Prevalence and characterization of *Listeria monocytogenes* isolated from retail-level ready-to-eat foods in South China

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ABSTRACT

*Listeria monocytogenes* is an important food-borne pathogen causing meningitis, meningoencephalitis and abortion. To assess the potential risk to consumer health, the presence of *L. monocytogenes* was investigated using qualitative and quantitative methods. Ten (6.33%) of 158 retail RTE food samples were positive for *L. monocytogenes* and the contamination levels were less than 10 MPN/g, while none of 65 dairy products was positive for *L. monocytogenes*. The 37 strains were grouped into five clusters and two singletons, five clusters and two singletons, and three clusters and one singleton by enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC-PCR) and RAPD fingerprint respectively, at similarity coefficient of 80%. The susceptibility test showed that 83.8% were susceptible to 15 antimicrobials; two were penicillin-resistant, and one was multidrug-resistant to kanamycin, tetracycline, sulfamethoxazole, rifampin, gentamycin, penicillin, and ampicillin. Virulent *L. monocytogenes* that possess partial antimicrobial resistance, and serotypes frequently associated with listeriosis were recovered from RTE foods. Consumers may, therefore, be exposed to potential risks of *L. monocytogenes* infection in South China. This study contributed to the prevalence and contamination levels of *L. monocytogenes* in RTE foods in South China for the first time, providing baseline information for Chinese regulatory authorities to formulate a regulatory framework for controlling *L. monocytogenes* to improve the microbiological safety of RTE foods.

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1. Introduction

*Listeria monocytogenes* is an important food-borne pathogen and had been isolated from feces of animals, food, and food-processing plants (Miettinen & Wirtanen, 2006). *L. monocytogenes* can cause severe listeriosis infections resulting in meningitis, meningoencephalitis, septicemia, abortion, and prenatal infection in individuals with weakened immune systems, such as fetuses, infants, the elderly, and immunocompromised individuals (Siegman-Igra et al., 2002; Wu, Zhang, Chen, & Yin, 2008). Mortality due to listeriosis is up to 20–30% in vulnerable groups (Lukinmaa, Miettinen, Nakari, Korkeala, & Siitonen, 2003).

For surveillance or tracing sources of *L. monocytogenes*, in recent years, a number of typing techniques, such as pulsed field gel electrophoresis (PFGE) (Gerner-Smidt et al., 2006), amplified fragment length polymorphism (AFLP) (Parisi et al., 2010), random amplified polymorphic DNA (RAPD) (Lawrence, Harvey, & Gilmour, 1993), multi-locus sequence typing (MLST) (Parisi et al., 2010), ribotyping (Pagadala et al., 2012), and enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC-PCR) (Chen, Pyla, Kim, Silva, & Jung, 2010), have been developed to differentiate *L. monocytogenes* strains. Of these techniques, PFGE has been considered to be the “gold standard” for subtyping *L. monocytogenes* due to its high reproducibility and discriminatory ability (Gerner-Smidt et al., 2006). However, PFGE is labor intensive and time-consuming. In contrast, ERIC-PCR is a relatively simple, highly reliable, and cost-effective method, and was demonstrated to generate clear DNA fingerprints within a single bacterial species (Jersek et al., 1999).

As listeriosis may result from ingestion of contaminated food products, the prevalence, quantitative data, and characterization of isolates in different types of food is important for risk assessment. Given its ability to tolerate wide pH, low temperature, and salt ranges, propagation of *L. monocytogenes* in ready-to-eat (RTE) foods may cause potential risk of listeriosis. Many countries have...
formulated a zero-tolerance policy for \( L. \) monocytogenes in certain RTE foods (Norrung, 2000). Generally, additional handling of RTE foods at the retail level, such as open-air slicing, weighing, and packaging, may increase the potential risks of cross contamination (Lin et al., 2006). However, presence of \( L. \) monocytogenes in RTE foods at retail level has received less attention in South China and little information on the prevalence and contamination levels of \( L. \) monocytogenes are available. The objective of this study was to investigate the occurrence of \( L. \) monocytogenes in RTE foods and dairy products at retail level in South China both qualitatively and quantitatively, and to characterize \( L. \) monocytogenes isolates by genotyping and phenotyping for risk analysis of \( L. \) monocytogenes from RTE food chains.

