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Prevalence, antibiotic resistance, and extended-spectrum and AmpC β-lactamase productivity of *Salmonella* isolates from raw meat and seafood samples in Ho Chi Minh City, Vietnam

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ABSTRACT

Salmonellosis is a type of foodborne disease caused by *Salmonella enterica* and is a frequent cause of childhood diarrhea in Vietnam. Of particular concern is the dissemination of multidrug-resistant *Salmonella*, as extended-spectrum β-lactamase (ESBL)-positive isolates were recently detected in children in Vietnam. In the present study, the prevalence and antibiotic resistance of *Salmonella* isolates obtained from 409 raw meat and seafood samples collected between October 2012 and March 2015 from slaughterhouses, wholesale fish market, and retail markets in Ho Chi Minh City, Vietnam were examined. A high rate of *Salmonella* contamination was detected in the pork (69.7%), poultry (65.3%), beef (58.3%), shrimp (49.1%), and farmed freshwater fish samples (36.6%). A total of 53 *Salmonella* serovars were found, of which *S.* Rissen, *S.* Weltevreden, *S.* London, *S.* Anatum, *S.* Typhimurium, and *S.* Corvallis were the most prevalent. In addition, 4 monophasic *S.* Typhimurium strains were identified using a PCR method for the detection of a specific IS*200* fragment within the *fliB-fliA* intergenic region. The *Salmonella* isolates had a high prevalence (62.2%) of resistance to antimicrobial agents, particularly tetracycline (53.3%), ampicillin (43.8%), chloramphenicol (37.5%), and trimethoprim/sulfamethoxazole (31.3%). Isolates with resistance to three or more classes of antimicrobials were found (41.1%). Especially, isolates such as *S.* monophasic Typhimurium, *S.* Schwarzengrund, *S.* Indiana, *S.* Newport, *S.* Saintpaul and *S.* Bovismorbificans exhibited resistance to 6 classes of antimicrobials (3.3%). All 7 *S.* Indiana strains were resistant to between 4 and 6 classes of antimicrobials, including ciprofloxacin, which is commonly used for the treatment of human *Salmonella* infections. Two fish isolates were confirmed to be CTX-M-55 ESBL-producing *Salmonella* serovars Bovismorbificans and Newport, and five CMY-2 AmpC-producing *Salmonella* isolates of serovars Braenderup (4) and Typhimurium (1) were detected in poultry samples. The findings from this study, which is the first report of ESBL- and AmpC-producing *Salmonella* isolates from food in Vietnam, indicate that multidrug-resistant *Salmonella* are widely disseminated not only in meats, but also in seafood, within the food distribution system of Vietnam. The presence of these multidrug-resistant strains is a public health concern and suggests that the use of antimicrobial agents in both humans and animals in Vietnam should be tightly controlled.

1. Introduction

Foodborne diseases, particularly those associated with *Salmonella*, are an emerging public health problem worldwide. For example, nontyphoidal *Salmonella* gastroenteritis accounts for 80.3 million global cases of foodborne illness each year (Majowicz et al., 2010). In southern Vietnam, *Salmonella* is the fourth most frequent causative organism of infant diarrhea (Anders et al., 2015). Although measures to prevent the transmission of *Salmonella* from foods to humans are warranted in Vietnam, a nationwide surveillance program for monitoring *Salmonella* food contamination has not been established (Ta et al., 2014).

Antimicrobials are widely used in humans and livestock as therapeutics, but are also administered for disease prevention and growth promotion. In Vietnam, high doses of antimicrobials are permissible in livestock, and several antimicrobial agents can be obtained without a doctor's prescription (Nga et al., 2014; Pham et al., 2013; Thai et al., 2012a). As the overuse and misuse of antimicrobials is generally considered to promote

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and accelerate the spread of antimicrobial-resistant bacteria, there is concern that human and animal pathogens may acquire antimicrobial resistance genes through horizontal gene transfer (Pham et al., 2013).

