Antimicrobial Susceptibility, Multilocus Sequence Typing, and Virulence of Listeria Isolated From A Slaughterhouse

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Antimicrobial susceptibility, Multilocus sequence typing, and virulence of *Listeria* isolated from a slaughterhouse

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Abstract

Background: *Listeria monocytogenes* is one of the deadliest foodborne pathogens, and the bacterium can tolerate severe environments through biofilm formation and antimicrobial resistance. The objective of this study was to investigate the antimicrobial susceptibility, resistance genes,virulence and molecular epidemiology about *Listeria* from meat processing environments .

Methods: This study evaluated the antibiotic resistance and virulence of *Listeria* isolates from slaughtering and processing plants. All isolates were subjected to antimicrobial susceptibility testing by using a standard microbroth dilution method. The carrying of resistant genes were identified by Polymerase Chain Reaction (PCR). The multilocus sequence typing (MLST) was determined subtyping of the isolates and to characterize possible routes of contamination from meat processing environments. The virulence of different STs of *L. monocytogenes* isolates were evaluated by Caco-2 cells invasion assay.

Results: A total of 59 *Listeria* isolates were identified from 320 samples, including 37 *L. monocytogenes* (62.71%). This study evaluated the virulence of *L. monocytogenes* and antibiotic resistance of *Listeria* isolates from slaughtering and processing plants. The susceptibility of these 59 isolates against eight antibiotics was analyzed, and the resistance levels to ceftazidime, ciprofloxacin, and lincomycin were as high as 98.31% *(L. m* 37; *L. innocua* 7; *L. welshimeri* 14), 96.61% (*L. m* 36; *L. innocua* 7; *L. welshimeri* 14), and 93.22% (*L. m* 35; *L. innocua* 7; *L. welshimeri* 13) respectively. Over 90% of the isolates were resistant to 3-6 antibiotics, indicating that *Listeria* isolated from meat processing environments has high antimicrobial resistance. Up to 60% of the isolates carried the tetracycline-resistance genes *tetA* and *tetM*. The frequencies of *ermA*, *ermB*, *ermC*, and *aac(6')-Ib* were 16.95%, 13.56%, 15.25%, and

6.78%, respectively. Notably, the resistant phenotype and genotype did not match exactly, suggesting that the mechanisms of antibiotic resistance of these isolates were likely related to the processing environment. Multilocus sequence typing (MLST) revealed that 59 *Listeria* isolates were grouped into 10 sequence types (STs). The dominant *L. monocytogenes* STs were ST5, ST9, and ST121 in the slaughtering and processing plant of Jiangsu province. Moreover, ST5 subtypes exhibited high invasion in Caco-2 cells compared with ST9 and ST121.

Conclusions: The results of this study predict a prevalence of *Listeria* contamination in the slaughtering and processing plant , and resistance of the ST5 subtypes isolates to the antimicrobials may cause potential public health risks.

Keywords: *Listeria*, antimicrobial resistance, antimicrobial resistance genes, virulence, MLST

Background

Listeria monocytogenes is one of the most important foodborne pathogens. The bacterium can infect humans and animals and can cause meningoencephalitis, abortion, and sepsis resulting in high rates of infection and mortality [1]. Due to its high degree of resistance in harsh conditions, *L. monocytogenes* is able to persist in food processing environments, such as meat, poultry, dairy, and seafood processing facilities, and the bacteria can proliferate during storage of chilled food products [1]. Sources and contamination patterns in various types of products have not yet been determined. Occurrence of *L. monocytogenes* in the food processing environment is variable, whereas its occurrence in food is generally around 5%−6% [2]. The food processing environment is easily contaminated by *L. monocytogenes* [3]. Molecular typing of isolates including Pulsed Field Gel Electrophoresis (PFGE) inside and outside a food processing facility can indicate potential sources of contamination from the external environment [4]. With the development of whole genome sequencing (WGS), multilocus sequence typing (MLST) has been widely used for the epidemiological investigation of *L. monocytogenes* and in source tracking of specific strains during outbreaks. Thus, the main ST subtypes can be analyzed more accurately [5].

Antimicrobial resistance is a global public health problem [6,7]. *L. monocytogenes* rarely develops acquired resistance to antibiotics. However, researchers have

reported that *L. monocytogenes* is resistant to antibiotics such as tetracycline, ciprofloxacin, erythromycin, and ampicillin [1]. Several studies have recently reported an increased rate of resistance to one or more clinically relevant antibiotics in environmental isolates [8,9,10] and less frequently in clinical strains [11,12]. Due to the seriousness of multidrug resistance and the transmission of resistance genes between bacteria and across species, the prevalence of antimicrobial resistance is rising.

However, precise information concerning the ancestral and evolutionary linkage and genetic diversity of *L. monocytogenes* is not presently available. The advent of subtyping techniques, such as PGFE and WGS, has enabled source tracking of *L. monocytogenes* during outbreak investigations, but these technologies are not yet used for general surveillance in food supply chains due to their cost, complexity of analysis, and the expertise required to interpret such data. In the present study we used MLST, which can determine source of processing environment contamination through the analysis of the slaughtering operations, to trace the presence of *L. monocytogenes* in isolates from food commodities. The method allows us to perform subtyping of the pathogen and to characterize possible routes of contamination.

Results

Occurrence of *Listeria spp.* in processing environment

The overall prevalence of *Listeria* in slaughter and processing environments tested during the year 2019 is shown in Table 1. Thirty-seven isolates of *L. monocytogenes*, 7 isolates of *L. innocua*, and 15 isolates of *L. welshimeri* were obtained. The 59

isolates were distributed in different areas: 7 from the slaughter area (8.75%), 9 from the cutting and deboning room (11.25%), 23 from the visceral area (28.75%), and 20 from the meat cooling and refrigeration area (25.00%). A total of 59 *Listeria* isolates were recovered from 320 analyzed samples (18.44%), including 37 *L. monocytogenes* (11.56%). The 59 isolates were distributed in different areas: 7 from the slaughter area (8.75%), 9 from the cutting and deboning room (11.25%), 23 from the visceral area (28.75%), and 20 from the meat cooling and refrigeration area (25.00%). A total of 59 *Listeria* isolates were recovered from 320 analyzed samples (18.44%), including 37 *L. monocytogenes* (11.56%). The highest percentage of *L. monocytogenes* strains (13) was found in samples taken from the cooling and refrigeration area (Table 1). Moreover, group 1/2b was the main serotype (12/37, Table 2), and the next highest were 1/2a (7/37) and 1/2c (7/37), respectively. The others were 3a (3/37), 3b (5/37), and 3c (3/37). From the above, serotypes 1/2b, 1/2a, and 3b were the main endemic *L. monocytogenes* in slaughtering environments.

Antimicrobial susceptibility testing

The susceptibility of the 59 isolates to eight antibiotics was examined using the micro-broth dilution method. The results showed that isolates were resistant to ceftazidime (MIC ≥ 32 μg/mL； 58/59, 98.31%), ciprofloxacin (MIC ≥ 64 μg/mL; 57/59, 96.61%), and lincomycin (MIC ≥ 4 μg/mL; 55/59, 93.22%) (Table 3). Resistance to tetracycline reached 16.95% (MIC ≥ 16 μg/mL; 10/59). Very few isolates were resistant to gentamicin (MIC 16μg/mL; 2/59) or ampicillin (MIC 32 μg/mL; 1/59). Noteworthy was the intermediate resistance against erythromycin

(MIC = 1-4μg/mL; 29/59) observed in these isolates . All of the isolates were highly susceptible to vancomycin (100%). Multidrug resistance showed that 58 strains were resistant to at least two antibiotics. Only one isolate (LM3-2) of *L. welshimeri* was susceptible to all antibiotics. The proportion of the strains resistant to three kinds of antibiotics was 76.27%, and the proportion for 3-6 antibiotics was 91.38%. The *L. welshimeri* isolate LM3-7 from the slaughter area was resistant to six antibiotics, and the resistance was the most serious in this study (Fig. 1, Fig. 2).

Prevalence of 11 resistance genes was assessed, and the results are summarized in Table 3. In the slaughtering and processing environment, the genes *tetA*, *tetM*, *ermA*, *ermB*, *ermC*, and *aac*(*6′*)-*Ib* were detected in different areas. The *tetS*, *mecA*, vanA, *vanB*, and *cfr* genes were not detected in all *Listeria* isolates. Tetracycline resistance genes *tetA* (61.3%) and *tetM* (45.3%) were the two most commonly detected antibiotic resistance genes. The erythromycin resistance gene cassette, including *ermA* (16.95%), *ermB* (13.56%), and *ermC* (15.25%), was present among *L. monocytogenes*, *L. welshimeri*, and *L. innocua*. Four isolates of *Listeria* were found to carry *aac*(*6′*)-*Ib* by detecting the resistance gene for aminoglycosides. However, the resistant genotypes and phenotypes were not exactly the same (Table 4, Fig. 3). In comparison, there were 58 strains of ceftazid-resistant isolates, but none of these isolates presented known resistance genes (Table 3).