2. Materials and methods

2.1. Sampling procedure

From July 2011 to August 2012, a total of 223 samples of 158 RTE foods were collected, including roast duck, roast spare ribs, cold vegetable dish in sauce, brine-soaked duck, roasted goose, salt baked chicken, braised pork, ham, fried drumsticks and 65 dairy products. The sampling sites were distributed through 12 different cities in South China. All of the samples were kept below 4 °C during transportation and testing was initiated within 2 h after receipt.

2.2. Qualitative detection

For qualitative detection, an enrichment method was used according to the National Food Safety Standards of China, with minor modifications (Fig. S1). In brief, samples were submitted to qualitative analysis for \( L. \) monocytogenes by homogenizing 25 g of the respective sample and 225 mL LB1 enrichment broth (Huankai, Guangzhou, China), for 30 s in stomacher bags (Huankai, Guangzhou, China). Homogenates were incubated at 30.0 °C for 24 h; thereafter, 0.1 mL LB1 enrichment culture was transferred to 10 mL LB2 enrichment broth at 30.0 °C for 24 h. A loopful of the LB2 enrichment culture was streaked onto \( Listeria \) selective agar plates (CHROM-agar, Paris, France) and incubated at 37 °C for 48 h. Three or four presumptive colonies, which were typically blue in color with a white halo, were selected for Gram staining, catalase test, and oxidase test. Confirmation of \( L. \) monocytogenes was performed using the Microgen ID \( Listeria \) identification system (Microgen, Camberley, UK) according to the manufacturer’s instructions. All isolates designated as \( L. \) monocytogenes by Microgen ID were additionally identified by duplex PCR targeting the genes hly (coding for listeriolysin O) and inlB (internalin B), as previously described (Xu, Wu, Zhang, Deng, & Zhou, 2009); the primers used are shown in Table S2.

2.3. MPN method for quantitative analysis

The most probable number (MPN) method described by Gombas, Chen, Clavero, and Scott (2003) was adapted for use in this study (Fig. S1). The MPN was determined on the basis of the number of positive tube(s) in each of the three sets and MPN table (USDA, 1998; USFDA, 1998).

2.4. Serotyping by multiplex PCR

The serotypes of 37 \( L. \) monocytogenes isolates and five reference strains (Table S1) were determined using multiplex PCR, as previously described by Doumith, Buchrieser, Glaser, Jacquet, and Martin (2004). The primers used are shown in Table S2. This assay differentiates isolates into five major serovars, each of which represents more than one serotype, including subtype I.1 (which includes serotypes 1/2a and 3a), subtype I.2 (serotypes 1/2c and 3c), subtype I.1 (serotypes 4b, 4d, and 4e), subtype I.2 (1/2b, 3b, and 7), and subtype II (serotypes 4a and 4c).

2.5. Determination of presence of virulence-related genes

Five PCRs were performed to detect the presence of seven virulence-related genes in the 37 \( L. \) monocytogenes strains and five reference strains (Table S1); two multiplex PCRs were designed to identify the actA, iap, plcA, and inlA genes, individual PCRs were carried out for prfA, plcB, mpl genes, respectively. The primers used for virulence-related genes identification are shown in Table S2 (Chen et al., 2009; Furrer, Candrian, Hoefelein, & Luethy, 1991; Notermans, Dufrenne, Leimeister-Wächter, Domann, & Chakraborty, 1991; Suárez, González-Zorn, Vega, Chico-Calero, & Vázquez-Boland, 2001).

2.6. Antimicrobial (AM) susceptibility test

Since no resistance criteria exist for \( Listeria \) antibacterial susceptibility testing in Clinical and Laboratory Standards Institute guidelines (Clinical and Laboratory Standards Institute, 2011) for tested AMs, criteria for staphylococci were applied. A panel of 15 antimicrobials at the specific concentration per disc were tested: ampicillin (AMP; 10 μg), cephalothin (CEP; 30 μg), chloramphenicol (CHL; 30 μg), ciprofloxacin (CIP; 5 μg), erythromycin (ERY; 15 μg), gentamicin (GEN; 10 μg), kanamycin (KAN; 30 μg), rifampin (RIF; 5 μg), doxycycline (DOX, 30 μg), levofloxacin (LEV; 5 μg), penicillin (PEN, 10 U), tetracycline (TET; 30 μg), vancomycin (VAN; 30 μg), sulfamethoxazole with trimethoprim (SXT; 23.75/1.25 μg), and sulbactam/ampicillin (SAM; 10/10 μg) (Oxoid, Basingstoke, UK). Antimicrobial susceptibility tests were performed as in a previous study (Kovačevic, Mesak, & Allen, 2012). Staphylococcus aureus ATCC 25923 and Escherichia coli ATCC 25922 were used as quality control strains for this study. Zones of inhibition were measured with a precision caliper to the nearest 0.01 mm. Isolates exhibiting resistance to at least two of the antimicrobial agents tested were considered to be multidrug-resistant strains.

