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Molecular characterization and antimicrobial susceptibility of *Listeria monocytogenes* isolated from foods and humans

Xudong Su [a], Jianmin Zhang [a, b], Weimin Shi [d], Xiaowei Yang [a], Yong Li [e], Haijian Pan [a], Dai Kuang [a], Xuebin Xu [f, **], Xianming Shi [a, *], Jianghong Meng [a, c, ***]

[a] Department of Food Science & Technology, School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai, China
[b] College of Veterinary Medicine, South China Agricultural University, Guangzhou, China
[c] Department of Food Science and Nutrition, University of Maryland, College Park, MD, USA
[d] Shanghai Municipal Ke-Ma-Jia Technology Center for Microbiology, Shanghai, China
[e] The Putuo District Center for Disease Control and Prevention, Shanghai, China
[f] Shanghai Municipal Center for Disease Control and Prevention, Shanghai, China

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ABSTRACT

A total of 120 *Listeria monocytogenes* isolates (107 from foods and 13 from humans) in Shanghai, China, were characterized by serogroup typing and virulence genes detection with PCR, antimicrobial susceptibility testing, and molecular subtyping using pulsed-field Gel Electrophoresis (PFGE). Isolates belonged to three *L. monocytogenes* serogroups, 1/2c, 3c (n = 47, 39.1%) was the most prevalent, followed by 1/2a, 3a (n = 44, 36.7%) and 1/2b, 3b, 7 (n = 29, 24.2%). Although the isolates were all susceptible to most antibiotics tested, 13 isolates were resistant to ceftriaxone and seven to tetracycline. None of the isolates were resistant to multiple antibiotics. The *tet* (M) genes were detected among tetracycline-resistant isolates. The isolates all harbored virulence genes *hly*, *prfA*, *plcA*, *plcB*, *inlA*, *inlB*, *inlI*; 116 isolates (96.7%) were positive for *inlC*; 78 (65.0%) for *inlG*; 119 (99.2%) for *inlJ*; 8 (6.67%) for *llsX*. A total of 74 distinct patterns were generated for the 119 isolates tested using *Apa*I and *Asc*I with Pulsed Field Gel Electrophoresis (PFGE). Three clinical isolates shared the same PFGE patterns with retail meet (chicken, duck, and beef) isolates. These findings indicated that food of animal origin likely serve as a major vehicle in transmitting human listeriosis in Shanghai. Active surveillance for *L. monocytogenes* is needed for better understanding the epidemiology of the pathogen, and for effective control and prevention of human listeriosis.

1. Introduction

Listeria monocytogenes is an important foodborne pathogen causing listeriosis with high mortality rates between 20% and 30% (Lukinmaa, Miettinen, Nakari, Korkeala, & Siitonen, 2003). High-risk populations include immunocompromised individuals, infants, and the elderly. Most human listeriosis cases are linked to contaminated food (Farber & Peterkin, 1991; Pinner et al., 1992; Scallan et al., 2011; Schuchat, Swaminathan, & Broome, 1991). Many types of food including meat, poultry, dairy, cheese and vegetable products have been implicated as vehicles of listeriosis (Ochiai et al., 2014; Ruckerl et al., 2014; Zhang et al., 2007).

Over the past two decades, numerous foodborne listeriosis outbreaks have been reported in United States, Australia, Germany, New Zealand, England, France, Japan, Austria, Colombia, Brazil (Gasanov, Hughes, & Hansbro, 2005; Schmid et al., 2014; Yan et al., 2010) and Denmark (http://news/world/europe/contaminated-sausages-kill-12-people-in-denmark-listeria-outbreak-9665952.html). In 2011, a multistate listeriosis outbreak associated caused 146 invasive illnesses, one miscarriage and 29 deaths. It was one of the deadliest foodborne disease outbreaks in the United States (Laksanalamai et al., 2012). In China, there are only a few published

* Corresponding author. MOST-USDA Joint Research Center for Food Safety, School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai, 200240, China.
** Corresponding author. Municipal Center for Disease Control & Prevention, Shanghai, 200336, China.
*** Corresponding author. 0112 Skinner Building, Department of Nutrition & Food Science, University of Maryland, College Park, MD, 20742, USA.
E-mail addresses: xbxu@scdc.sh.cn (X. Xu), xmshi@sjtu.edu.cn (X. Shi), jmeng@umd.edu (J. Meng).

