Food Control 57 (2015) 308–313

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

# Prevalence, enumeration, and characterization of *Salmonella* isolated from aquatic food products from retail markets in China

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ARTICLE INFO

*Article history:*
Received 22 December 2014
Received in revised form
10 March 2015
Accepted 11 March 2015
Available online 26 April 2015

*Keywords:*
*Salmonella*
Prevalence
Aquatic food products
Serotype
Virulence

ABSTRACT

This study aimed to estimate the extent and level of *Salmonella* contamination of aquatic food products in China, and to determine serotype, virulotype, and the antimicrobial resistance profiles of recovered *Salmonella* isolates. Out of 554 samples collected from July 2011 to May 2014, 86 (15.5%) tested positive for *Salmonella*. The highest contamination rate occurred in oysters (23.1%, 6/26), followed by freshwater fish (18.6%, 43/231), shrimp (13.0%, 13/100), and saltwater fish (12.2%, 24/197). The contamination levels generally corresponded to a most probable number (MPN)/g of 0.3–10, although one sample exceeded 110 MPN/g. Among the 103 isolates, *S.* Typhimurium, *S.* Wandsworth, *S.* Thompson, and *S.* Derby were the most prevalent serovars. Sixty-eight isolates (66.0%) were resistant to at least one antimicrobial, and 35 (34.0%) were resistant to more than three. High rates of resistance were observed for tetracycline (35.9%), ampicillin (28.2%), nalidixic acid (26.2%), trimethoprim-sulfamethoxazole (25.2%), chloramphenicol (20.4%) and streptomycin (18.4%). Of note, *S.* Thompson isolates exhibited resistance to multiple extended-spectrum cephalosporins, ciprofloxacin, and other antimicrobials. PCR analysis of 15 virulence genes showed that *ssaQ*, *mgtC*, *siiD*, *sopB*, and *bcfC* were present in all 103 isolates, whereas the remaining loci were variably distributed. *S.* Typhimurium, *S.* Enteritidis, and *S.* Weltevreden isolates exhibited a wider range of pathogenicity determinants compared with the other strains. Our study provides a comprehensive surveillance on prevalence of *Salmonella* in aquatic food products from China and indicates its potential risk to public health. These data are valuable for epidemiological studies, risk management, and public health strategies.



## 1. Introduction

Aquatic food products are consumed worldwide for their high nutritional value and potential health benefits. In recent years, aquatic food products are becoming more popular in China, and consumption has increased significantly. Simultaneously, the safety of these foods, particularly as related to microbiological contamination, has become a source of concern.

*Salmonella* is one of the most commonly reported causes of aquatic food related illnesses and outbreaks worldwide (Amagliani, Brandi, & Schiavano, 2012; CSPI, 2009; EFSA, 2010). *Salmonella* is not a component of the normal flora of aquatic animals, and the presence of these bacteria in aquatic food products is a result of fecal contamination from polluted water, or cross-contamination during transportation or storage (Amagliani et al., 2012). Adequate cooking can kill *Salmonella*; however, unlike other foods, such as meat and poultry, that are usually fully cooked, aquatic food products are often consumed raw or prepared in ways that do not kill organisms. Therefore, in these cases, *Salmonella* may present a risk to human health.

To date, more than 2600 serotypes of *Salmonella* have been reported (Guibourdenche et al., 2010). Many serovars have been associated with particular disease potentials (Achtman et al., 2012), and serotype determination is important for epidemiological surveillance and disease assessment. Additionally, various serovars and strains of *Salmonella* that cause specific disease syndromes are distinguished by the complements of virulence genes they carry (Capuano, Mancusi, Capparelli, Esposito, & Proroga, 2013; Graziani et al., 2008). The detection of virulence genes (virulotyping) has

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http://dx.doi.org/10.1016/j.foodcont.2015.03.046
0956-7135/

recently been widely used to characterize and define the pathogenicity of *Salmonella* isolates (Capuano et al., 2013; Huhen et al., 2010). Most of the virulence genes with reported contributions to pathogenicity are located in *Salmonella* pathogenicity islands (SPIs), fimbrial clusters, plasmids and prophages. The distribution of these virulence genes have been investigated in strains of some frequently detected serovars in different countries and serovar-specific repertoires of genes have been identified (Capuano et al., 2013; Gong et al., 2014; Huehn et al., 2010).

Generally speaking, the fluoroquinolones and extended-spectrum cephalosporins are the antibiotics of choice for the treatment of complicated infections caused by *Salmonella* (Cui et al., 2009; Hohmann, 2001). However, in the last few years the number of *Salmonella* isolates with resistance to these clinically important antimicrobial agents has increased in China and other countries (Deng et al., 2012; Li et al., 2013; Newell et al., 2010), threatening the efficacy of therapy. Of particular concern is the increasing isolation of *Salmonella* strains showing multidrug resistance (MDR). These MDR *Salmonella* strains have been found to be of many serotypes such as *S.* Derby, *S.* Newport, *S.* Heidelberg, *S.* Typhimurium and its monophasic variant *S.* 1,4,[5],12:i:- (Hoelzer et al., 2010; Hur, Jawale, & Lee, 2012). The pandemic MDR clones of these serovars have caused numerous large outbreaks in humans around the world (Barco et al., 2014; Hoelzer et al., 2010). The emergence and transmission of resistant *Salmonella* poses a serious health threat.