2.7. ERIC-PCR

Genomic DNA was extracted from \( L. \) monocytogenes using a Bacterial Genomic DNA Purification Kit (Dongsheung Biotech, Guangzhou, China) according to the manufacturer’s instruction. Genomic DNA concentration was determined at 260 nm using a Nano Drop® ND-1000UV-Vis Spectrophotometer (Thermo Fisher Scientific, MA, USA). The ERIC primers were from Versalovic, Koehler, and Lupski (1991). ERIC-PCR typing was performed on the \( L. \) monocytogenes strains using the protocol described by Jeršek et al. (1999) with some modifications. The PCR mixture (25-μL) contained 1 unit GoTaq® Hotstart polymerase (Promega, WI, USA), 0.5 μM of each primer, 2.5 mM MgCl₂, 0.2 mM each dNTP, and 40 ng of template genomic DNA. Amplifications were performed with a DNA thermocycler (Applied Biosystems, CA, USA) with the following temperature profile: an initial denaturation at 94 °C for 3 min; 35 cycles each consisting of 30 s at 94 °C, 30 s at 46 °C, 30 s at 49 °C, and 3 min at 72 °C, and a final extension at 72 °C for 10 min. The ERIC-PCR products were separated by electrophoresis in a 1.5% agarose gel with Goldview staining (0.005%, v/v), and photographed using a UV Imaging System (GE Healthcare, WI, USA). The images were captured in TIFF file format for further analysis.
2.8. Random amplified polymorphic DNA typing

The 10-mer primers OPM-01 (5′-GTGGTGCTG-3′) and UBC155 (5′-CTGGCGCCTG-3′) were designed and tested in other studies (Farber & Addison, 1994; Lawrence et al., 1993). Reaction mixtures (25-μL) were prepared, each containing one unit of Taq polymerase, 2.5 mM MgCl₂, 0.2 mM each dNTP, 1.6 μM primer, and 40 μg L. monocytogenes DNA template. For optimal amplifications with primer OPM-01 and UBC-155, PCR was carried out with GoTaq® HotStart polymerase (Promega, WI, USA) and Taq DNA polymerase (Thermo Fisher, MA, USA), respectively. The reaction mixtures were cycled with a hot-lid cycler (Applied Biosystems, CA, USA) through the following temperature profile: an initial 5 cycles at 94 °C for 5 min, 35 °C for 5 min, 72 °C for 5 min; then 30 cycles each consisting of 1 min at 94 °C, 2 min at 35 °C, and 2 min at 72 °C; and a final extension at 72 °C for 10 min. Ten microliters of all amplicons were resolved by electrophoresis in a 2.0% agarose gel with Goldview staining (0.005%, v/v), and gels were photographed under UV transillumination (GE Healthcare, WI, USA); images were saved as TIFF format files for genotype analysis.

2.9. Fingerprint data analysis

The observed bands in the gels were evaluated based on the presence (coded 1) or absence (coded 0) of polymorphic fragments for the ERIC and RAPD products. Cluster analysis was performed with NTSYS-pc (Version 2.10), a numerical taxonomy and multivariate analysis software package (Rohlf, 2000), based on a Dice’s similarity coefficient (SD), with a 1% position tolerance and the unweighted pair group method using arithmetic averages (UPCMA).

3. Results

3.1. Qualitative and quantitative analysis

Ten (6.33%) samples positive for L. monocytogenes were detected among 158 RTE foods, but no L. monocytogenes was isolated from 65 dairy products in South China after qualitative and quantitative analyses. As shown in Table 1, among the positive samples, five (6.25%) were from deli poultry samples, four (26.7%) were from cold vegetable dish in sauce samples, and one from roast spare ribs. No L. monocytogenes strain was isolated from cooked beef or cold noodles in sauce (Table 1). The MPN values of all seven positive samples did not exceed 10 MPN/g; that of six of seven positive samples were less than 1 MPN/g (Table 2). All 37 isolates (Fig. S6) were positive for hly, the gene encoding listeriolysin O, and inlB, which indicated that all of the isolated strains belonged to L. monocytogenes.