The occurrence of extended-spectrum β-lactamase (ESBL)- and AmpC-β-lactamase (AmpC)-producing bacteria, which are able to hydrolyze third-generation cephalosporins, has increased worldwide during the last decade (Peter-Getzlaff et al., 2011; Trang et al., 2013). As plasmid-encoded ESBL and AmpC genes are easily transferred between bacteria (Overdevest et al., 2011), such horizontal transmission represents a serious threat to global public health, as resistance to β-lactams severely limits the choice of effective antimicrobial agents, particularly in the case of coresistance to other antimicrobial classes, such as the fluoroquinolones (Liebana et al., 2013).

ESBL- and AmpC-producing *Salmonella* isolates have been detected worldwide, including the People's Republic of China (Wu et al., 2013; Yang et al., 2014), Germany (Rodríguez et al., 2009), the United States (Winokur et al., 2001), and Brazil (Biffi et al., 2014). In Vietnam, the antimicrobial resistance of *Salmonella* isolates from pork, poultry, and beef has been extensively examined (Ta et al., 2014; Thai et al., 2012a; Tu et al., 2015); to date, however, ESBL- and AmpC-producing *Salmonella* have not been detected in the food distribution system within Vietnam. Recently, ESBL-producing *Salmonella* isolates were detected in stool samples obtained from children with diarrhea in Ho Chi Minh City (HCMC), Vietnam (Thompson et al., 2015), although the origin of these *Salmonella* isolates was not determined.

Vietnam is the third largest worldwide producer of aquaculture products (Anonymous, 2009), and Vietnamese meals typically include servings of fish and shrimp. However, the prevalence of *Salmonella* in fish and shrimp consumed in Vietnam has only been examined in a few studies (Phan et al., 2005; Van et al., 2007). More recently, ESBL-producing *Salmonella* was detected in seafood imported into the United States from the People's Republic of China and Vietnam (Bae et al., 2015). This finding suggests that antimicrobial-resistant strains of *Salmonella*, including ESBL- and AmpC-producing stains, may be distributed among fish and shrimp in HCMC, Vietnam.

In the present study, we investigated the prevalence of *Salmonella* in raw meat, which is the main source of *Salmonella* infection, and raw seafood samples collected at various points within the food distribution system of HCMC, Vietnam. The aims of this study were to determine the *Salmonella* contamination rate, prevalence of antimicrobial-resistant *Salmonella*, and phenotype and genotype of ESBL- and AmpC-producing *Salmonella* isolates from raw meat and seafood samples collected from slaughter houses, wholesale fish market, and retail stores.

2. Materials and methods

2.1. Food sampling

A total of 409 raw meat and seafood samples were collected from slaughter houses (30 poultry, 30 pork, and 30 beef samples), a wholesale fish market (12 farmed shrimp and 28 farmed freshwater fish samples), and retail stores (42 poultry, 69 pork, 54 beef, 41 farmed shrimp, and 73 farmed freshwater fish samples) in HCMC, Vietnam between October 2012 and March 2015. Each sample (≥100 g) was placed in a sterile plastic bag and transported on ice to the microbiology laboratory at the Institute of Public Health Ho Chi Minh City, Vietnam. All samples were tested within 24 h of their collection.

2.2. Salmonella isolation and serotyping

For the pre-enrichment of *Salmonella*, 25 g of each food sample was homogenized in a sterile bag containing 225 ml buffered peptone water (Merck, Darmstadt, Germany), and the resulting homogenate was incubated at 37 °C for 16 to 20 h. After incubation, 0.1 ml of each culture sample was added to 10 ml Rappaport-Vassiliadis broth (Merck) and further incubated at 42 °C for 20 to 24 h. A loopful of each culture was streaked onto xylose lysine desoxycholate agar (Merck) and Chromagar *Salmonella* (CHROMagar Microbiology, Paris, France), which were then further incubated at 37 °C for 20 to 24 h. Three suspected *Salmonella* colonies were streaked onto Trypticase soy agar (Merck) and further incubated at 37 °C for 18 h. Biochemical characteristics of the isolated colonies were examined using triple-sugar iron agar (Merck) and lysine indole motility medium (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan). Isolates that showed biochemical characteristics consistent with typical nontyphoidal *Salmonella* were confirmed by the agglutination test using *Salmonella* O and H antisera (Denka Seiken Ltd., Tokyo, Japan; SSI Diagnostica, Hillerød, Denmark) according to the Kauffmann and White scheme (Grimont and Weill, 2007).

