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Prevalence and characterization of *Listeria monocytogenes* isolated from retail food in Henan, China

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ABSTRACT

Listeria monocytogenes, an important foodborne pathogen, is the causal agent of listeriosis. In this study, a total of 954 food samples originating from raw meat, cooked meat products, seafood, and vegetables purchased from supermarkets and open-air markets in Henan province, China, were analyzed for the presence of *L. monocytogenes*. All *L. monocytogenes* isolates were subjected to serotyping, pulsed-field gel electrophoresis (PFGE), and antimicrobial resistance. The overall percentage of *L. monocytogenes* prevalence was 6.2% ($n = 59$) with the highest rate of 7.4% for cooked meat products followed by raw meat (6.7%). The isolates belonged to five serotypes (1/2a, 1/2b, 1/2c, 4b, and 4c), with serotype 1/2a being predominant (55.9%). PFGE revealed a low genetic diversity among the isolates, irrespective of their sources, suggesting that dominant clones are widespread in different food products in Henan. Resistance to cefotaxime (30.5%) and ciprofloxacin (13.5%) was most often, whereas resistance to tetracycline, trimethoprim/sulfamethoxazole, and erythromycin was observed less frequently. The presence of *L. monocytogenes* in food products and antimicrobial resistance among the isolates represents a potential public health risk. Our results indicate that effective hygienic measures and bacteriological controls are necessary in China to reduce the contamination of retail food samples by *L. monocytogenes*.

1. Introduction

Listeriosis, a serious foodborne and onward disease caused by the ubiquitous bacterium *Listeria monocytogenes*, represents an important public health problem. The pathogen is able to cause several types of infections in humans, and invasive listeriosis is more frequently associated with immunocompromised individuals, elderly, pregnant women, and neonates (Jalali & Abedi, 2008). *L. monocytogenes* is widely distributed in the natural environment, and it can survive under adverse environmental conditions (Todd & Notermans, 2011). Food is considered to be the main vector of *L. monocytogenes* in epidemic and sporadic cases of systemic human listeriosis. Meat, milk, ready-to-eat food as well as fresh vegetables and seafood are reported as important sources of contamination (Meyer et al., 2012; Osaili, Alaboudi, & Nesiar, 2011; Yan et al., 2010).

Although listeriosis may be caused by all 13 serotypes of *L. monocytogenes*, only 1/2a, 1/2b, and 4b are frequently isolated from clinical samples, with serotype 4b causing by far the most cases of human listeriosis (Borucki & Call, 2003; Pan, Breidt, & Gorski, 2010; Swaminathan & Gerner-Smidt, 2007). However, serotype 1/2a seems to be the most prevalence in food (Garrido, Vitas, & Garcia-Jalon, 2009; Zhang et al., 2007).

L. monocytogenes is generally susceptible to a wide range of antimicrobials (Charpentier & Courvalin, 1999). Currently, the effective treatment of listeriosis is usually based on the administration of ampicillin or penicillin plus gentamicin or the combination trimethoprim/sulfamethoxazole (Safdar & Armstrong, 2003). However, the resistant isolates of *L. monocytogenes* have been recovered from food, environment, and human listeriosis (Chen, Pyla, Kim, Silva, & Jung, 2010; Conter et al., 2009; Morvan et al., 2010). The increasing prevalence of resistance to clinically important antimicrobial agents among *L. monocytogenes* isolates has also been observed in China and other countries (Chen, Zhang, Mei, Jiang, & Fang, 2009; Granier et al., 2011; Sakaridis et al., 2011; Yan et al., 2010). Therefore, it is necessary to implement the monitoring system to be aware of the antimicrobial resistance profiles of *L. monocytogenes* in various sources from different areas.

In China, the prevalence of *L. monocytogenes* in retail food is up to 25.3% during 2000–2007 and *L. monocytogenes* is recognized as a

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serious risk to food safety (Chen et al., 2009). However, there is lack of detailed information regarding the prevalence, serotyping, PFGE profiles, and antimicrobial susceptibility of *L. monocytogenes* from food samples in China. Thus, the objectives of this study were to analyze retail food samples for the presence of *L. monocytogenes*, determine serotypes and PFGE patterns, and investigate the antimicrobial susceptibility profile of the isolates.

2. Materials and methods

2.1. Sample collection

A total of 954 food samples, composed of raw meat (pork, beef, mutton and chicken), cooked meat products, seafood, and vegetables, were purchased from supermarkets and open-air markets from August 2007 to September 2009 in Henan province, located in the central part of China. All samples were aseptically collected and transported in cooler with ice packs to the laboratory immediately. Samples were kept at 4 °C and analyzed within 24 h.