Table 1
The occurrence of L. monocytogenes in RTE foods.

<table>
<thead>
<tr>
<th>Product category</th>
<th>Total tests</th>
<th>L. monocytogenes No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooked pork</td>
<td>36</td>
<td>1/36 (2.8)</td>
</tr>
<tr>
<td>Cooked beef</td>
<td>3</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Deli poultry</td>
<td>80</td>
<td>5/80 (6.3)</td>
</tr>
<tr>
<td>Cold vegetable dish in sauce</td>
<td>15</td>
<td>4/15 (26.7)</td>
</tr>
<tr>
<td>Cold noodles in sauce</td>
<td>24</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Dairy products</td>
<td>65</td>
<td>0 (0.0)</td>
</tr>
</tbody>
</table>

shown in Fig. S2, except for subtype III, all serotypes were identified among the 37 L. monocytogenes isolates; 19 (51.3%) and 10 (27.0%) L. monocytogenes strains belonged to serovar II.1, and serovar II.2, respectively; serovar I.1 and I.2 had four isolates each. Serovar II.1 was the predominant serotype among the RTE isolates.

3.3. Detection of virulence-related genes in L. monocytogenes isolates

All 37 isolates presented in this study were identified as L. monocytogenes, based on biochemical tests and duplex PCR, and were examined for the presence of seven virulence marker genes by PCR. All 37 strains harbored inlA, plcA, actA, mpl, inlJ, and prfA sequences (Figs. S7–S11). Except for strain 427-1, all isolates also harbored plcB.

3.4. Antimicrobial susceptibility test

The susceptibility tests suggested that 31 of 37 (83.8%) isolates were sensitive to all antimicrobials tested in the panel, three (8.1%) L. monocytogenes strains were resistance to PEN and two isolates had intermediate resistance to PEN. Only one isolate exhibited intermediate resistance to DOX, and three L. monocytogenes strains showed intermediate resistance to AMP (Table 3). All of the L. monocytogenes isolates were susceptible to CHL, VAN, LEV, CIP, ERY, and SAM (Table 3). However, it was surprising to note that the strain 191-1, isolated from a fried drumstick sample, was a multidrug-resistant strain, which had resistance to seven AMs, namely KAN, TET, SXT, RIF, GEN, PEN, and AMP.

Table 2
Results of qualitative and quantitative methods for RTE products.

<table>
<thead>
<tr>
<th>No. of samples</th>
<th>Qualitative method</th>
<th>Quantitative method (MPN/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>41</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>191</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>206</td>
<td>-</td>
<td>0.36</td>
</tr>
<tr>
<td>208</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>359</td>
<td>+</td>
<td>0.3</td>
</tr>
<tr>
<td>409</td>
<td>-</td>
<td>0.61</td>
</tr>
<tr>
<td>427</td>
<td>-</td>
<td>0.3</td>
</tr>
<tr>
<td>428</td>
<td>-</td>
<td>0.36</td>
</tr>
<tr>
<td>509</td>
<td>-</td>
<td>0.3</td>
</tr>
<tr>
<td>678</td>
<td>+</td>
<td>3.6</td>
</tr>
</tbody>
</table>

3.5. ERIC-PCR

ERIC-PCR resulted in three to eight amplification bands, with a size range from 300 to 2135 bp, which were specific for the 37 L. monocytogenes isolates and five reference strains of L. monocytogenes (Fig. S3). Bands with molecular sizes of 500, 1000, and 1800 bp were common to most isolates. Most of the isolates from the same positive sample yielded similar fingerprint profiles. At a relative similarity coefficient of 65%, the 37 L. monocytogenes isolates and five reference strains fell into three major clusters, designated as A, B, and C according to the different serovars. Cluster A included serovar I.1 (1/2a, 3a) and I.2 (1/2c, 3c); cluster B included serovar I.1 (4b, 4d, 4e) and I.2 (1/2b, 3b, 7); while cluster C only included strain 191-1, which had been isolated from a fried drumstick sample and which had multidrug-resistance to various antimicrobials. Based on a similarity coefficient of 80%, we identified five clusters and two singletons; only one cluster included different serovar strains, the other clusters showed genetic similarity ranging from 94% to 100% (Fig. 1).
3.6. RAPD genotyping analysis