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reports on the characterization of *L. monocytogenes* isolates from food (Chao, Zhou, Jiao, Qian, & Xu, 2007; Yan et al., 2010; Zhang et al., 2013). Studies on both food and clinical isolates of *L. monocytogenes* are lacking.

Numerous studies have been conducted on the adhesion, invasion, and/or virulence regulation of *L. monocytogenes*. The roles of virulence and surface proteins (i.e., PrfA, ActA, InlA, InlB, InlC) of *L. monocytogenes* in pathogenesis have been well characterized in different animal hosts or cell types. The virulence of *L. monocytogenes* is in part related to its serotype and lineage (Roche et al., 2012). It is estimated that about 96% of the human listeirosis cases are caused by serovars 1/2a, 1/2b, and 4b (Swaminathan and Gerner-Smidt, 2007). During 2000, the National Surveillance Network for Food Contamination and Foodborne Diseases System was launched in China (Yan et al., 2010), but there is a paucity on data regarding the prevalence, types of serovars, and virulent profiles of *L. monocytogenes*.

Listeriosis requires antimicrobial therapy, and the treatment of choice consists of a β-lactam antibiotic, normally ampicillin, alone or in combination with an aminoglycoside, classically gentamicin. However *L. monocytogenes* srains resistant to one or more antibiotics have been recovered from food and sporadic cases of human listeriosis (Bertrand et al., 2005; Harakeh et al., 2009; Yucel & Balci, 2010). Although the knowledge on *L. monocytogenes* has improved, limited information on antibiotic resistance is available.

The aims of this study were to characterize *L. monocytogenes* isolates from foods and humans by serogrouping and lineage identification, virulence factor detection and antimicrobial susceptibility test. The research findings should help determine a possible link between human cases and source of the pathogen, and could be used to evaluate the potential risk of *L. monocytogenes* among contaminated foods in Shanghai, China.

2. Materials and methods

2.1. Bacterial strains

A total of 120 *L. monocytogenes* isolates recovered from 2004 to 2013 in Shanghai, China, were examined in the study, including 30 isolates from duck, 28 from chicken, 27 from beef, 18 from pork, 2 from vegetables, 1 from fish, and 1 from yogurt as well as 13 clinical isolates that were collected from patients admitted to three hospitals in Shanghai. The clinical isolates were recovered from 11 blood and two cerebrospinal fluid (CSF) specimens.

2.2. Molecular serogrouping and lineage identification

Multiplex PCR assays were performed to determine serogroups (1/2a, 3a; 1/2c, 3c; 1/2b, 3b, 7; 4a, 4c and 4b, 4d, 4e) (Doumith, Buchrieser, Glaser, Jacquet, & Martin, 2004), and lineages (I, II and III) of *L. monocytogenes* isolates (Ward et al., 2004).

2.3. Antimicrobial susceptibility tests and characterization of tetracycline resistance

Minimum inhibitory concentrations (MICs, μg/mL) were determined using the agar microdilution method according to guidelines recommended by the Clinical and Laboratory Standard Institute (CLSI, 2010). The following antimicrobials were tested: penicillin (PEN), ampicillin (AMP), Ampicillin-sulbactam (SAM), gentamicin (GEN), chloramphenicol (CHL), ceftriaxone (CRO), cephalothin (CEP), ciprofloxacin (CIP), Vancomycin (VAN), erythromycin (ERY), tetracycline (TET), Rifampin (RIF), trimethoprim/sulfamethoxazole (SXT). *Escherichia coli* ATCC25922, *Staphylococcus aureus* ATCC29213 and *Enterococcus faecalis* ATCC29212 were used as quality control organisms in the MICs determinations. The susceptibility tests were performed using the agar dilution method described by Barbosa et al. (Barbosa et al., 2013). Breakpoints for these antimicrobials were employed according to CLSI guidelines for Gram-positive bacteria (CLSI, 2010) and CLSI documents M31-A3 for *L. monocytogenens* (CLSI, 2008). Each experiment was performed in duplicate.