MLST

A total of 59 *Listeria* isolates were classified into 11 sequence types (STs) (Fig. 4). Seventeen *L. monocytogenes* belonged to ST5 (17/37, 45.95%). Other STs belonged

to ST9 (10/37) and ST121 (10/37). The remaining 22 non-*L. monocytogenes* isolates were grouped into ST540, ST602, ST637, ST537, ST10057, ST168, and ST1084. The most endemic ST was ST5, which was isolated from four areas. ST121 was widely distributed in the meat cooling and refrigeration area (D). Seven *L. innocua* isolates were divided into five STs, three of which belonged to ST537. Fifteen *L. welshimeri* isolates were divided into ST1005, ST1084, and ST168. The 17 isolates of *L. monocytogenes* ST5 were serotype 1/2b, and five belonged to serotype 3b. Among the 10 ST9 isolates, three belonged to 3c, and seven belonged to 1/2c. The serotypes of ST121 consisted of seven isolates of 1/2a and three isolates of 3a. We found that isolates classified as the same serogroup could be differentiated into different STs. This finding may be applied to other isolates of *L. monocytogenes.*

3.4 Virulence genes and invasion assays

In this study, the virulence and invasiveness of *L. monocytogenes* were evaluated by invasion assays. Seven virulence associated genes, *prfA*, *plcA*, *gyrB*, *plcB*, *inlA*, *hly*, and *sigB,* were detected by PCR. Each of the seven virulence-associated genes was detected in all *L. monocytogenes* strains. The invasion efficiency of the isolates ranged from 0.002% to 1.295%. The results showed that the isolates within the same STs had different levels of invasiveness against Caco-2 cells. The invasion frequencies of ST5 and ST121 ranged from 0.004% to 1.159% and 0.012% to 1.295%, respectively. The invasion frequency of ST9 was relatively lower than that of ST5 and ST121, from 0.002% to 0.669%. The average invasion frequency of ST9 was 0.1406%, whereas the values for ST5 and ST121 were 0.4419% and 0.4332%,

164 respectively (Fig. 5). The ST5 isolates mainly came from the cutting and deboning
165 room and visceral area regions and showed higher levels of invasiveness.

166 **Discussion**

167 *L. monocytogenes*, ubiquitous in the environment, is the causative agent of listeriosis.
168 Although incidence of the disease is low compared to those by other foodborne
169 pathogens, the disease outcome is often more serious [13]. Food safety regulations in
170 many countries have tended to adopt a zero tolerance policy for *L. monocytogenes* in
171 ready-to-eat (RTE) food products, as human listeriosis outbreaks have been most
172 often associated with RTE products that are consumed without prior cooking. RTE
173 meat products contaminated with *Listeria* might be the result of cross-contamination
174 during processing and handling during storing, slicing, weighing, and packaging [14].
175 In this study, we investigated the resistance and STs of the *Listeria* isolated from the
176 slaughter and processing environment in Jiangsu province, China. Fifty-nine *Listeria*
177 strains were found in 320 samples from the slaughterhouse (18.44%), including 37 of
178 *L. monocytogenes* (37), seven of *L. innocua* (7), and 15 of *L. welshimeri* (15).
179 However, from previous reports, 19 cheese factories (55.8%) were contaminated by
180 *Listeria* spp., demonstrating a higher contamination rate compared with our results.
181 Of these, 20.6% were *L. monocytogenes* positive, while in our data the proportion
182 reached 62.71%. Moreover, *L. monocytogenes* was found on 4.9% of product contact
183 surfaces and 18.8% of floor drains [15]. In Romania, meat processing plants were
184 contaminated at higher rates of *L. monocytogenes* (18.8% and 26.5%) [16,17,18].
185 Vongkamjan et al. also demonstrated that *L. monocytogenes* occurred (35%) in

environmental samples from one seafood processing plant. Therefore, the environmental surfaces appear to be easier to contaminate than the food matrices [19]. In our study, prevalence of *L. monocytogenes* in meat cooling and refrigeration areas (D) was significantly higher than in other areas in 2018. The suggestion that *L. monocytogenes* grows well at low temperatures should be remembered. Therefore, periodic surveillance and sanitation should be strictly implemented to improve the hygiene conditions of the slaughter and processing environment to achieve higher food safety levels.

Previous studies have reported that *L. monocytogenes* from seafood processing plants belonged to serotypes 1/2b, 3b, 4b, 4d, and 4e [15,20,21], and serotypes 1/2b, 1/2a, 4b, and 1/2c are usually found in meat products and meat processing plants [22]. Skowron et al. found that most (38.6%) isolates in a fish processing plant belonged to the group 1/2a–3a [13]. Among our results, the majority of the isolates belonged to serotypes 1/2b (35.14%), 1/2a, and 1/2c (18.92%), which are the serotypes that are predominantly found in food. Serotype 4b is mainly from listeriosis patients and was not found in these isolates.

The antimicrobial resistance of *L. monocytogenes* is usually low (2%-3%) [20,23]. However, several studies have shown that up to 7.1% of strains resistant to antibiotics is not uncommon in fish processing plants [13]. In our results, the commonly used antibiotics CAZ, CIP, and LIN were generally ineffective against resistant *L. monocytogenes* isolates. We speculate that the reason is that antibiotics have been used in the breeding process. With the emergence of strains carrying

antibiotic resistance genes, such genes can be transferred between strains via plasmids. Multidrug resistance tests indicated that 90% of the isolates were resistant to more than two antibiotics, meaning that antimicrobial resistance in *Listeria* is still at a low prevalence compared with the meat processing environment [7]. In recent years, a growing body of evidence suggests that the resistant bacteria produced in the processing environment may affect antibiotic resistance transfer in human pathogens through the food products. Although many *L. monocytogenes* strains from humans are susceptible to antimicrobials, our results illustrate how new isolates can become resistant to commonly used antimicrobials.

In the present study, we first described the multiple resistance genes *tetA*, *tetM*, *ermA*, *ermB*, *ermC*, and *aac(6')-Ib* of *L. monocytogenes* isolated from the slaughter and processing environment. In general, *tetB* and *tetM* were frequently detected in mobile plasmids [24]. In our study, *tetA* and *tetM* were the major phenotypes, and these were significantly enhanced compared with previous studies, suggesting a potential connection of *tetA* and *tetM* with multidrug resistance bacteria. MDR *L. monocytogenes* isolated from frozen food products has shown harboring the multiple resistance *ermB* and *tetS* genes [8]. Moreover, certain antibiotic resistance genes such as *tetM* can be transferred among bacterial communities in various environments [25,26,27]. Horizontal gene transfer among humans and the environment is possible. The *cfr* gene was not identified in any of the 59 isolates, although this gene is commonly found in *staphylococci* isolates from humans and animals [28]. Many reports of *cfr* genes come from China, mostly from animals.

However, there are few reports concerning the *cfr* gene in *Listeria*. Our analysis of resistant *Listeria* phenotypes and resistance genotypes found that the coincidence rate was inconsistent (Table 2), which may be due to the existence of multiple resistance mechanisms. In present study, *Listeria* isolates were resistant to ampicillin (1.69%), erythromycin (49.15%), gentamicin (3.39%), and tetracycline (16.95%). The present findings were in partial correlation with that of Yadav *et al.* [29] who reported resistance to ampicillin, erythromycin, gentamicin and tetracycline to be 22.92%, 16.67%, 31.25%, 10.42%, respectively. Kumar *et al.* [30] reported that the multidrug-resistant *Listeria* isolated from meat and fish had observed sensitivity (66.66%) for ciprofloxacin. However, our research shows that the sensitivity of ciprofloxacin was 3.39%. In present investigation there were 91.38% strains of *Listeria spp.* that were resistant to 3-6 antibiotics. So it is of great concern that this expanding range of antibiotics now includes those drugs that is used for treatment of human and animal listeriosis. The high number of multiple drug resistant strains of *Listeria* found in this study seems to suggest that mobile genetic elements encoding resistance to a wide range of antibiotics in this genus have appeared and are spreading. The resistance mechanisms of bacteria are very complex. The location of resistance genes (on plasmids or chromosomes), genetic structure, expression level, interactions between different resistance genes, and formation of bacterial biofilms affect bacterial resistance to antibiotics. The resistance of bacteria to a drug may result from the combination of several resistance genes and resistance mechanisms [31]. Some studies have shown that *L. monocytogenes* can obtain resistance genes

252 from the environment through plasmids and transposons, leading to the gradual
253 increase of *L. monocytogenes* resistance [32]. Due to differences in the actual gene
254 expression levels or antibiotic metabolism, strains carrying the same antibiotic
255 resistance genotype may have different resistant phenotypes.

256 MLST plays an important role in analysis of the mode of contamination and
257 transmission routes of *Listeria*. Different studies have shown that the currently
258 separated STs in the processing environment of China are ST5, ST199, ST8, ST9,
259 ST121, ST3, and ST224. Wang et al. found that the most common STs of *L.*
260 *monocytogenes* in China were ST8 and ST9, and the main ST of *L. monocytogenes*
261 isolated from chicken and pork was ST9 [33]. Compared with European countries,
262 the STs in the food processing environment mainly include ST1, ST9, ST87, ST5,
263 ST7, ST37, ST570, and ST204. However, our study found that the major STs were
264 ST5, ST9, and ST121. Almost all of ST9 and ST121 isolated were from the visceral
265 area (C) and the meat cooling and refrigeration area (D). ST5 was isolated from all
266 areas, indicating that ST5 plays an important role in the entire processing
267 environment. The C and D areas in the slaughtering processing environment
268 contained large numbers of ST9 and ST121, indicating that ST9 and ST121 from
269 RTE meat products may originate from processing raw meat in the processing
270 environment. In a study of 300 clinical, food, and environmental sources of isolates
271 from 42 countries on five continents, CC9 was the fourth most common CC in the
272 world, ranking third in Europe after CC1, CC2 and CC3 [34]. In our study, among
273 the seven housekeeping gene alleles, more than five identical alleles were present

from a clonal complex. The main clonal complexes present in this study were CC5, CC9, and CC121. In the *L. monocytogenes* strains CC9 and CC121, premature stop codons leading to truncation of the virulence gene *inlA* are often present. The *L. monocytogenes* STs are assigned to the latter cells and are considered to be more suitable for environmental conditions. Lineage II bacteria, including the most dominant worldwide strain CC121, are the main STs reported in human sporadic listeriosis. ST5, ST9, and ST121 included resistant isolates and resistance genes, suggesting that monitoring of potentially pathogenic STs should be strengthened. ST5 has been associated with human listeriosis outbreaks, and ST9 is predominant in China [33,35,36], indicating that *L. monocytogenes* that exist in the slaughtering and processing environment share a common source with humans. In present study, *L. inocua* (7 isolates) and *L. welshimeri* (15 isolates) were isolated from 320 samples of slaughterhouse. Antunes *et al.* [37] found that *Listeria spp.* were present in all 63 (100%) poultry samples, including *L. innocua* (32 isolates), *L. welshimeri* (8 isolates). Yadav *et al.* [29] reported that the 20 strains of *L. inocua* were isolated from 2417 animals and its surrounding environment samples. Thus it can be seen, the pattern of susceptibility between *L. monocytogenes* and *L. innocua* is important, owing to the fact that both species are usually found in the same food or food processing environment [7].