All of the *L. monocytogenes* isolates were typed with OPM-01 and UBC-155 (Figs. S4, S5); both primers had comparable differentiating power in ERIC-PCR (Fig. 1). Using the primer OPM-01, the 37 *L. monocytogenes* isolates and five reference strains were grouped into five clusters and two singletons (i.e., vii), prevalent clusters were iv, v, and i, comprising 18, 7, and 7 isolates, respectively, whereas clusters ii and vi included only one isolate each (Fig. 2A).

Three clusters and one singleton were discriminated among the 37 *L. monocytogenes* isolates and five reference strains using the UBC-155 primer, at a relative similarity coefficient of 80%; these were designated as clusters I, II, III, and IV. Surprisingly, the 37 *L. monocytogenes* isolates and five reference strains were grouped into three clusters at a relative genetic similarity of 59% (Fig. 2B). It was interesting to observe that three clusters were differentiated according to serovar, similar to the clustering observed with ERIC-PCR genotyping. Cluster a comprised 11 strains, all of which belonged to serovar I.1 and I.2; cluster b

### Table 3
Results of antimicrobial resistance of *L. monocytogenes* isolates in the study.

<table>
<thead>
<tr>
<th>Antimicrobial agents</th>
<th>Breakpoints (mm)</th>
<th>Susceptible (S)</th>
<th>Intermediate (I)</th>
<th>Resistant (R)</th>
<th>No. of isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kanamycin</td>
<td>≥18</td>
<td>14–17</td>
<td>≤13</td>
<td>36 (97.3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>≥19</td>
<td>15–18</td>
<td>≥14</td>
<td>36 (97.3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Sulfamethoxazole with trimethoprim</td>
<td>≥16</td>
<td>11–15</td>
<td>≤10</td>
<td>36 (97.3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>≥16</td>
<td>13–15</td>
<td>≤12</td>
<td>37 (100)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>≥18</td>
<td>13–17</td>
<td>≤12</td>
<td>32 (86.8)</td>
<td>2 (5.4)</td>
</tr>
<tr>
<td>Penicillin</td>
<td>≥29</td>
<td>–</td>
<td>≤28</td>
<td>33 (89.2)</td>
<td>3 (8.1)</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>≥29</td>
<td>–</td>
<td>≤28</td>
<td>37 (100)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>≥17</td>
<td>15–16</td>
<td>≤14</td>
<td>37 (100)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>≥19</td>
<td>16–18</td>
<td>≤15</td>
<td>37 (100)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>≥15</td>
<td>13–14</td>
<td>≤12</td>
<td>36 (97.3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>≥21</td>
<td>16–20</td>
<td>≤15</td>
<td>37 (100)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>≥23</td>
<td>14–22</td>
<td>≤13</td>
<td>37 (100)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>≥18</td>
<td>15–17</td>
<td>≤14</td>
<td>36 (97.3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Rifampin</td>
<td>≥20</td>
<td>17–19</td>
<td>≤16</td>
<td>36 (97.3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Sulbactam/ampicillin</td>
<td>≥15</td>
<td>12–14</td>
<td>≤11</td>
<td>37 (100)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

*No. of isolates (%)* indicates the percentage of susceptible (S), intermediate (I), and resistant (R) isolates among the total number of isolates.

*Table 3: Results of antimicrobial resistance of *L. monocytogenes* isolates in the study.*

Explanatory note: Breakpoints for Enterococcus spp.*

![Fig. 1. The characterization of *L. monocytogenes* isolates from RTE foods in South China. ND: not detect; SUS: susceptibility; KAN: kanamycin; TET: tetracycline; SXT: sulfamethoxazole with trimethoprim; RIF: rifampin; GEN: gentamicin; PEN: penicillin; AMP: ampicillin.](image-url)
included 30 strains, which comprised 20 serovar II.1 strains and 10 serovar II.2 strains, while only one strain belonged to cluster c.

