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The association of *Salmonella enterica* from aquatic environmental and clinical samples in Taiwan

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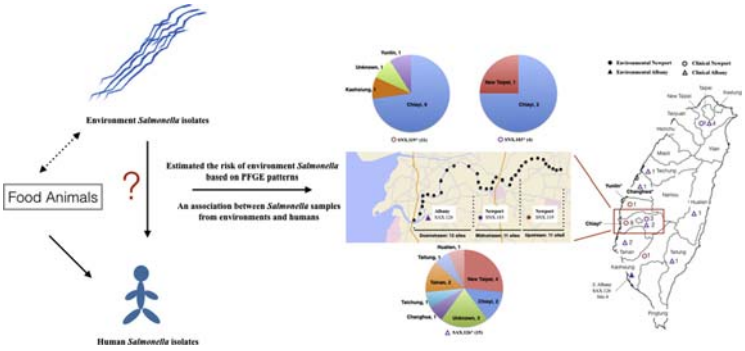
HIGHLIGHTS

- *Salmonella* detections were 31.7% and 42.2% of the samples from two rivers.
- Serovar Newport and Albany were found in clinical and environmental isolates.
- Serovar Newport was not uniformly distributed cross the cities.
- Environmental PFGE genotypes can also obtained from a local hospital.

GRAPHICAL ABSTRACT



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ABSTRACT

Salmonella is one of the most common pathogens of waterborne and foodborne disease-causing pathogens. In this study, we collected 172 surface water samples from Puzih River and Kaoping River between the years 2010 and 2011. *Salmonella* was detected in 31.7% (32/101) and 42.2% (30/71) of the samples from the two rivers, respectively. From these positive samples, 44 *Salmonella* isolates were obtained from these positive samples and were characterized using serotyping and pulsed-field gel electrophoresis (PFGE) genotyping. The isolates were found with 17 serovars and 32 PFGE patterns. *Salmonella enterica* Newport, Bareilly, Kedougou, Albany and subspecies IIIb 50:k:z were the five most common serovars in aquatic environmental *Salmonella* isolates. In addition, of the total clinical samples from Chiayi and Kaohsiung, 33.7% (60/178) Newport serovars were isolated. After conducting categorical analysis, we found that the serovar Newport was not uniformly distributed cross the cities. The serovar Newport was over-represented ($p < 0.001$) among human isolates in Chiayi and Kaohsiung. To investigate the association between *Salmonella* isolates from aquatic environment and human samples, we compared the environmental PFGE patterns of the test samples with those of 2438 clinical isolates, obtained from 51 hospitals across the country between 2010 and 2011. Of the 32 PFGE genotypes of environmental isolates, 8 genotypes were identical to those of clinical samples. Serovar Newport isolates with PFGE patterns SNX.119 and SNX.183 obtained from Puzih River samples were also identified in human samples at a local hospital. These suggest that there is a link between environmental and human clinical *Salmonella*. Identification of *Salmonella* serovars and genotypes present in surface water provides an indication of the specific *S. enterica* serovars and

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genotypes present in humans. This is the first study to investigate the *Salmonella* serovars and genotypes present in aquatic environment and humans in Taiwan.

1. Introduction

Salmonellosis is an important public health problem, which causes substantial morbidity worldwide. *Salmonella* is categorized into two species (*Salmonella enterica* and *S. bongori*), six subspecies, and >2600 serotypes (Issenhuth-Jeanjean et al., 2014). *S. enterica* is the one of the most commonly pathogens causing human infections, which are mainly attributed to the consumption of non-potable water or contaminated foods, particularly beef, pork, eggs, sea-food and fresh produce (Gomez et al., 1997; Medus et al., 2006). According to the Centers for Disease Control and Prevention in the U.S., *Salmonella* is one of the most common pathogenic foodborne bacteria, it caused 39,126 (19%) of the 92,093 illnesses with a confirmed or suspected single etiology, and was the reason for 30% of the deaths in the United States from 1998 to 2008 (Gould et al., 2013). Non-typhoidal *Salmonella* (NTS) is a zoonotic pathogen and a common cause of bacteremia in children and adults worldwide (Mtove et al., 2010). The annual incidence of NTS-associated cases in Taiwan (19.3/100,000 person-years) (Chen et al., 2012) was higher than those in the U.S. (15.9/100,000 person-years) (Crim et al., 2014) and similar to Europe (21.9/100,000) (Bartels et al., 2014). *Salmonella* spp. and subspecies can be found in various vertebrates and enters the river and drinking water systems directly via feces of infected humans or wild and farm animals as well as household sewage runoff (Kidgell et al., 2002; Martinez-Urtaza et al., 2004; Polo et al., 1999). The irrigation water may be a potential source of contamination of the *Salmonella* in fresh produce (Li et al., 2014); hence, it is important to detect and monitor the diversity and distribution of *Salmonella* in surface watershed. Various molecular serotyping methods can be used to monitor the diversity of *Salmonella* (e.g., multilocus sequence typing, ribotyping, and pulsed field gel electrophoresis (PFGE)) (Bouchet et al., 2008; Ho et al., 2017; Zou et al., 2012).