Conclusions

In conclusion, the presence of serogroups 1/2a, 3a and 1/2b, 3b, as well as the resistance and pathogenic STs, is associated with human listeriosis. The findings in

this study illustrate a potential public health risk in the slaughtering and processing environment. Antimicrobial resistance is increasing in foodborne pathogens. We found three new STs in Jiangsu province, highlighting the need to fill out the MLST database by increasing the surveillance of *L. monocytogenes* worldwide.

Materials and methods

Sample collection and isolation of *Listeria*

A total of 320 environmental swabs were collected from a pig slaughtering and processing environment in 2019. The slaughtering and processing region can be divided into four areas: slaughter, carcass partition, visceral separation, and meat cooling and cryopreservation. Sampling included both food contact surfaces (FCS) and non-food contact surfaces (NFCS), including flooring, tables, walls, conveyor belts, trays, carts, and sinks (Rückerl et al., 2014). 320 environmental samples were collected in two times in four regions, including 80 from slaughter (flooring, carcass surface with pig hair, blood, sinks), 80 from carcass partition (carcass, cutting knife, conveyor) before their cleaning, 80 from visceral separation (cutting knife, tables, trays, walls), 80 from meat cooling and cryopreservation (flooring, tables, trays, carts, belts, walls). About 100 cm2 of plane surfaces were swabbed two to five times using sterile cotton-tipped applicators moistened with 0.1% peptone water. The two to five swabs were pooled as one sample. Effluent was collected using sterile sampling bags. All of the samples were loaded in a refrigerated vehicle and transported to the lab within 24 h. *Listeria* was isolated according to the National Standard of China GB 4789.30-2016. For detection of *Listeria spp.*, 25 g of

318 slaughterhouse sample was enrichment in semi-concentrated Fraser Broth
319 (Merck,Germany) (Primary Selective Broth) at 37 °C for 24 h followed by
320 transferring the 0.1 ml of initial base solution to 10 ml of Fraser Broth (secondary
321 selective broth) and incubation at 37 °C for 24h. Secondly enrichments were
322 streaked onto Oxford agar (Merck, Germany) and Palcam agar (Merck, Germany)
323 and incubated at 35 °C for 48 h. The plates were examined for *Listeria* colonies
324 (black colonies with black sunken center) and at least three suspected colonies were
325 subcultured onto Trypton Soy agar supplemented with 0.6% of yeast extract (TSAYE)
326 (Merck, Germany) and incubated at 37 °C for 24 h. All of the isolates were
327 confirmed to morphological characteristics of colonies and single bacterial cells after
328 the Gram staining, catalase test and motility test (in *Listeria* Motility Medium
329 (Merck, Germany) after the incubation at 25 °C for 2-5 days). Serotyping of *L.*
330 *monocytogenes* was carried out by the serum agglutination test according to the
331 *Listeria* antisera of antigen 0 and flagellar antigen H (Denka Seiken Co. LTD.).

332 **Antimicrobial susceptibility testing**

333 Minimum inhibitory concentrations of *Listeria* isolates were determined by using the
334 micro-broth dilution method recommended by the Clinical and Laboratory Standard
335 Institute (CLSI, 2014). The following antimicrobial agents (Solarbio Ltd., China)
336 were used in this study (range in μg/mL): gentamicin (GEN; 1-128), ampicillin
337 (AMP; 2-128), ceftazidime (CAZ; 2-128), ciprofloxacin (CIP; 0.25-64), tetracycline
338 (TET; 1-64), erythromycin (ERY; 0.25-16), lincomycin (LIN; 0.25-32), and
339 vancomycin (VAN; 1-128). *Streptococcus pneumonia* ATCC 49619 was selected as a

quality control strain. *tetA*, *tetM*, *tetS*, *ermA*, *ermB*, *ermC*, *aac*(*6'*)-*Ib*, *mecA*, *vanA*, *vanB*, and *cfr* were selected as specific resistance genes and were identified by PCR (Table 5).

Multilocus sequence typing (MLST)

MLST based on seven housekeeping genes (*abcZ, bglA, cat, dapE, dat, ldh*, and *lhkA*) was performed according to the method of Wang *et al*. [33] The scheme and genotypic data are available at http://bigsdb.web.pasteur.fr/listeria/. Minimum spanning tree analysis was inferred using BioNumerics (Version 5.10, Applied Maths, Belgium).

Virulence gene and invasion assays in vitro

Virulence genes *prfA*, *plcA*, *gyrB*, *plcB*, *inlA*, *hly*, and *sigB* of *L. monocytogenes* were identified by PCR as described previously [31,38]. Primers and the size of each amplified product are listed in Table 6. Invasiveness of these isolates was measured by using the human colorectal adenocarcinoma cell line Caco-2. In brief, Caco-2 cells (3.0×10^5 cells per well) were cultured in 24-well plates in Dulbecco's modified Eagle medium (DMEM) (Gibco; Invitrogen, Carlsbad, CA, USA) containing 10% calf serum (Invitrogen) at 37°C in an incubator supplemented with 5% carbon dioxide (CO_2). Isolates of *L. monocytogenes* were grown in BHI broth under cultivation conditions of 30°C for 18 h. Cell monolayers were infected with 1.0×10^7-2.0×10^7 *L. monocytogenes* cells/well for 30 min, followed by three washes with Dulbecco's phosphate-buffered saline (DPBS). After incubating for 45 min, monolayers were overlaid with DMEM containing 100 μg/mL gentamycin to kill

362 extracellular bacteria. After incubating for 90 min, cells were washed three times
363 with DPBS. Then, 1 mL of ice-cold distilled water was added, and viable
364 intracellular bacteria were enumerated by plating appropriate dilutions of the cell
365 lysate on BHI agar. At least three independent invasion assays were performed for
366 each isolate. Invasion efficiency was calculated as the percentage of the inoculum
367 recovered from the infected Caco-2 cells by the enumeration of intracellular bacteria
368 [31].
369
370
371
372

Table 1 Isolation frequency of *Listeria* from pig slaughter factory

Sample type	No. of samples	No. of *Listeria*	No. of positive samples (%)
Slaughter area (A)	80	*L. monocytogenes* (6) *L. innocua* (1)	8.75 (7)
Cutting and deboning room (B)	80	*L. monocytogenes* (7) *L. innocua* (2)	11.25 (9)
Visceral area (C)	80	*L. monocytogenes* (11) *L. innocua* (1) *L. welshimeri* (11)	28.75 (23)
Meat cooling and refrigeration area (D)	80	*L. monocytogenes* (13) *L. innocua* (3) *L. welshimeri* (4)	25.00 (20)
Total	320	*L. monocytogenes* (37) *L. innocua* (7) *L. welshimeri* (15)	18.44 (59)

Phylogenetic groups of tested *L. monocytogenes* strains (n=59)

Table 2 Serotypes and isolation regions of *L. monocytogenes* isolates

Group	Number of *L. monocytogenes* isolates [n(%)]				
	Slaughter area (A)	Cutting and deboning room (B)	Visceral area (C)	Meat cooling and refrigeration area (D)	Total
1/2a	ND	ND	LM3-11	LM1, LM2, LM3, LM6, LM7, LM8,	7 (18.91%)
1/2b	LMA1, LMA8, LMA9, LMA13, LMA-II	LMB4, LMB-I	LMC4, LMC9, LMC15, LMC-I	LMD3, LMD10	13 (35.14%)
1/2c	ND	LM2-18	LM3-2-2,LM3-19, LM3-20-2	LM1T7, LM2T3, LM2W3	7 (18.92%)
3a	LM1-9	ND	LMC11	LM4	3 (8.11%)
3c	ND	ND	LMX-3, LMC7	LM1W3	3 (8.11%)
3b	ND	LMB5, LMB9, LMB10, LMB13	ND	ND	4 (10.81%)
Total	6	7	11	13	37 (100%)

ND represents "None determined

389

390 **Table 3 Antimicrobial-resistance profiles of *Listeria* isolates from the four areas（n=59）**

Antibitocs (ug/ml)		Source and no. of resistant strains (%)				
		Slaughter area *n*=7	Cutting and deboning room *n*=9	Visceral area *n*=23	Meat cooling and refrigeration area *n*=20	Total
GEN	R≥16	0 (0.00%)	0 (0.00%)	*L. welshimeri* (1) (4.35%)	*L. welshimeri* (1) (5.00%)	2 (3.39%)
	I=8	0 (0.00%)	0 (0.00%)	*L.m* (1) (4.35%)	0 (0.00%)	1 (1.69%)
	S≤4	*L. innocua* (1) *L. m*(6) (100.00%)	*L. innocua* (2) *L. m* (7) (100.00%)	*L. innocua* (1) *L. welshimeri* (9) *L. m* (11) (91.30%)	*L. innocua* (3) *L. welshimeri* (3) *L. m*(13) (95.00%)	56 (94.92%)
CAZ	R≥32	*L. innocua* (1) *L. m*(6) (100.00%)	*L. innocua* (2) *L. m* (7) (100.00%)	*L. innocua* (1) *L. welshimeri*(10) *L. m* (11) (95.65%)	*L. innocua* (3) *L. welshimeri* (4) *L. m* (13) (100.00%)	58 (98.31%)
	I=16	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
	S≤8	0 (0.00%)	0 (0.00%)	*L. welshimeri* (1) (4.35%)	0 (0.00%)	1 (1.70%)
AMP	R≥32	*L. m* (1) (14.29%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (1.69%)
	I=16	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
	S≤8	*L. innocua* (1) *L. m* (5) (85.71%)	*L.innocua* (2) *L. m* (7) (100.00%)	*L. innocua* (1) *L. welshimeri* (11) *L. m* (11) (100.00%)	*L. innocua* (3) *L. welshimeri* (4) *L. m* (13) (100.00%)	58 (98.31%)
CIP	R≥4	*L. innocua* (1) *L. m* (6) (100.00%)	*L. innocua* (2) *L. m* (6) (88.89%)	*L. innocua* (1) *L. welshimeri*(10) *L. m* (11) (95.65%)	*L. innocua* (3) *L. welshimeri* (4) *L. m* (13) (100.00%)	57 (96.61%)