2.2. Isolation of L. monocytogenes

Isolation of *L. monocytogenes* was performed using standard procedures described in the National Standards of the People's Republic of China (GB/T 4789.30-2003). Briefly, twenty-five gram of each sample was aseptically taken, added to 225 ml Listeria enrichment broth I (LB_1; Huankai Ltd., Guangzhou, Guangdong, China), and homogenized in a stomacher bag for 60 s at normal speed. Following 24 h of incubation at 30 °C, 0.1 ml of primary enrichments was transferred to 10 ml of Listeria enrichment broth II (LB_2; Huankai) and incubated at 30 °C for 24 h. Secondary enrichments were plated on PALCAM agar (Huankai) and CHROMagar Listeria agar (CHROMagar Co., Paris, France) and incubated for 24 h at 37 °C. The suspected colonies were identified using API Listeria kit (BioMérieux, Marcy l'Etoile, France). All isolates designated as *L. monocytogenes* were additionally analyzed by PCR to amplify the *lmo0733* gene with the specific primers (5′-CGCAAGAAGAAATTGCCATC-3′ and R: 5′-TCCGCGTTAGAAAAATTCCA-3′) as previously described (Filiousis, Johansson, Frey, & Perreten, 2009).

2.3. Serotyping

L. monocytogenes isolates were serotyped with *Listeria* O and H antisera (Denka Seiken, Tokyo, Japan), according to the manufacture's instructions.

2.4. PFGE

PFGE of all isolates was performed using the standard CDC PulseNet protocol in general with minor modification (Graves & Swaminathan, 2001).

2.5. Antimicrobial susceptibility testing

Ciprofloxacin, moxifloxacin, levofloxacin, tetracycline, erythromycin, gentamycin, chloramphenicol, streptomycin, trimethoprim/sulfamethoxazole, and cefotaxime obtained from Sigma were included in the susceptibility study. MICs were determined using the broth microdilution method described previously (Yan et al., 2010). Interpretation for susceptibility status was based on the standards of the Clinical and Laboratory Standards Institute (CLSI). *Staphylococcus aureus* ATCC29213, *Enterococcus faecalis* ATCC29212, and *Escherichia coli* ATCC25922 were used as control strains.

3. Results

3.1. Prevalence of L. monocytogenes in food products

In the present study, a total of 59 *L. monocytogenes* isolates were recovered, representing 6.2% of the food samples tested (Table 1). The pathogen occurred among all kinds of food products and the prevalence of *L. monocytogenes* was highest in cooked meat products (7.4%), followed by raw meat (6.7%), seafood (2.7%), and vegetables (1.7%). Among raw meat samples, pork (9.8%) was more contaminated than chicken (8.9%), beef (2.9%), and mutton (1.1%).

3.2. Serotyping

All isolates were grouped into five different serotypes 1/2a, 1/2b, 1/2c, 4b, and 4c (Fig. 1). The predominant serotypes were 1/2a (33, 55.9%), followed by 1/2c (12, 20.3%), 1/2b (11, 18.6%), 4b (2, 3.4%), and 4c (1, 1.7%). Serotype 1/2a was most frequently present in pork and chicken samples as well as cooked meat products. Half of the isolates of serotype 1/2c were detected from pork samples, while the majority of the isolates belonging to serotype 1/2b were found in chicken samples. The two isolates of serotype 4b were recovered from cooked meat samples.

3.3. PFGE

The genetic fingerprint of the 59 *L. monocytogenes* isolates was determined using PFGE and 17 distinct PFGE profiles were identified (Fig. 1). The result showed that 9 PFGE types occurred at least two times, accounting for 86.4% of the isolates characterized. A total of 8 PFGE types occurred only once and accounted for 13.6% of the isolates. PFGE type P5 predominated and included 15 strains (8 serotype 1/2a isolates and 7 serotype 1/2c isolates), followed by type P3 (8 serotype 1/2a isolates and 1 serotype 4c isolate).

3.4. Antimicrobial resistance

The results of resistance analysis of the isolated *L. monocytogenes* strains against 10 antimicrobial agents are presented in Table 2. Thirty-four (57.6%) of the *L. monocytogenes* strains exhibited resistance to at least one antimicrobial. All isolates were susceptible to moxifloxacin, levofloxacin, gentamycin, and chloramphenicol. Resistance to cefotaxime (30.5% of the isolates were resistant) and resistance to ciprofloxacin (13.5%) were observed most often, whereas resistance to tetracycline, trimethoprim/sulfamethoxazole, and erythromycin was observed less frequently. Intermediate susceptibility was observed for cefotaxime (11.9%), ciprofloxacin (3.4%), erythromycin (3.4%), and streptomycin (1.7%). Among the resistant isolates, four, one, and one isolates, respectively, were resistance to two, three, and five antimicrobials.

Table 1
Prevalence of the *Listeria monocytogenes* in different food products.

Food type	No. of samples	No. of positive samples (%)
Raw meat	645	43 (6.7)
Pork	183	18 (9.8)
Beef	136	4 (2.9)
Chicken	235	20 (8.9)
Mutton	91	1 (1.1)
Cooked meat products	176	13 (7.4)
Seafood	75	2 (2.7)
Vegetables	58	1 (1.7)
Total	954	59 (6.2)