Seven isolates of tetracycline-resistant *L. monocytogenes* were detected with primers amplifying resistance genes *tet* (A), *tet* (B), *tet* (C), *tet* (D), *tet* (E), *tet* (G), *tet*(L), *tet*(M), *tet*(S) (Call, Bakko, Krug, & Roberts, 2003) and *tet* (K) (Gevers, Danielsen, Huys, & Swings, 2003).

2.4. Detection of virulence markers

Virulence genes *hly*, *plcA*, *plcB* (Volokhov, Rasooly, Chumakov, & Chizhikov, 2002), *prfA* (Cooray et al., 1994), *actA*, *inlA*, *inlB*, *inlG*, *inlI* (Chen et al., 2009), *inlC*, *inlJ* (Liu, Lawrence, Austin, & Ainsworth, 2007) were detected using PCR assays. *Listeriolysin S*-positive isolates were confirmed using PCR targeting *llsX* gene (Clayton, Hill, Cotter, & Ross, 2011).

2.5. PFGE

PFGE was performed according to the protocol on *L. monocytogenes* described by the U.S. Centers for Disease Control (http://www.pulsenetinternational.org/protocols/). Briefly, agarose-embedded DNA was digested with 50 U *Asc*I and *Apa*I for 2 h in a water bath at 37 °C. Restriction fragments were separated by electrophoresis in 0.5 × TBE buffer at 14 °C for 18 h using a Chef Mapper electrophoresis system (Bio-Rad, Hercules, CA). Running parameters were as follows: 6 V/cm; angle: 120°; temperature: 14 °C initial switch: 4 s; final switch: 40 s; length: 19 h. The gels were stained with DuRed. DNA bands were visualized with UV trans-ilumination (Bio-Rad, Hercules, CA). *Salmonella* enteric serovar Braenderup H9812 restricted with *Xba*I was used as the molecular weight size standard. Bands above or below this range were not included in the analysis. PFGE results were analyzed using the BioNumerics software, (Applied-Maths, Kortrijk, Belgium).

3. Results

3.1. Distribution of L. monocytogenes serogroups and lineages

Three serogroups were identified among 120 *L. monocytogenes* isolates (Table 1). Serogroup 1/2c, 3c (n = 47, 39.1%) was the most prevalent, followed by serogroups 1/2a, 3a (n = 44, 36.7%) and 1/2b, 3b, 7 (n = 29, 24.2%). Serogroup 1/2a, 3a was more frequently present in duck (31.8%, 14 of 44) and beef (29.5%, 13 of 44) (Fig. 1). In contrast, 48.3% chicken isolates (14 of 29) belonged to serogroup 1/2b, 3b, 7. Serogroup 1/2c, 3c was more frequently present in duck, beef, pork and chicken. The human isolates were split into serogroups 1/2a, 3a (6 of 13) and 1/2b, 3b, 7 (6 of 13) and remaining one isolate belonged to serogroup 1/2c, 3c (Fig. 1).

Based on variable gene content, 120 isolates were divided into lineages I and II (Table 1, Fig. 1). The majority of the isolates belonged to lineage II (n = 91, 75.83%), and only a quarter to lineage I (n = 29, 24.17%). Serogroup1/2b, 3b, 7 isolates were part of lineage I, and serogroup 1/2a, 3a, and 1/2c, 3c isolates were under lineage II.

3.2. Prevalence of virulence markers

All isolates harbored *hly*, *plcA*, *plcB*, *prfA*, *actA*, *inlA* and *inlB* as well as *inlI* (Fig. 2). Additionally, 116 contained *inlC*, 78 had *inlG* and 119 had *inlJ*. Eight isolates of serogroup 1/2b, 3b (6.67%) contained