	I=2	0 (0.00%)	0 (0.0%)	*L. welshimeri* (1) (4.35%)	0 (0.0%)	1 (1.69%)
	S≤1	0 (0.00%)	*L. m* (1) (11.11%)	0 (0.00%)	0 (0.00%)	1 (1.69%)
TET	R≥16	*L. m* (1) (14.29%)	*L. innocua* (2) *L. m* (1) (33.33%)	*L. innocua* (1) *L. welshimeri* (2) *L. m* (1) (17.39%)	*L. innocua* (1) *L. m* (1) (10.00%)	10 (16.95%)
	I=8	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
	S≤4	*L. innocua* (1) *L. m* (5) (85.71%)	*L. m* (6) (72.7%)	*L. welshimeri* (9) *L. m* (10) (82.61%)	*L. innocua* (2) *L. welshimeri* (4) *L. m* (12) (90.00%)	49 (83.05%)
ERY	R≥8	0 (0.00%)	0 (0.00%)	*L. welshimeri* (1) *L. m* (1) (8.70%)	*L. welshimeri* (1) *L. m* (1) (10.00%)	4 (6.78%)
	I=1-4	*L. m* (2) (28.57%)	*L. innocua* (2) *L. m* (4) (66.67%)	*L. innocua* (1) *L. welshimeri* (7) *L. m* (3) (47.83%)	*L. welshimeri* (2) *L. m* (8) (50.00%)	29 (49.15%)
	S≤0.5	*L. innocua* (1) *L. m* (4) (71.43%)	*L. m* (3) (33.33%)	*L. welshimeri*(3) *L. m* (7) (43.48%)	*L. innocua* (3) *L. welshimeri*(1) *L. m* (4) (40.00%)	26 (44.07%)
LIN	R≥4	*L. innocua* (1) *L. m* (5) (85.71%)	*L. innocua* (2) *L. m* (7) (100.00%)	*L. innocua* (1) *L. welshimeri* (9) *L. m* (11) (91.30%)	*L. innocua* (3) *L. welshimeri* (4) *L. m* (12) (95.00%)	55 (93.22%)
	I=1-2	*L. m* (1) (14.29%)	0 (0.00%)	*L. welshimeri* (2) (8.70%)	*L. m* (1) (5.00%)	4 (6.78%)
	S -	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
VAN	R≥32	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
	I=8-16	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
	S≤4	*L. innocua* (1) *L. m* (6) (100.00%)	*L. innocua* (2) *L. m* (7) (100.00%)	*L. innocua* (1) *L. welshimeri* (11) *L. m* (11) (100.00%)	*L. innocua* (3) *L. welshimeri* (4) *L. m* (13) (100.00%)	59 (100.00%)

GEN-gentamicin,CAZ-ceftazidime,AMP-ampicillin,CIP-ciprofloxacin,TET-tetracycline,ERY-erythromycin,LIN-lincomycin, VAN-vancomycin

Table 4 Correlation rate of phenotype and genotype of the *Listeria spp.*

Antibiotics	Resistant strains	Resistance genes	Resistance genes strains
Tetracycline	9	*tetA*	36
		tetM	24
		tetS	0
Ciprofloxacin	57	*aac(6’)-Ib*	4
Eryphilin	4	*ermA*	10
		ermB	8
		ermC	9
Ceftazidime	58	*mecA*	0
Vancomycin	0	*van A*	0
		van B	0

Table 5 Primer used in this study for amplification of resistance genes of *L. monocytogenes*

Category	Gene	Primer	Size (bp)	Accession number	Reference
Tetracycline	*tetA*	F:GCTACATCCTGCTTGCCTTC R:CATAGATCGCCGTGAAGAGG	220	NG_048154.1	[8]
	tetM	F:GTGGACAAAGGTACAACGAG R:CGGTAAAGTTCGTCACACAC	974	NC_013929.1	
	tetS	F:CATAGACAAGCCGTTGACC R:ATGTTTTTGGAACGCCAGAG	1050	NC_013929.1	
Aminoglycosides	*aac(6’)-Ib*	F:TTGCGATGCTCTATGAGTGGCTA R:CTCGAATGCCTGGCGTGTTT	544	NZ_CP016990.1	
Macrolides	*ermA*	F:AAGCGGTAAAACCCCTCGAG R:TCA AAGCCTGTCGGATTGG	651	MH_830363.1	[39]
	ermB	F:GAAAAGGTACTCAACCAAATA R:CATTTGTTAAATTCATGGCAATGA	639	NG_047798.1	
	ermC	F:TCAAAACATAATATAGATAAA R:GCTAATATTGTTTAAATCGTCAAT	641	NG_047806.1	
ESBLs	*mecA*	F:TAGAAATGACTGAACGTCCG R:TTGCGATCAATGTTACCGTAG	154	NG_047937	[40]
Vancomycin	*van A*	F:GGGAAA ACGACAATTGC R:GTACAA TGCGGCCGTTA	732	NC_011916.1	[8]
	van B	F:TTGATGTGGCTTTCCCGGTT R:ACCCGATTTCGTTCCTCGAC	544	NC_011916.1	
Multi-drugefflux pump gene	*cfr*	F:CGATTTGAGGATATGAAGGTTCT R:AAATTAGGATCCGTAAACGAAT	416	NG_047631.1	[19]

427

428 **Table 6 Primer used in this study for amplification of virulence genes of *L. monocytogenes***

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Gene	Primer	Size(bp)	Accession number	Reference
prfA	F:AGCGAGAACGGGACCATC R:TTGACCGCAAATAGAGCC	285		
plcA	F:CCCAGAACTGACACGAGC R:GCAGCATACTGACGAGG	293		[31]
gyrB	F:AGACGCTATTGATGCCGATGA R:GTATTGCGCGTTGTCTTCGA	91		
plcB	F:ATTAACCAAACCACTGGCTCA R:TTGATAAGCAGTCTGGACAAT	502	EU372057.1	
inlA	F:ATAAGTGATATAAGCCCAG R:TTTATCCGTACTGAAATTCC	606		[38]
hly	F:GTTGCAAGCGCTTGGAGTGAA R:ACGTATCCTCCAGAGTGATGG	420		
sigB	F:CCAAAAGTATCTCAACCTGAT R:CATGCATTTGTGATATATCGA	642		

Figure legends

Fig. 1 Antimicrobial susceptibility of *L. monocytogenes* isolates from processing plants to eight antibiotics. Gentamicin (GEN), ampicillin (AMP), ceftazidime (CAZ), ciprofloxacin (CIP), tetracycline (TET), erythromycin (ERY), lincomycin (LIN) and vancomycin (VAN) were selected as test antibiotics. A:non-resistance, B:one-resistance, C:two-resistance, D:three-resistance, E:four-resistance, F: five-resistance, G:six-resistance, H:seven-resistance, I:eight-resistance. *Streptococcus pneumonia* ATCC 49619 was selected as the quality control strain.

Fig. 2 Resistant analysis of *L. welshimeri*, *L. inocua* and *L. monocytogenes*. Gentamicin (GEN), ampicillin (AMP), ceftazidime (CAZ), ciprofloxacin (CIP), tetracycline (TET), erythromycin (ERY), lincomycin (LIN) and vancomycin (VAN) were selected as test antibiotics. *Streptococcus pneumonia* ATCC 49619 was selected as the quality control strain.

Fig. 3 Resistance genotypes of 59 *Listeria* isolates. Eleven resistance genes *tetA*, *tetM*, *tetS*, *ermA*, *ermB*, *ermC*, *aac*(6')-*Ib*, *mecA*, *vanA*, *vanB,* and *cfr* were selected as specific resistance genes and were identified by PCR within *Listeria* spp. . Primers used in this study are listed in Table 5.