Taiwan's Centers for Disease Control (TCDC) have screened clinical samples and collected *Salmonella* isolates since 2004 and has established a database with a large number of antimicrobial susceptibility data and >20,000 *Salmonella* pulsed-field gel electrophoresis (PFGE) data. PFGE is accepted as the standard molecular subtyping method in *Salmonella* for disease surveillance and disease outbreak investigation (Murase et al., 1995; Zou et al., 2012). Standardized PFGE protocols are established and applied to foodborne bacterial disease surveillance (Swaminathan et al., 2001). Using a standardized PFGE protocol, PFGE patterns can be compared across laboratories for real-time disease surveillance and tracking reservoirs of infection in humans. The PFGE database maintained by TCDC has been successfully used to track the major animal reservoirs for human salmonellosis in Taiwan (Kuo et al., 2014).

The *Salmonella* contamination occurred in surface water used as a drinking water source or for recreational purposes and irrigation (Gannon et al., 2004; Till et al., 2008). Diversity of *Salmonella* in surface water and in the animal-based agriculture river system has been investigated in U.S. and in Brazil (Li et al., 2014; Palhares et al., 2014). Natural water bodies and watersheds are sources for *Salmonella* dissemination and routes for transmission among hosts in the environment (Walters et al., 2013). In Taiwan, Puzih River is an important water source for agricultural activities around the area, and Kaoping River downstream weir is the water source for facilities carrying out drinking water treatment; hence, the latter serves as the major drinking water source for a metropolitan area. We previously investigated and analyzed the seasonal distribution of *Salmonella* in surface water (Puzih River) (Huang et al., 2014) and found that the 70% of surface water samples test positive for *Salmonella* during the summer of 2009. These above reports show the potential risk of surface water to human health. However, few reports show the association between environmental and clinical *Salmonella* isolates.

The aim of this study was to examine the occurrence of *Salmonella* in Puzih River and Kaoping River, and the distribution of *Salmonella* serovars and genotypes in these two rivers and in human clinical samples. Comparison of the PFGE patterns among *Salmonella* isolates from environmental aquatic samples and humans can help elucidate the link between environmental and clinical *Salmonella*; consequently, this will enable us to understand the risks to human health caused by *Salmonella*, which in turn is associated with indirect consumption of contaminated water.

2. Materials and methods

2.1. Sample collection

The sampling sites included 34 sites on the upstream, midstream, and downstream stretches of the Puzih River (an important water source for activities such as agriculture around the area), and 5 sites at the Kaoping River downstream weir (areas from which untreated water is collected at five facilities carrying out drinking water treatment) (Fig. 1). Our previous report showed the high occurrences of *Salmonella* in surface water occurred during season of summer and fall (Huang et al., 2014). Altogether, 172 samples were collected from Puzih River and Kaoping River in southern Taiwan, between June and September in 2010, and during August 2011. The sampling depth is approximately 20–30 cm from the water surface. At each sampling location, 2000 ml of water was taken in two sterilized polypropylene bottle. A total of 2438 clinical *S. enterica* isolates were obtained from 51 hospitals across the country between the years 2010 and 2011 by TCDC.

2.2. Detection and isolation of Salmonella

Salmonella isolation from rivers was performed as described in our previous study (Hsu et al., 2014; Huang et al., 2014). Briefly, 1 l of water sample from each river was filtered through a GN-6 membrane filter of 45-mm diameter and 0.22 μm pore size (Pall, Mexico City, Mexico). The filter membrane was washed with 100 ml of sterilized phosphate-buffered saline, and the suspension was centrifuged at 2600 ×*g* for 30 min. After centrifugation, the top supernatant was discarded, and the remaining 10 mL concentrate was used for subsequent experiments. One milliliter of the concentrate was added to 5 ml buffered peptone water (BPW) (Neogen, Lansing, MI, USA) incubated at 37 °C for 16 h as a pre-enrichment step. Five hundred microliter of the BPW culture was inoculated in 4.5 ml Rappaport-Vassiliadis (RV) broth (Neogen) and incubated at 42 °C for 16–18 h. Subsequently, 1 mL culture was collected for DNA extraction and detected for the presence of *Salmonella* using *invA* gene primer sets (Chiu and Ou, 1996). One hundred microliter of the incubated RV broth was smeared on CHROMagar™ *Salmonella* agar plates (CHROMagar, Paris, France) for 18–24 h at 37 °C. Suspect colonies were transferred to Xylose Lysine Deoxycholate (XLD) agar (Merck, Gernsheim, Germany) and incubated for 18–24 h at 37 °C. *Salmonella* colonies were further confirmed and identified using PCR by detecting the *invA* gene, as described previously (Chiu and Ou, 1996).

2.3. Serotype prediction and PFGE analysis of S. enterica isolates from water and human samples

From 62 *Salmonella*-positive samples (*invA* positive), a total of 44 *Salmonella* isolates were recovered and characterized by serotype