In China, surveillance reports of *Salmonella* associated with aquatic food products are scarce compared with those pertaining to poultry and meat. As such, risk assessments relating to the safety of these products are hampered by the lack of available data. Therefore, to provide such data for assessing the risk of *Salmonella* to Chinese public health, various raw aquatic food products were collected from retail markets in different cities in China. The prevalence and level of *Salmonella* were then determined for each of the samples, and the isolates were further characterized for their serotypes, virulotypes, and antimicrobial resistance profiles.

## 2. Materials and methods

### 2.1. Sample collection

From July 2011 to May 2014, 554 raw aquatic food samples were collected from retail markets; these included freshwater fish (231), saltwater fish (197), shrimp (100), and oysters (26). The sampling sites were distributed throughout the 24 provincial capitals of China (Fig. S1). Each sample was weighed, marked, placed in a separate sterile bag, and immediately transported to the laboratory in an icebox.

### 2.2. Detection and enumeration

All of the samples were subjected to qualitative and quantitative analysis for *Salmonella*. Qualitative analyses were performed according to National Food Safety Standards of China document GB 4789.4-2010. A 25 g sample was randomly collected from each aquatic food product and was pre-enriched in 225 ml of buffered peptone broth (Huankai, Guangzhou, China). 1 ml cultures were incubated in 10 ml of selenite cystine broth (SC) (Huankai) at 37 °C and 10 ml of tetrathionate brilliant green broth (TTB) at 42 °C for 24 h, respectively. Loopfuls of SC and TTB cultures were streaked onto xylose-lysine-tergitol 4 (XLT4) selective agar plates (Difco, Detroit, MI, USA) and chromogenic *Salmonella* agar plates (Huankai), then incubated at 37 °C for 24 h. Presumptive colonies were picked from each plate, stabbed into a triple sugar iron slant (Huankai), and incubated at 37 °C for 24 h. Isolates with typical *Salmonella* phenotypes were further confirmed using API 20E test strips (bioMerieux, Marcy-l'Etoile, France). The level of *Salmonella* in the samples was determined using the most probable number (MPN) method as described by Abley, Wittum, Zerby, and Funk (2012).

### 2.3. Serotyping

All the *Salmonella* isolates were serotyped by slide agglutination using commercial O and H antisera (Tianrun Bio-Pharmaceutical, Ningbo, China, and S&A Reagents Lab, Bangkok, Thailand), according to the manufacturer's instructions.

### 2.4. Antimicrobial susceptibility testing

Antimicrobial susceptibility was evaluated using the Kirby–Bauer disk diffusion method for 19 antimicrobial agents, in accordance with the Clinical Laboratory Standards Institute guidelines (CLSI, 2006). These antimicrobials were ampicillin (AMP), amoxicillin-clavulanic acid (AMC), cephalothin (KF), cefazolin (KZ), cefoxitin (FOX), ceftriaxone (CRO), cefotaxime (CTX), ceftazidime (CAZ), cefoperazone (CFP), cefepime (FEP), chloramphenicol (C), tetracycline (TE), nalidixic acid (NA), ciprofloxacin (CIP), amikacin (AK), gentamicin (CN), streptomycin (S), kanamycin (K) and trimethoprim-sulfamethoxazole (SXT) (Oxoid, Basingstoke, UK). In this work, we defined an isolate as having "clinically important resistance" if it was resistant to one or more of the following agents: AMP, AMC, CRO, CTX, CAZ, FEP, CIP, AK, CN, and SXT (Deng et al., 2012).

### 2.5. Detection of virulence-related genes

Fifteen genes with reported contributions to virulence were selected. Five targets (*avrA*, *ssaQ*, *mgtC*, *siiD*, *sopB*) were located on the *Salmonella* pathogenicity islands (SPIs) 1–5, five targets (*gipA*, *sodC1*, *sopE1*, *gtgB*, *sspH1*) were on prophages, three (*spvC*, *pefA*, *rck*) were located on a virulence plasmid, and two (*bcfC*, *steB*) were located in the fimbrial cluster. Amplification was performed using primers and conditions previously published (Capuano et al., 2013; Gong et al., 2014; Huehn et al., 2010). The primers used are shown in Table S1. *S.* Paratyphi A CMCC50093, *S.* Typhimurium ATCC14028, *S.* Typhimurium CMCC50115, *S.* Enteritidis CMCC50041, *S.* Enteritidis CMCC50335, *S.* Typhi CMCC50071, and *S.* Typhi CMCC50098 were used as positive controls.

## 3. Results

### 3.1. Prevalence and enumeration of Salmonella in aquatic food products collected from retail markets

Out of the 554 samples, 86 (15.5%) tested positive for *Salmonella*. Oysters had the highest prevalence (23.1%), followed by freshwater fish (18.6%), shrimp (13.0%), and saltwater fish (12.2%). Out of the 31 samples that tested positive by the MPN method, 24 samples (77.4%) were less than 1 MPN/g and six (19.4%) reached 10 MPN/g. One freshwater fish sample exceeded 110 MPN/g (Table 1).

### 3.2. Serotype distribution

One-hundred and three *Salmonella* isolates were recovered from the 86 positive samples (Table S2). Notably, two different serovars were simultaneously identified in 13 samples, and three different serovars were detected in two freshwater fish samples. Forty distinct serovars were identified, including *S.* Typhimurium (n = 10), *S.* Wandsworth (n = 10), *S.* Thompson (n = 8), *S.* Derby (n = 8), *S.* Pomona (n = 6), *S.* Litchfield (n = 4), *S.* Weltevreden (n = 4), *S.* Enteritidis (n = 4), *S.* Braenderup (n = 4), *S.* Saintpaul