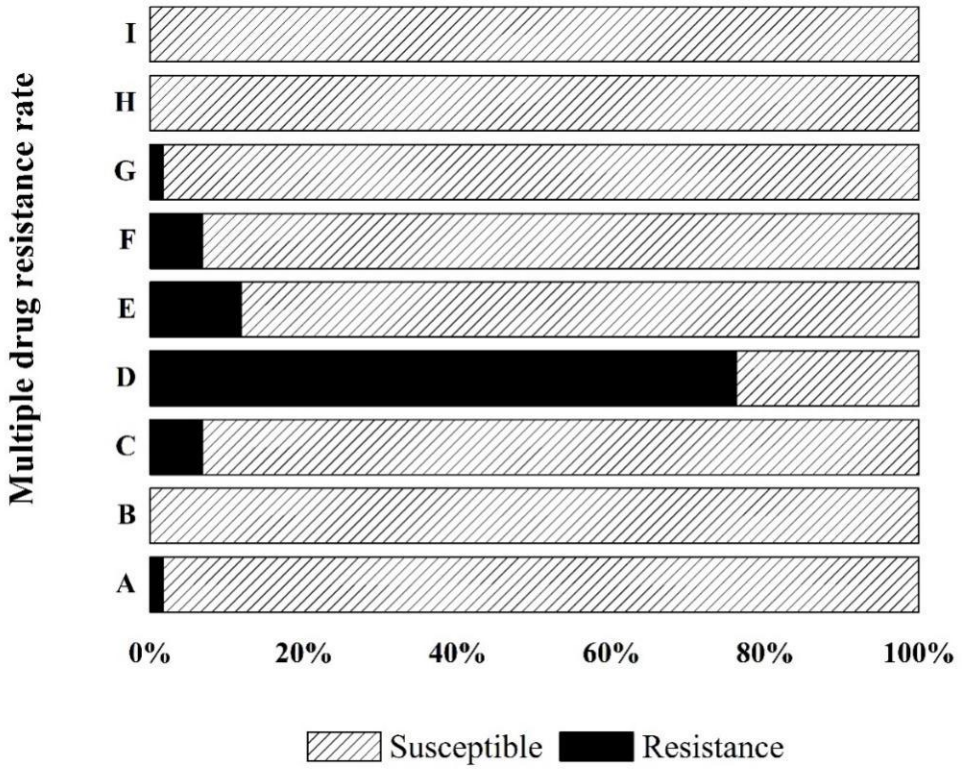
Fig. 4 Serotypes, resistance, source, and STs of the *Listeria* isolates from the processing environment. MLST performed based on seven housekeeping genes (*abcZ, bglA, cat, dapE, dat, ldh and lhkA*) according to the previous method. Genotypic data are available at http://bigsdb.web.pasteur.fr/listeria/. Minimum spanning tree analysis was inferred using BioNumerics (Version 5.10, Applied Maths, Belgium).

ND represents : None determined.

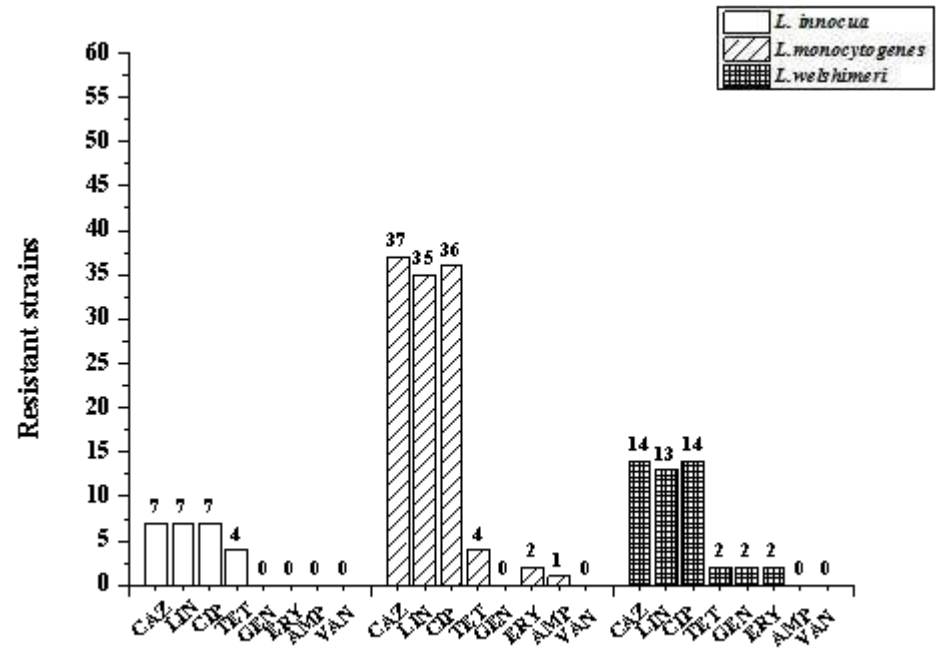
Fig. 5 Invasion level of *L. monocytogenes* isolates against the human colorectal adenocarcinoma cell line Caco-2 cells. In vitro invasion was performed in the Caco-2 cell line (3.0×10^5 cells per well) infected with 1.0×10^7 - 2.0×10^7 *L. monocytogenes* cells/well. After contact for 90 min, viable intracellular bacteria

were enumerated by plating appropriate dilutions of the cell lysate on BHI agar. Error bars represent standard deviations of the mean. ATCC19114 strain was included as an invasion control. Significant difference compared with ATCC19114; *** P<0.01; ** P<0.05; *P>0.05.

Fig. 1



477 **Fig.2**



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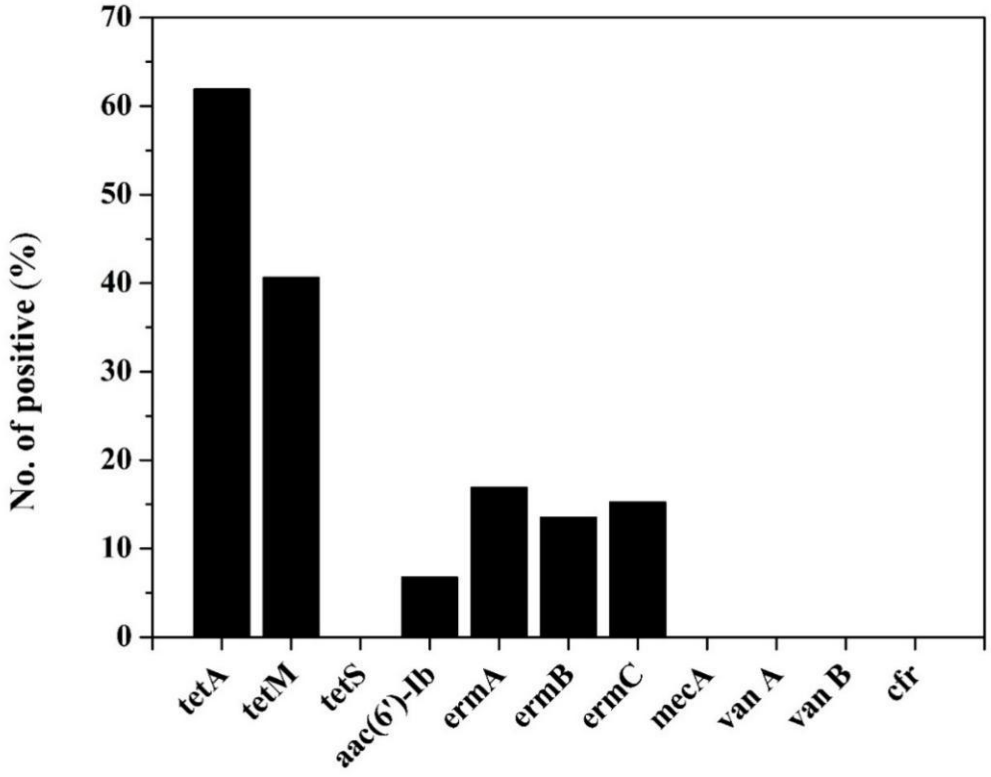
479 **Fig.3**



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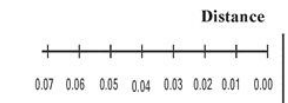
482 **Fig. 4**



