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Occurrence and antimicrobial susceptibility of *Listeria monocytogenes* isolates from retail raw foods

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ABSTRACT

The occurrence and counts of *Listeria monocytogenes* were investigated in a total of 526 retail raw food samples. All *L. monocytogenes* isolates were further analyzed by serotyping and antimicrobial susceptibility assays. The molecular basis of tetracycline resistance of each isolate and the genetic relatedness were determined. *L. monocytogenes* isolates were found in 12.4% (65/526) of the samples, with counts below 10^2 CFU/g. *L. monocytogenes* was most commonly isolated from pork (20%, 20/100), seafood (13.8%, 15/109), chicken (13.2%, 14/106), and beef (10.3%, 11/107). In addition, *L. monocytogenes* was also detected in 4.8% (5/104) of raw mutton samples. Four serogroups were identified among the 65 *L. monocytogenes* isolates, with serogroups 1/2a-3a (60%) and 4b-4d-4e (24.6%) being dominant. Most *L. monocytogenes* isolates were resistant to cefotaxime (54.6%), fosfomycin (51.5%), and clarithromycin (36.4%). Some isolates showed intermediate resistance to streptomycin (12.1%), norfloxacin (13.6%), ciprofloxacin (13.6%), and nitrofurantoin (9.1%). Multiple resistances were observed in 72.3% of isolates. Genetic relatedness analysis revealed that there were no prominent associations between specific food types, serotypes, antimicrobial susceptibility profiles and Pulsed-field gel electrophoresis (PFGE) patterns. In addition, these isolates were multiresistant and belonged to the epidemiologically important serotypes 1/2a and 4b, implying a potential public health risk.

1. Introduction

Listeria monocytogenes is a gram-positive, facultative intracellular pathogen that can cause serious human and animal infections, including abortion and septicemia (Rocourt & Cossart, 1997). Epidemiological studies have demonstrated that *L. monocytogenes* is an important foodborne pathogen (Farber & Peterkin, 1991). Foodborne listeriosis linked to *L. monocytogenes* was first reported in 1981 (Schlech et al., 1983). Since then, several outbreaks of foodborne listeriosis associated with this bacterium have been documented all over the world (Denny & McLauchlin, 2008; Warriner & Namvar, 2009). *L. monocytogenes* isolates associated with outbreaks of listeriosis have been detected in various kinds of products including dairy products, raw meat, vegetables and seafood (Bell & Kyriakides, 2005; Schlech, 2000; Yücel, Çitak, & Önder, 2005). The high occurrence of *L. monocytogenes* in foods (Farber et al., 1991; Jorgensen & Huss, 1998) and the high mortality rate (up to 30%) associated with listeriosis have contributed to *L. monocytogenes* being considered a pathogen of major concern (Jalali & Abedi, 2008).

Serogroup determination is usually used for the microbiological characterization and a rapid initial screening in epidemiological investigations of *L. monocytogenes*. Furthermore, serotyping is widely used for long-term microbiological surveillance of human listeriosis (Doumith, Buchrieser, Glaser, Jacquet, & Martin, 2004). To date, 13 serotypes of *L. monocytogenes* have been identified, but only serotypes 1/2a, 1/2b, 1/2c, and 4b are frequently isolated from foods and patients, with serotypes 1/2a, 1/2b, 3b, and 4b causing most cases of human listeriosis (Farber et al., 1991).

Currently, ampicillin or penicillin G combined with gentamicin is the reference therapy for human listeriosis, whereas trimethoprim-sulfamethoxazole, vancomycin and erythromycin

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are regarded as second-choice drugs to treat listeriosis in pregnant women (Hof, 2004). However, since the first isolation of a multi-resistant strain in France in 1988 (Poyart-Salmeron, Trieu-Cuot, Carlier, & Courvalin, 1990), the number of strains resistant to one or more antibiotics used for treating listeriosis has continually increased (Granier et al., 2011; Morvan et al., 2010; Yan et al., 2010). The levels of resistance vary and are influenced by antimicrobial use and geographical differences. Therefore, it is necessary to monitor the occurrence and the antibiotic susceptibility of *L. monocytogenes* on a worldwide basis. China is one of the major producers and consumers of food-producing animals; however, there is a paucity of information on the prevalence and antimicrobial susceptibility of *L. monocytogenes*.

Therefore, this study investigated the occurrence, the serotypes, the antimicrobial susceptibility, and the molecular basis of tetracycline resistance of *L. monocytogenes* isolates from fresh raw foods.

2. Materials and methods

2.1. Samples, L. monocytogenes detection and enumeration

Four hundred and sixty-three raw samples of pork ($n = 100$), beef ($n = 107$), mutton ($n = 104$), chicken meat ($n = 106$), and seafood ($n = 109$) were randomly collected from six cities in Heilongjiang province in the Northeast of China from 2008 to 2009. Detection and enumeration of *L. monocytogenes* were carried out as recommended by ISO 11290-1 and 11290-2 methods, respectively (1996, 1998). All culture media and selective supplements were from Oxoid Ltd. (Oxoid, Hampshire, UK) unless otherwise mentioned.