2.3. Antimicrobial susceptibility testing

All *Salmonella* isolates were tested for antimicrobial susceptibility using the disk diffusion method, and the results were interpreted according to the standards of the Clinical and Laboratory Standards Institute (CLSI, 2013). The antimicrobial disks (BD, Sparks, MD) used in the assay contained the following 12 antimicrobial agents: 10 μg ampicillin (AMP), 30 μg tetracycline (TET), 30 μg kanamycin (KAN), 30 μg chloramphenicol (CHL), 10 μg gentamicin (GEN), 23.75 μg trimethoprim and 1.25 μg sulfamethoxazole (SXT), 5 μg ciprofloxacin (CIP), 30 μg nalidixic acid (NAL), 200 μg fosfomycin (FOF), 30 μg cefoxitin (FOX), 30 μg cefotaxime (CTX), and 30 μg ceftazidime (CAZ). The isolates were classified as susceptible, intermediate, or resistant to a particular antibiotic based on the size of the zones of growth inhibition and the criteria of the CLSI. *Escherichia coli* ATCC 25922 was used as a quality control organism in this assay. Isolates exhibiting resistance to three or more antimicrobial classes were defined as multidrug resistant (MDR).

Salmonella isolates showing resistance to CAZ or CTX were confirmed for ESBL production by the double-disk diffusion method (CLSI, 2013) using antimicrobial disks containing 30 μg CTX, 30 μg CTX plus 10 μg clavulanic acid, 30 μg CAZ, and 30 μg CAZ plus 10 μg clavulanic acid. FOX-resistant strains were classified as putative AmpC-producing *Salmonella* (Mataseje et al., 2009).

2.4. PCR detection of β-lactamase genes

The genes responsible for ESBL and AmpC activities in ESBL- and AmpC-producing isolates, respectively, were screened using multiplex PCR analyses with the primers shown in Table 1 for the detection of bla_{CTX-M}, bla_{TEM}, and bla_{SHV} genes for ESBL (Le et al., 2015) and family-specific plasmid-mediated *ampC* genes for AmpC (Pérez-Pérez and Hanson, 2002). DNA templates were prepared by boiling bacterial colonies suspended in TE buffer (pH 8.0) (Nacalai Tesque, Kyoto, Japan) for 10 min. PCR was performed using Qiagen Multiplex PCR Plus kits (Qiagen, Hilden, Germany) according to the manufacturer's guidelines. The PCR program consisted of a denaturation step at 95 °C for 5 min, followed by 25 cycles at 95 °C for 30 s, 60 °C for 90 s, and 72 °C for 90 s, and a final extension step at 68 °C for 10 min. PCR products were analyzed by gel electrophoresis using 2% agarose (Lonza Rockland, Inc., Rockland, ME) and a 100-bp DNA ladder (Nacalai Tesque) as a molecular weight marker.

2.5. Sequencing of PCR-amplified β-lactamase genes

PCR amplicons of $bla_{CTX-M-1}$, bla_{CMY}, and bla_{TEM} group genes were generated and sequenced using the primers described in Table 1. All PCR products were purified using the Illustra Exo Pro Star kit (GE Healthcare Life Sciences, Little Chalfont, UK). Direct sequencing of PCR products was performed using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) and the same primers used for the initial PCR. Sequence analysis was performed on an Applied Biosystems 3130XL Genetic Analyzer (Applied Biosystems) with HiDi