Isolate code	Species	Serotypes	Resistant	Source	ST
LM8	*L.monocytogenes*	1/2a	LIN,CAZ,CIP	D	ST121
LMC11	*L.monocytogenes*	4ab	LIN,CAZ,CIP,TET	C	ST121
LM7	*L.monocytogenes*	4c	LIN,CAZ,CIP	D	ST121
LM6	*L.monocytogenes*	1/2a	LIN,CAZ,CIP	D	ST121
LM4	*L.monocytogenes*	3a	LIN,CAZ,CIP	D	ST121
LM3-11	*L.monocytogenes*	1/2a	LIN,CAZ,CIP	C	ST121
LM3	*L.monocytogenes*	1/2b	LIN,CAZ,CIP	C	ST121
LM2	*L.monocytogenes*	3c	LIN,CAZ,CIP	D	ST121
LM1-9	*L.monocytogenes*	3a	LIN,CAZ,CIP	A	ST121
LM1	*L.monocytogenes*	4c	LIN,DAZ,CIP	D	ST121
LM1T7	*L.monocytogenes*	4ab	LIN,CAZ,CIP,TET	D	ST9
LM1W3	*L.monocytogenes*	4ab	LIN,CAZ,CIP	D	ST9
LM2-18	*L.monocytogenes*	4b	LIN,CAZ,CIP	B	ST9
LM2T3	*L.monocytogenes*	4c	LIN,CAZ,CIP	D	ST9
LM2W3	*L.monocytogenes*	4ab	LIN,CAZ,CIP	D	ST9
LM3-2-2	*L.monocytogenes*	3a	LIN,CAZ,CIP	C	ST9
LM3-19	*L.monocytogenes*	4b	LIN,CAZ,CIP	C	ST9
LM3-20-2	*L.monocytogenes*	1/2a	LIN,CAZ,CIP	C	ST9
LMC7	*L.monocytogenes*	3a	LIN,CAZ,CIP	C	ST9
LMX-3	*L.monocytogenes*	1/2a	LIN,CAZ,CIP,ERY,TET	C	ST9
LMA1	*L.monocytogenes*	4a	LIN,CAZ,CIP,TET,	A	ST5
LMA8	*L.monocytogenes*	4ab	LIN,CAZ,CIP	A	ST5
LMA9	*L.monocytogenes*	1/2c	LIN,CAZ,CIP	A	ST5
LMA13	*L.monocytogenes*	1/2a	LIN,CAZ,CIP	A	ST5
LMA-II	*L.monocytogenes*	4ab	LIN,CAZ,CIP	A	ST5
LMB4	*L.monocytogenes*	4ab	LIN,CAZ,CIP	B	ST5
LMB5	*L.monocytogenes*	3a	LIN,CAZ,CIP	B	ST5
LMB9	*L.monocytogenes*	3a	LIN,CAZ,CIP	B	ST5
LMB10	*L.monocytogenes*	3a	LIN,CAZ	B	ST5
LMB13	*L.monocytogenes*	4ab	LIN,CAZ,CIP	B	ST5
LMB-I	*L.monocytogenes*	3a	LIN,CAZ,CIP	B	ST5
LMC4	*L.monocytogenes*	4ab	CAZ,CIP	C	ST5
LMC9	*L.monocytogenes*	4ab	LIN,CAZ,CIP	C	ST5
LMC15	*L.monocytogenes*	1/2b	CAZ,CIP	C	ST5
LMC-I	*L.monocytogenes*	4ab	LIN,CAZ,CIP,AMP	C	ST5
LMD3	*L.monocytogenes*	4b	LIN,CAZ,CIP,ERY	C	ST5
LMD10	*L.monocytogenes*	4c	LIN,CAZ,CIP	D	ST5
LM2-14	*L. innocua*	-	LIN,CAZ,CIP,TET	B	ST540
LMD1	*L. innocua*	-	LIN,CAZ,CIP	D	ST602
LM1W5	*L. innocua*	-	LIN,CAZ,CIP	D	ST637
LM1T2	*L. innocua*	-	LIN,CAZ,CIP,TET	D	ST537
LM2-18-2	*L. innocua*	-	LIN,CAZ,CIP	B	ST537
LM3-1	*L. innocua*	-	LIN,CAZ,CIP,TET	C	ST537
LMA2	*L. innocua*	-	LIN,CAZ,CIP,TET	A	ST637
LM3-5	*L. welshimeri*	-	LIN,CAZ,CIP	C	ST1005
LM3-17	*L. welshimeri*	-	LIN,CAZ,CIP	C	ST1005
LM3-2	*L. welshimeri*	-	LIN,CAZ,CIP	C	ST1005
LM1W9	*L. welshimeri*	-	LIN,CAZ,CIP	D	ST1005
LM1W6	*L. welshimeri*	-	LIN,CAZ,CIP	D	ST1005
LM3-15-2	*L. welshimeri*	-	LIN,CAZ,CIP	C	ST1084
LM3-20	*L. welshimeri*	-	LIN,CAZ,CIP	C	ST1084
LM3-15	*L. welshimeri*	-	LIN,CAZ,CIP	C	ST1084
LM3-7	*L. welshimeri*	-	LIN,CAZ,CIP,GEN,ERY,TET	C	ST1084
LM1T0	*L. welshimeri*	-	LIN,CAZ,CIP,TET	C	ST168
LM3-8	*L. welshimeri*	-	LIN,CAZ,CIP	C	ST168
LM3-16	*L. welshimeri*	-	LIN,CAZ,CIP	C	ST168
LM3-16-2	*L. welshimeri*	-	CAZ,CIP	C	ST168
LM3-18	*L. welshimeri*	-	CAZ,CIP	C	ST168
LMD-I	*L. welshimeri*	-	LIN,CIP,GEN,ERY	D	ST168

483

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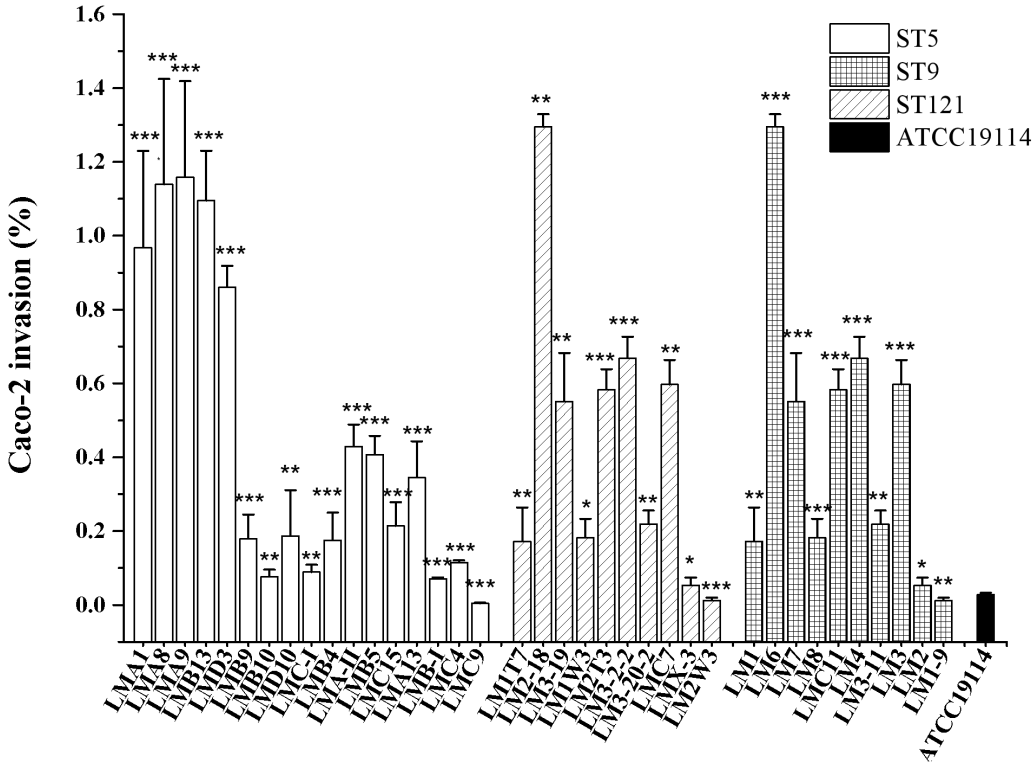
Fig. 5


Caco-2 invasion (%)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
ST5
ST9
ST121
ATCC19114
LMA1
LMA8
LMA9
LMB13
LMD3
LMB9
LMB10
LMD10
LMC1
LMB4
LMA11
LMB5
LMC15
LMA13
LMB1
LMC4
LMC9
LMIT7
LM2-18
LM3-19
LM1W3
LMIT3
LM3-22-2
LM3-20-2
LMC7
LMX-3
LM2W3
LM1
LM6
LM7
LM8
LMC11
LM4
LM3-11
LM3
LM2
LM1-9
ATCC19114

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Author contributions:

Hui Zhang and Liting Wu conceived and designed research. Liting Wu, Mengya Zhu and Tao He conducted experiments. Hongduo Bao, Yan Zhou and Ran Wang contributed new reagents or analytical tools. Zhengquan Yang，Maoda Pang and Yuan Tian analyzed data. Liting Wu and Hui Zhang wrote the manuscript. All authors read and approved the manuscript.

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Competing interests

There are no conflicts of interest (financial, professional or personal) related to this manuscript.

Availability of data and materials

Not applicable.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Not applicable.

