) were positive for *Salmonella*. These included 59 from clam (27.2% of the total positives observed); 45 from spiral shell (20.7%); 36 from bullfrog (16.6%); 27 from fish (12.4%); 13 from *S. strictus* (6.0%); 13 from shellfish (6.0%); 10 from shrimp (4.6%); and 14 from miscellaneous other samples (6.5%). Thirty-eight *Salmonella* serovars were identified, including: Aberdeen (18.4%); Wandsworth (12.0%); Thompson (9.2%); Singapore (5.5%); Stanley (4.6%); Schwarzengrund (4.6%); Hvittingfoss (4.1%); Typhimurium (4.1%); Pomona (3.7%); and Saintpaul (2.8%) (Table 1). Other serovars, ranging from 0.46%–2.30% of the samples, were Newport, Irumu, Indiana, Virehow, Senftenberg, Enteritidis, Derby, Montevideo, Tennessee, Idikan, Potsdam, Braenderup, Hadar, Infantis, Kottbus, Anatum, Litchfield, Bovismorbificans, Agona, Meleagridis, Give, Kedougou, Corvallis, Liverpool, Rissen, Mouline, Weltevreden and Uganda.

Table 1
Salmonella serovars recovered from retail aquaculture products in Shanghai, China.

Serovar	Number of isolates								Total number (%)
	Clam	Spiral shell	Bullfrog	Fish	<i>Solen strictus</i>	Other shellfish	Shrimp	Others	
Aberdeen	9	24	0	3	3	0	1	0	40 (18.4)
Wandsworth	5	4	11	4	1	0	1	0	26 (12.0)
Thompson	7	5	4	1	0	1	0	2	20 (9.20)
Singapore	3	1	1	1	1	2	1	2	12 (5.50)
Stanley	3	0	0	2	0	2	1	2	10 (4.60)
Schwarzengrund	2	1	1	2	2	1	0	1	10 (4.60)
Hvittingfoss	4	0	1	1	2	1	0	0	9 (4.10)
Typhimurium	3	2	0	0	0	1	2	1	9 (4.10)
Pomona	4	0	0	1	1	1	0	1	8 (3.70)
Saintpaul	0	0	3	1	0	1	0	1	6 (2.80)
Others	19	8	15	11	3	3	4	4	67 (30.9)
Total	59	45	36	27	13	13	10	14	217 (100)

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