L. monocytogenes detection consisted of a resuscitation step in which 25 g of sample were transferred to sterile plastic bags containing 225 mL of half-Fraser (HF) broth without supplements, homogenized using a Stomacher 400-laboratory blender (Seward Medical, London, UK), and incubated at 20 °C for 1 h. After this resuscitation step, selective supplement SR-166 was added to the HF broth, following incubation at 30 °C for further 23 h. Then, 0.1 mL of the HF broth was transferred to tubes containing 10 mL of Fraser Broth (FB plus supplement SR 156) and incubated at 35 °C for 48 h. The culture was streaked onto duplicate plates of *Listeria* selective agar (Oxford) and Palcam agar, supplemented with SR 140E and SR 150E, respectively. The plates were incubated for 48 h at 35 °C and observed for the presence of typical *Listeria* colonies. Presumptive *Listeria* colonies were selected and submitted to Gram staining and biochemical tests. *L. monocytogenes* was confirmed by the API-Listeria system (bioMérieux, France). Results were expressed as absence or presence of *L. monocytogenes* per 25 g.

For enumeration of *L. monocytogenes*, the same HF broth used for *L. monocytogenes* detection was used. After the resuscitation period and before the addition of the supplement SR-166, aliquots of HF broth were taken and submitted to serial decimal dilutions in 0.1% peptone water and 0.1 mL of each dilution was plated on Palcam agar in duplicate. After incubation at 35 °C for 48 h, Presumptive *Listeria* colonies were confirmed as described above and counted. The results were expressed as the number of *L. monocytogenes* per gram (CFU/g).

2.2. Serotyping

Strains identified as *L. monocytogenes* were serotyped using a multiplex PCR method (Doumith et al., 2004), with some modifications. Briefly, each isolate was inoculated onto Trypticase Soy Agar (TSA; BD, Sparks, MD) supplemented with 0.6% of yeast extract, and the places were incubated for 48 h at 37 °C. Three to five bacterial colonies from each purified isolate were emulsified in 50 μl of 0.25% sodium dodecyl sulfate and 0.05 N sodium hydroxide and heated at 99 °C for 15 min. Then, 100 μl of deionized distilled H_2O was added to the mixture, 1 μl of which was used as the PCR template. PCR was performed in a final volume of 20 μl containing 1 μl of template DNA and 10 μl of 2 × PCR Mix (MBI Fermentas). The five primer sets were added at the following final concentrations: 1 μM for *lmo*0737, ORF2819, and ORF2110; 1.5 μM for *lmo1118*; and 0.2 μM for *prs*. PCR was performed with an initial denaturation step at 94 °C for 3 min; 35 cycles of 94 °C for 0.40 min, 53 °C for 1.15 min, and 72 °C for 1.15 min; and one final cycle of 72 °C for 7 min in a thermal cycler (Applied Biosystems). Five microliters of the reaction mixture was mixed with 3 μl of loading buffer and separated on a 2% agarose gel in a TBE buffer (90 mM Trizma base, 90 mM boric acid, 2 mM EDTA, pH 8.3). The PCR products were visualized by ethidium bromide staining.

2.3. Antimicrobial susceptibility testing

Susceptibility to the following antimicrobial agents was determined for all *L. monocytogenes* strains by the microdilution broth method. The antibiotics used were (concentration range in μg/ml): ampicillin (0.03–32), penicillin (0.03–32), cefazolin (0.12–128), cephalothin (0.06–64), cefotaxime (0.12–128), fosfomycin (0.125–128), vancomycin (0.06–64), trimethoprim-sulfamethoxazole (0.6–640), nitrofurantoin (0.25–256), tetracycline (0.125–128), kanamycin (0.125–128), gentamicin (0.03–32), streptomycin (0.25–256), norfloxacin (0.03–32), ciprofloxacin (0.03–32), clarithromycin (0.03–32), and erythromycin (0.06–64). The results were interpreted according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2006a,b). Because there are no CLSI breakpoints for fosfomycin that are applicable to *Listeria* or *Staphylococcus*, the breakpoint for sensitivity was defined as ≤64 μg/mL, as reported by Troxler, von Graevenitz, Funke, Wiedemann, and Stock (2000). *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, and *Enterococcus faecalis* ATCC 29212 were used as quality control strains.

2.4. Detection of tetracycline resistance determinants and the Tn916 transposon

Tetracycline-resistant and tetracycline-susceptible *L. monocytogenes* strains were screened for the four tetracycline resistance genes. The tetracycline resistance determinants *tet*(L), *tet*(M), *tet*(K) and *tet*(S) were amplified by PCR with specific primers as previously described (Ng, Martin, Alfa, & Mulvey, 2001). To investigate whether the Tn*916* transposon was present in the *tet*(M)-positive isolates, the *int*-Tn gene, which encodes for the integrase of Tn*916*-Tn*1545*, was also amplified as described by Morvan et al. (2010).

2.5. Pulsed-field gel electrophoresis (PFGE)

PFGE was carried out as described previously (Graves & Swaminathan, 2001) except that genomic DNA from all of the isolates was digested with *Asc* I. The electrophoretic parameters used were as follows: initial switch time, 4.0 s; final switch time, 40.0 s; run time, 22 h; angle, 120°; gradient, 6.0 V/cm; temperature, 14 °C; and ramping factor, linear. *Xba* I-digested plugs of *Salmonella serovar* Braenderup H9812 standards served as size markers. PFGE patterns were analyzed with BioNumerics version 5.1 (Applied Maths, Sint-Martens-Latem, Belgium). If a difference in the PFGE pattern was observed, a new pulsotype was assigned. The definition of a PFGE cluster was based on a similarity cutoff of 100% (Latorre et al., 2009) using the Dice coefficient (with an optimization of 1% and a position tolerance of 0.5%–1%), and the relationships were