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655

Figures

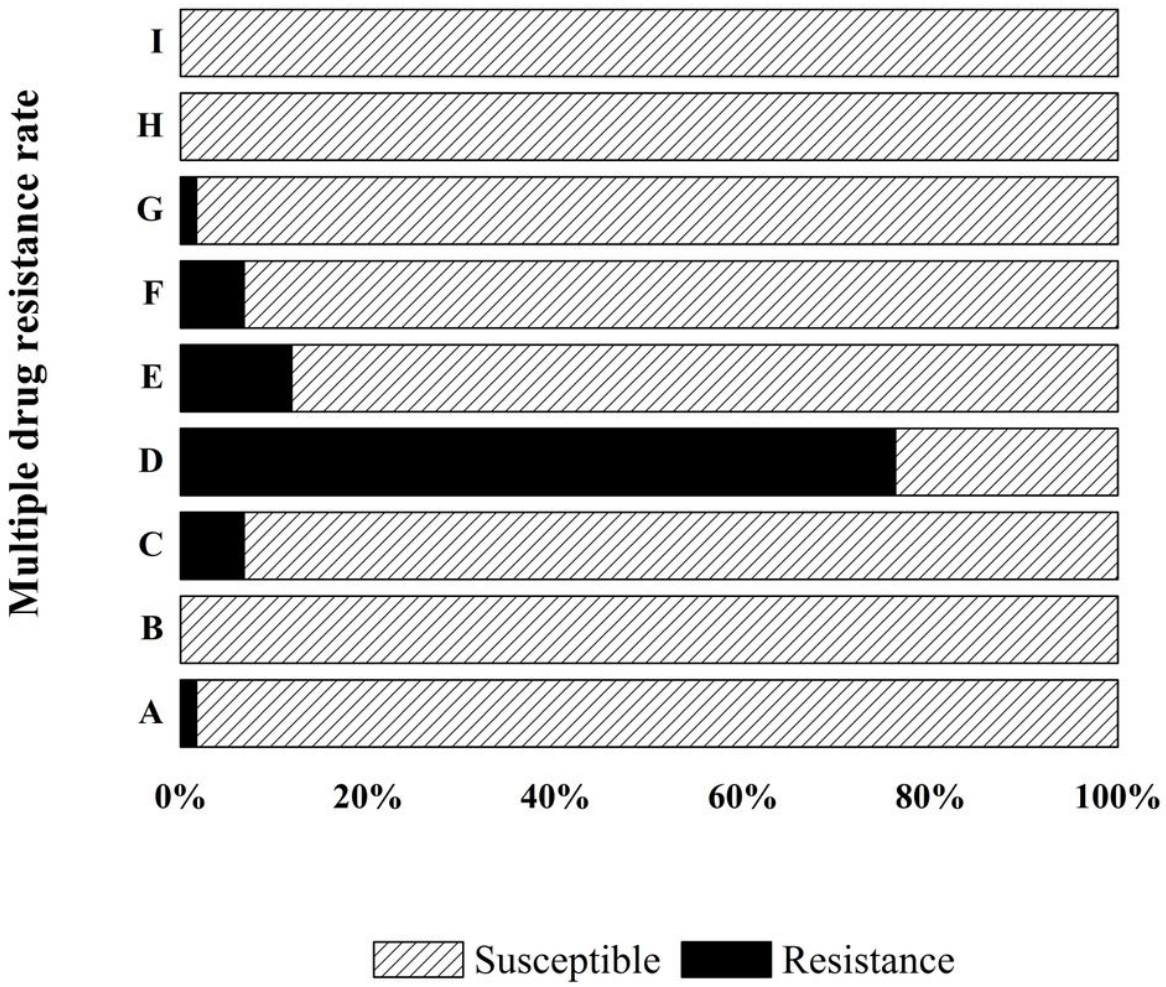

Figure 1

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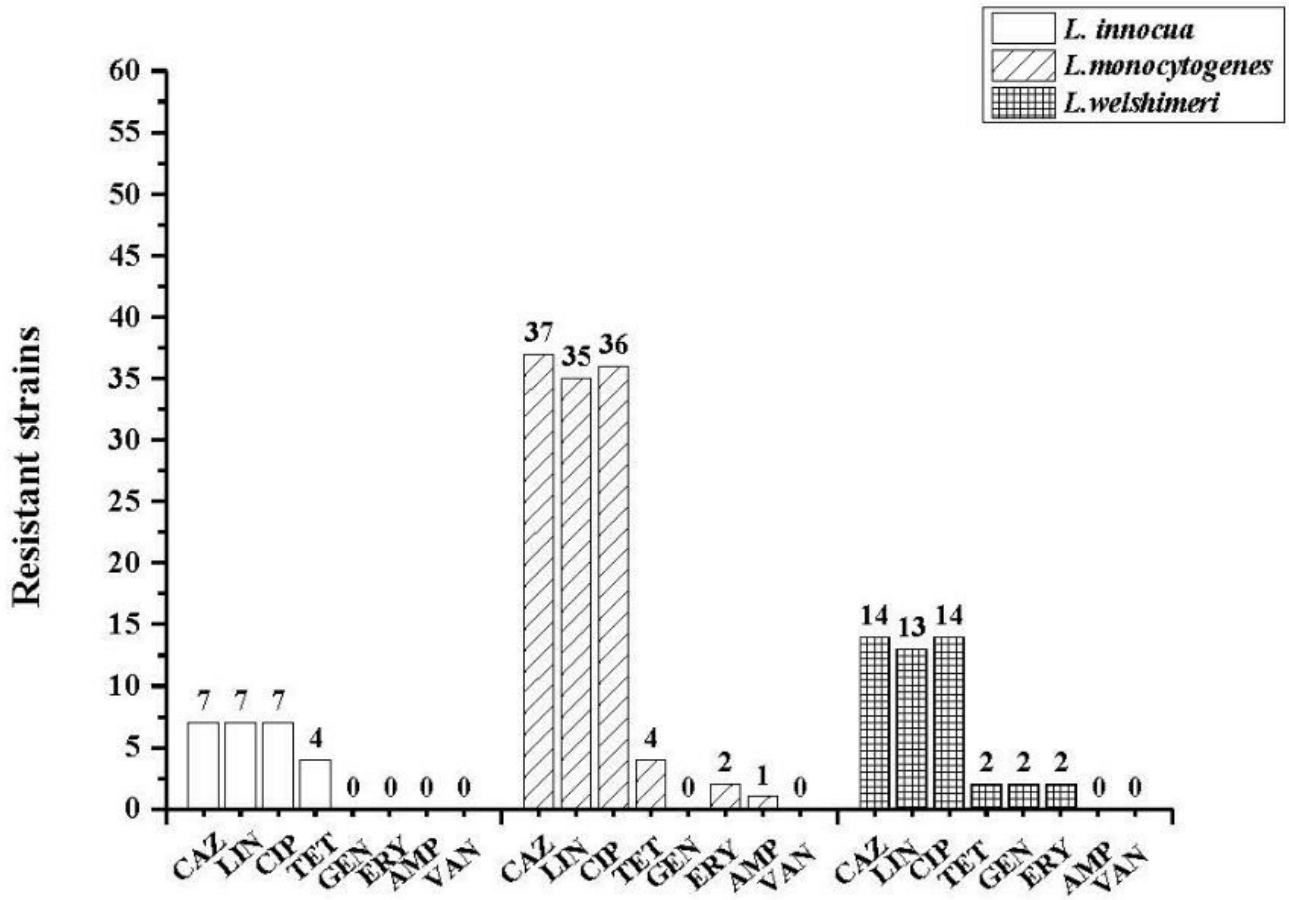

Figure 2

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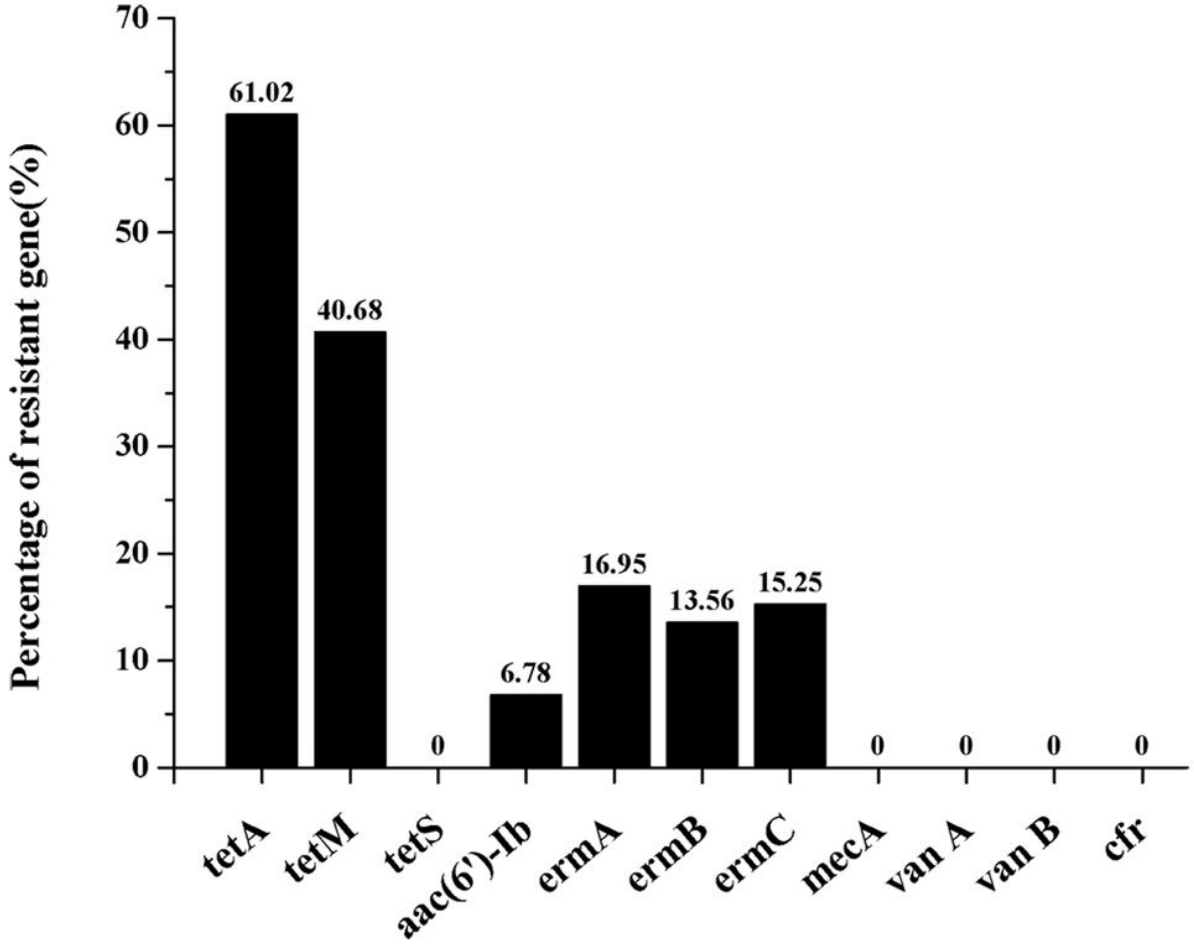

Figure 3

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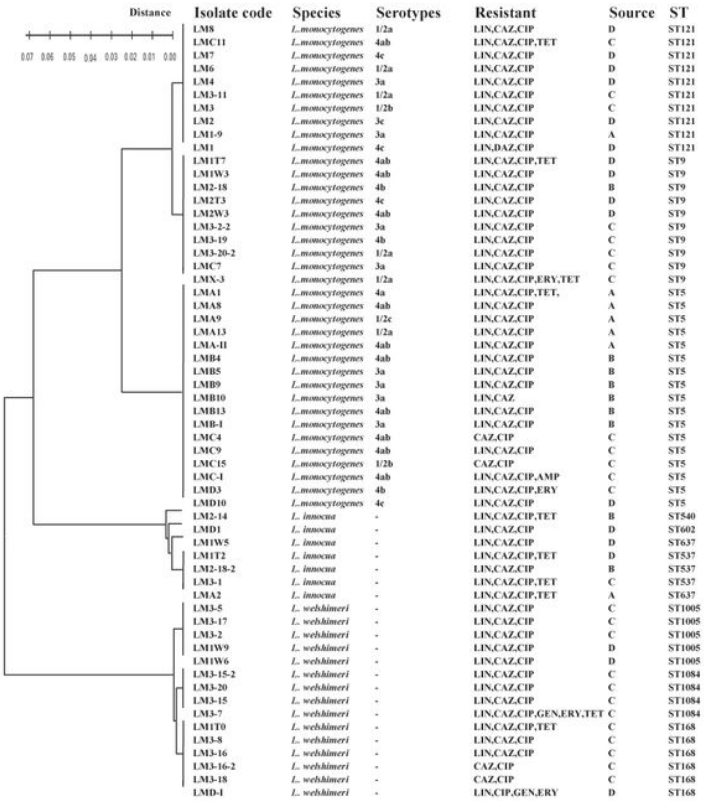