4. Discussion

4.1. Risk analysis

To date, limited information has been available on the prevalence and contamination levels of L. monocytogenes present in RTE food in China. To our knowledge, no study on the prevalence and risk analysis of L. monocytogenes in retail-level RTE foods from South China has been reported yet. Our results showed that 10 samples from 158 RTE foods were positive for the presence of L. monocytogenes, indicating a prevalence of 6.33%. These data were consistent with previous studies (Chao, Zhou, Jiao, Qian, & Xu, 2007; Okada, Monden, Igimi, & Yamamoto, 2012). Since, at retail level in China, RTE foods are normally processed at 100°C, the MPN value of all seven positive samples was less than 10 MPN/g indicating that the contamination of L. monocytogenes in those food products may arise from the additional handling and processing, such as open-air slicing, weighing, and packaging. No L. monocytogenes was isolated from 65 dairy products, which was consistent with some studies previously conducted in China (Chao et al., 2007; Yan et al., 2010), but was different to results from some other countries (Harakeh et al., 2009; Hong et al., 2007). Therefore, our study provided some new information on the prevalence of L. monocytogenes in retail-level RTE foods from South China.

L. monocytogenes with several putative virulence genes associated with the infection cycle may be potentially pathogenic for consumers, especially genes responsible for expression of phosphatidylinositol-specific phospholipase C (PI-PLC), internalin-like proteins, and listeriolysin O (Kaur, Malik, Vaidya, & Barbuddhe, 2007; Vázquez-Boland et al., 2001). The results from our study showed that 97.3% of the 37 L. monocytogenes isolates harbored all the virulence-associated genes examined, including inlA, prfA, plcA, plcB, mpl, iap, and actA; only one strain lacked plcB. The observation indicated most of the isolates may have full pathogenic potential. However, the presence of seven virulence-related genes in a L. monocytogenes isolate does not always imply that it is capable of causing disease (Jiang, Xu, Chen, Shuai, & Fang, 2006; Van Stelten, Simpson, Ward, & Nightingale, 2010). Further studies will, therefore, be required to examine whether the strains isolated in this study are pathogenic.

4.2. Serotyping

L. monocytogenes are divided into 13 serotypes based on somatic (O) and flagellar (H) antigens, including three lineages, viz. I, II, and III. Doumith et al. (2004) have developed a rapid multiplex PCR for serotyping of L. monocytogenes, by which the three lineages were defined into five distinct phylogenetic groups, each correlated with serovars: I.1 (1/2a–3a), I.2 (1/2c–3c), II.1 (4b–4d–4e), II.2 (1/2b–3b–7), and III (4a–4e). Since serotypes 3a, 3b, 4d, and 4e were relatively rare in foodborne L. monocytogenes, serovar I.1, I.2, and II.2 were considered as serotype 1/2a, 4b, and 1/2b, respectively. L. monocytogenes strains have different abilities to cause human listeriosis according to their serotypes. Most strains isolated from patients with outbreak listeriosis to date belong to serotype 4b.
(Gianfranceschi, Gattuso, D’Ottavio, Fokas, & Aureli, 2007; Orsi, den Bakker, & Wiedermann, 2011). In the present study, serovar II.1 (4b–4d–4e) was the predominant serotype among strains isolated from RTE foods, which is in accordance with the previous studies (Aurora, Prakash, & Prakash, 2009; Zhang et al., 2007). This result suggested that consumers are exposed to potential risks of \emph{L. monocytogenes} infection in South China. Because of the poor discriminatory power of serotyping for clinical isolates, a number of molecular typing methods with high discriminatory power have been developed for typing \emph{L. monocytogenes} strains.

4.3. ERIC-PCR

ERIC-PCR is widely applied for typing of foodborne pathogens, such as \emph{L. monocytogenes} (Chen et al., 2010) and \emph{Cronobacter sakazukii} (Ye et al., 2009). In this study, a total of 37 test and five reference \emph{L. monocytogenes} strains were divided into three clusters, according to different lineages, at a similarity level of 65%. Cluster A included five strains belonging to serovar I.1 and six strains belonging to serovar II.2, respectively. Cluster B comprised 10 strains belonging to serovar II.2 and 20 strains that belonged to serovar II.1, respectively; strain 191-1 was the only isolate in cluster C (Fig. 1). As shown in Fig. 1, identical ERIC-PCR profiles were observed for some isolates with different serotypes, as has also been reported in a previous study (Soni, Singh, Singh, & Dubey, 2013). This result suggested that the target sequences of DNA amplified by ERIC primers are not serotype-specific. In addition, \emph{L. monocytogenes} strains belonging to the same serovar could not be distinguished efficiently by ERIC-PCR; this observation was consistent with a previous study conducted by Jersek et al. (1999). Thus, it is necessary to apply another molecular typing method for further differentiation of \emph{L. monocytogenes}, although ERIC-PCR was chosen for analysis of genetic diversity.

4.4. RAPD

RAPD is a very simple and rapid molecular subtyping technique with high discriminatory power; it has been used as a powerful tool in the differentiation of foodborne pathogens. In the present study, thirty-seven \emph{L. monocytogenes} strains and five reference strains were divided into seven and four clusters by OPM-01 and UBC-155 primers, respectively, at a similarity coefficient of 80%. Similar to ERIC-PCR fingerprint analysis, RAPD analysis could not discriminate \emph{L. monocytogenes} isolates belonging to serovar II.1 (Fig. 2), which may be due to the homogenous nature of serogroup four strains (Aurora et al., 2009). The discriminatory power of RAPD is comparable to that of ERIC-PCR. However, it was found that a higher discriminatory power could be achieved by using a combination of ERIC-PCR and RAPD fingerprint analysis (Fig. 1). It is noteworthy that some isolates belonging to the same serovar, serovar II.1, possessed an identical pattern when analyzed by the three different typing methods, such as strains 191-2, 427-1, and 359-1, which were from distinct districts. This implies a potential common epidemiological lineage among these three different regions (Figs. 1 and 2).

4.5. Antimicrobial susceptibility

Since the first antibiotic resistant and multidrug-resistant \emph{L. monocytogenes} strains were isolated from patients in 1988 (Poyart-Salmoner, Carlier, Trieu-Cuot, Courtieu, & Courvalin, 1990), antimicrobial resistant strains were commonly recovered from food, natural environment, and clinical cases of listeriosis. Surveillance of the emerging antimicrobial resistance profiles of \emph{L. monocytogenes} in RTE foods is of utmost importance. The results of this study suggested a low incidence of antimicrobial resistance in \emph{L. monocytogenes} strains isolated from RTE foods in South China. Three (8.1%) \emph{L. monocytogenes} strains were resistant to PEN and two isolates had intermediate resistance to PEN. This resistance was lower than the studies that showed 40% or 90% penicillin-resistance levels (Harakeh et al., 2009; Srinivasan et al., 2005); these strains had been isolated from dairy-based products and from the environment. AMP in combination with GEN, or SXT, is necessary for the treatment of invasive listeriosis infections (Hof, Nüchterlein, & Kretschmar, 1997; Schlech, 2000). In the current study, except for multidrug-resistant strain 191-1, no AMP-resistant strain was identified. However, three strains exhibited intermediate resistance to AMP in the present study. These results suggested the potential for AMP-resistant strains to emergence under some conditions. Only one multidrug-resistant strain was recovered in the current study, which was isolated from a fried drumstick sample. The acquired resistance to TET and CIP may be related to the excessive use of these antimicrobials in animal feed and as a drug of second choice in the treatment of human diseases (Harakeh et al., 2009). Several studies have elucidated that antimicrobial resistance may be acquired by \emph{L. monocytogenes} via conjugation (Charpentier, Gerbaud, & Courvalin, 1999; Lyon, Berrang, Fedorka-Cray, Fletcher, & Meinersmann, 2008); self-transferable plasmids (Charpentier & Courvalin, 1999; Poyart-Salmoner et al., 1990), and vertical and horizontal gene transfer (Charpentier et al., 1999). Surprisingly, this multi-resistant strain exhibited an unique amplified DNA pattern by both ERIC-PCR and RAPD typing (Figs. S3–S5), suggesting that this multidrug-resistant strain may have acquired resistance by vertical and horizontal gene transfer or from other microorganisms by conjugative mobilization.

5. Conclusions

In summary, the findings of this study suggested that the high prevalence of \emph{L. monocytogenes} in RTE foods may reflect poor hygienic practices at the final processing of RTE foods at retail level. RTE foods may serve as potential vehicles for transmission of virulent \emph{L. monocytogenes}. On the basis of genotype and phenotype analyses, the presence of serotype 4b in RTE foods pose a potential risk for causing listeriosis in consumers in South China. Chinese regulatory authorities should consider formulating a regulatory framework for controlling \emph{L. monocytogenes} to improve the microbiological safety of RTE foods.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.foodcont.2013.09.061.

References