Isolate code	Species	Serotypes	Resistant	Source	ST
LM8	*L.monocytogenes*	1/2a	LIN,CAZ,CIP	D	ST121
LMC11	*L.monocytogenes*	4ab	LIN,CAZ,CIP,TET	C	ST121
LM7	*L.monocytogenes*	4c	LIN,CAZ,CIP	D	ST121
LM6	*L.monocytogenes*	1/2a	LIN,CAZ,CIP	D	ST121
LM4	*L.monocytogenes*	3a	LIN,CAZ,CIP	D	ST121
LM3-11	*L.monocytogenes*	1/2a	LIN,CAZ,CIP	C	ST121
LM3	*L.monocytogenes*	1/2b	LIN,CAZ,CIP	C	ST121
LM2	*L.monocytogenes*	3c	LIN,CAZ,CIP	D	ST121
LM1-9	*L.monocytogenes*	3a	LIN,CAZ,CIP	A	ST121
LM1	*L.monocytogenes*	4c	LIN,DAZ,CIP	D	ST121
LM1T7	*L.monocytogenes*	4ab	LIN,CAZ,CIP,TET	D	ST9
LM1W3	*L.monocytogenes*	4ab	LIN,CAZ,CIP	D	ST9
LM2-18	*L.monocytogenes*	4b	LIN,CAZ,CIP	B	ST9
LM2T3	*L.monocytogenes*	4c	LIN,CAZ,CIP	D	ST9
LM2W3	*L.monocytogenes*	4ab	LIN,CAZ,CIP	D	ST9
LM3-2-2	*L.monocytogenes*	3a	LIN,CAZ,CIP	C	ST9
LM3-19	*L.monocytogenes*	4b	LIN,CAZ,CIP	C	ST9
LM3-20-2	*L.monocytogenes*	1/2a	LIN,CAZ,CIP	C	ST9
LMC7	*L.monocytogenes*	3a	LIN,CAZ,CIP	C	ST9
LMX-3	*L.monocytogenes*	1/2a	LIN,CAZ,CIP,ERY,TET	C	ST9
LMA1	*L.monocytogenes*	4a	LIN,CAZ,CIP,TET,	A	ST5
LMA8	*L.monocytogenes*	4ab	LIN,CAZ,CIP	A	ST5
LMA9	*L.monocytogenes*	1/2c	LIN,CAZ,CIP	A	ST5
LMA13	*L.monocytogenes*	1/2a	LIN,CAZ,CIP	A	ST5
LMA-II	*L.monocytogenes*	4ab	LIN,CAZ,CIP	A	ST5
LMB4	*L.monocytogenes*	4ab	LIN,CAZ,CIP	B	ST5
LMB5	*L.monocytogenes*	3a	LIN,CAZ,CIP	B	ST5
LMB9	*L.monocytogenes*	3a	LIN,CAZ,CIP	B	ST5
LMB10	*L.monocytogenes*	3a	LIN,CAZ	B	ST5
LMB13	*L.monocytogenes*	4ab	LIN,CAZ,CIP	B	ST5
LMB-I	*L.monocytogenes*	3a	LIN,CAZ,CIP	B	ST5
LMC4	*L.monocytogenes*	4ab	CAZ,CIP	C	ST5
LMC9	*L.monocytogenes*	4ab	LIN,CAZ,CIP	C	ST5
LMC15	*L.monocytogenes*	1/2b	CAZ,CIP	C	ST5
LMC-I	*L.monocytogenes*	4ab	LIN,CAZ,CIP,AMP	C	ST5
LMD3	*L.monocytogenes*	4b	LIN,CAZ,CIP,ERY	C	ST5
LMD10	*L.monocytogenes*	4c	LIN,CAZ,CIP	D	ST5
LM2-14	*L. innocua*	-	LIN,CAZ,CIP,TET	B	ST540
LMD1	*L. innocua*	-	LIN,CAZ,CIP	D	ST602
LM1W5	*L. innocua*	-	LIN,CAZ,CIP	D	ST637
LM1T2	*L. innocua*	-	LIN,CAZ,CIP,TET	D	ST537
LM2-18-2	*L. innocua*	-	LIN,CAZ,CIP	B	ST537
LM3-1	*L. innocua*	-	LIN,CAZ,CIP,TET	C	ST537
LMA2	*L. innocua*	-	LIN,CAZ,CIP,TET	A	ST637
LM3-5	*L. welshimeri*	-	LIN,CAZ,CIP	C	ST1005
LM3-17	*L. welshimeri*	-	LIN,CAZ,CIP	C	ST1005
LM3-2	*L. welshimeri*	-	LIN,CAZ,CIP	C	ST1005
LM1W9	*L. welshimeri*	-	LIN,CAZ,CIP	D	ST1005
LM1W6	*L. welshimeri*	-	LIN,CAZ,CIP	D	ST1005
LM3-15-2	*L. welshimeri*	-	LIN,CAZ,CIP	C	ST1084
LM3-20	*L. welshimeri*	-	LIN,CAZ,CIP	C	ST1084
LM3-15	*L. welshimeri*	-	LIN,CAZ,CIP	C	ST1084
LM3-7	*L. welshimeri*	-	LIN,CAZ,CIP,GEN,ERY,TET	C	ST1084
LM1T0	*L. welshimeri*	-	LIN,CAZ,CIP,TET	C	ST168
LM3-8	*L. welshimeri*	-	LIN,CAZ,CIP	C	ST168
LM3-16	*L. welshimeri*	-	LIN,CAZ,CIP	C	ST168
LM3-16-2	*L. welshimeri*	-	CAZ,CIP	C	ST168
LM3-18	*L. welshimeri*	-	CAZ,CIP	C	ST168
LMD-I	*L. welshimeri*	-	LIN,CIP,GEN,ERY	D	ST168

Figure 4

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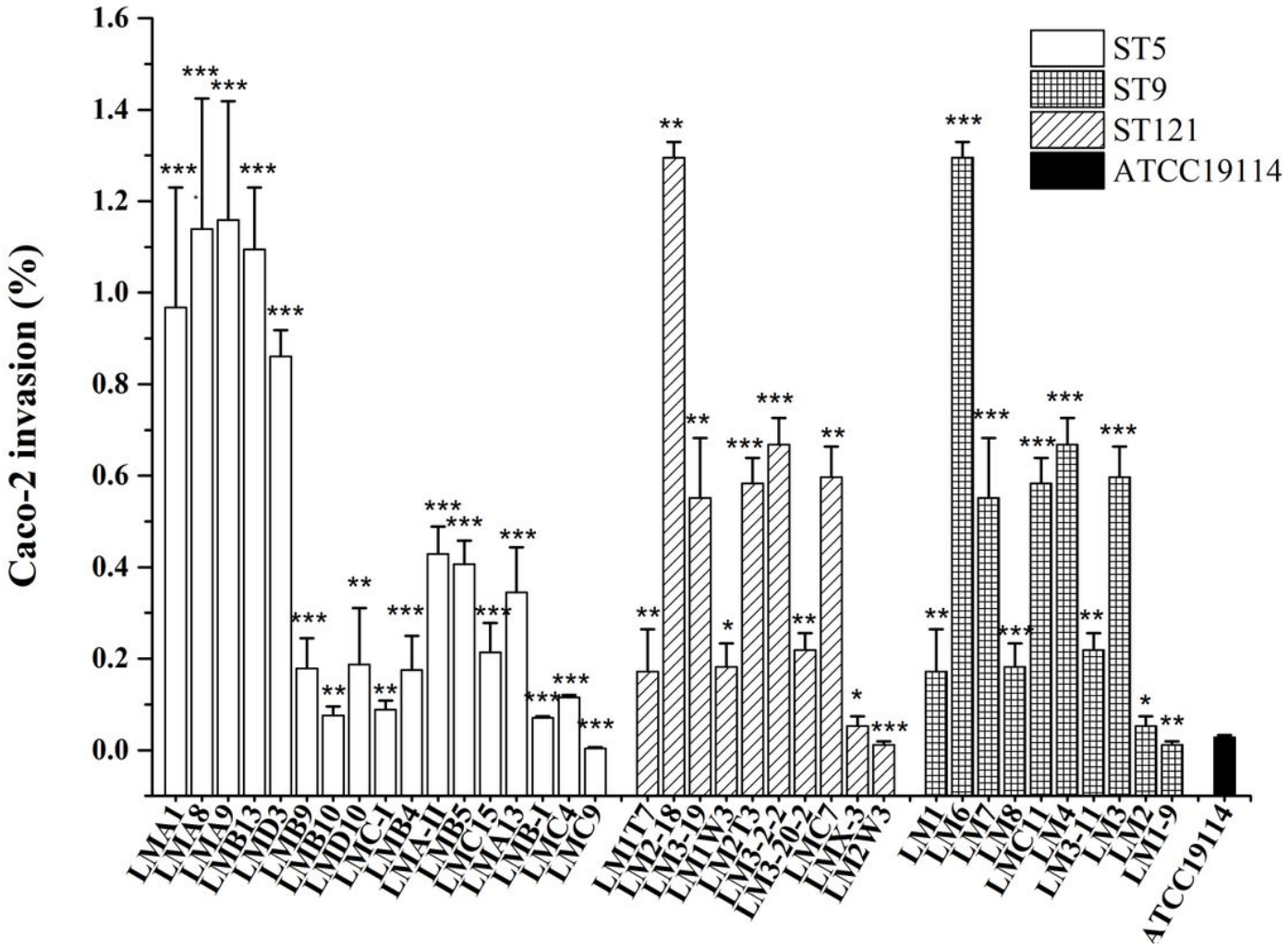

Figure 5

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