Letter to the Editor

Combination Use of PFGE and Drug-resistant Analysis in the Epidemiological Investigation of *Listeria Monocytogenes*

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Listeria monocytogenes is the pathogen of listeriosis and it causes severe infections like septicemia, encephalitis, and meningitis, especially in immunocompromised individuals, newborns, and pregnant women. Its wide distribution in the environment and ability to survive or even grow under adverse conditions has made *L. monocytogenes* an important public health concern and in food industry[1]. Many reports of invasive listeriosis were reported in developed countries in past[2], but outbreaks and sporadic listeriosis have been reported rarely in China, althouth *L. monocytogenes* has been isolated from almost all Chinese cities through Surveillance Network of Foodborne Diseases.

Tracing *L. monocytogenes* contamination routes in the food processing industry has recently been the focus of several laboratories[3]. Fingerprinting by pulsed-field gel electrophoresis (PFGE) has been proven to be very useful for precise characterization of *L. monocytogenes*[4].

The extensive use of antimicrobials has led to the emergence of antimicrobial-resistant bacteria in the environment. *L. monocytogenes* isolates may acquire resistance by obtaining mobile genetic components such as plasmids and conjugative transposons[5]. It is apparent that the genome sequence of drug-resistant strain and drug-sensitive strain is different. It has not been reported that whether insertion and deletion of drug-resistant genes changes the PFGE patterns.

The purposes of this study is to investigate the homology and drug-resistance of *L. monocytogenes* isolated from Hubei Province in China and to compare the diversity of drug-resistance among PFGE subtypes and, to use the combination of the PFGE and the drug-resistant analysis for subtyping.

42 strains of *L. monocytogenes* isolated from Hubei Province in China, *L. monocytogenes* ATCC.BAA-679™, *L. monocytogenes* CMCC54004 and *Salmonella enterica* serotype (CDC H9812) were used as control strains.

Serotyping was performed by multiplex PCR described previously[6] .

All isolates were analyzed by PFGE according to the standard CDC PulseNet protocol[7]. Firstly, cultures were harvested from brain heart infusion agar plates (Difco/BD, Sparks, MD) after incubation at 37 °C for 18 h and re-suspended in TE, pH 8.0 (10 mmol/L Tris, 1 mmol/L EDTA). The optical density was adjusted to 1.3 at 610 nm. Bacterial cells were then embedded in 1% agarose plugs (SeaKem Gold agarose; Cambrex, Rockland, ME), lysed, washed, and digested with restriction enzymes *Asc*I (Merk) at 30 °C for at least 5 h. *Xba*I-digested *Salmonella enterica serotype Braenderup* (CDC H9812) DNA was used as a reference size standard. Then, restricted DNA fragments were separated for 19 h at 14 °C in 1% agarose gels by using CHEF MAPPER System (Bio-Rad USA) at 6 V/cm with switch times of 4 s to 40 s. Band pattern images were captured with GEL Doc 2000 (Bio-Rad USA), and analyzed with the BioNumerics Version 4.0 software (Applied Maths, Saint-matins-Latem, Belgium).

Drug-resistance tests were performed as described previously[8]. The bacterial suspensions at a density of McFarland 0.5 were smeared on Mueller Hinton Agar (MHA) (Oxoid) with 5% off fiber sheep blood. Then E-test strips (AB Bio-disk, Solna, Sweden) with ampicillin, gentamicin, amikacin, erythromycin, sulfamethoxazole, sulfadiazine, ciprofloxacin, vancomycin, tetracydline, doxycyclinge, streptomycin, chloramphenicol, and iminepeinern, were tightly affixed on the M-H agar surface, and incubated at 37 °C for 18 h. Minimum inhibitory concentration (MIC) of various antibiotics was read with the help of a magnifying lens, based on which the judgment of sensitivity, intermediate sensitivity and resistance was determined afterwards.

All the isolates were divided into two groups, 31 strains were serotype 1/2a, and the other 11 strains

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were serotype 1/2b, by multiplex PCR amplifying and serological assaying.

The PFGE analysis with the use of endonuclease *Asc*I showed 20 subtypes among the 42 isolates of *L. monocytogenes* from Hubei Province (Figure 1). PFGE-types J, M, and O occurred at least four times, accounting for 40.5% of the characterized isolates. PFGE-type J was the most widely distributed one, as it was shared by 7 samples including those from aquatic products, poultry and meat. The next subtype was PFGE-type M shared by 6 samples including those from poultry, meat and FFC-food. Furthermore, 11 isolates showed unique PFGE profiles, which accounted for 26.2% of the total strains.

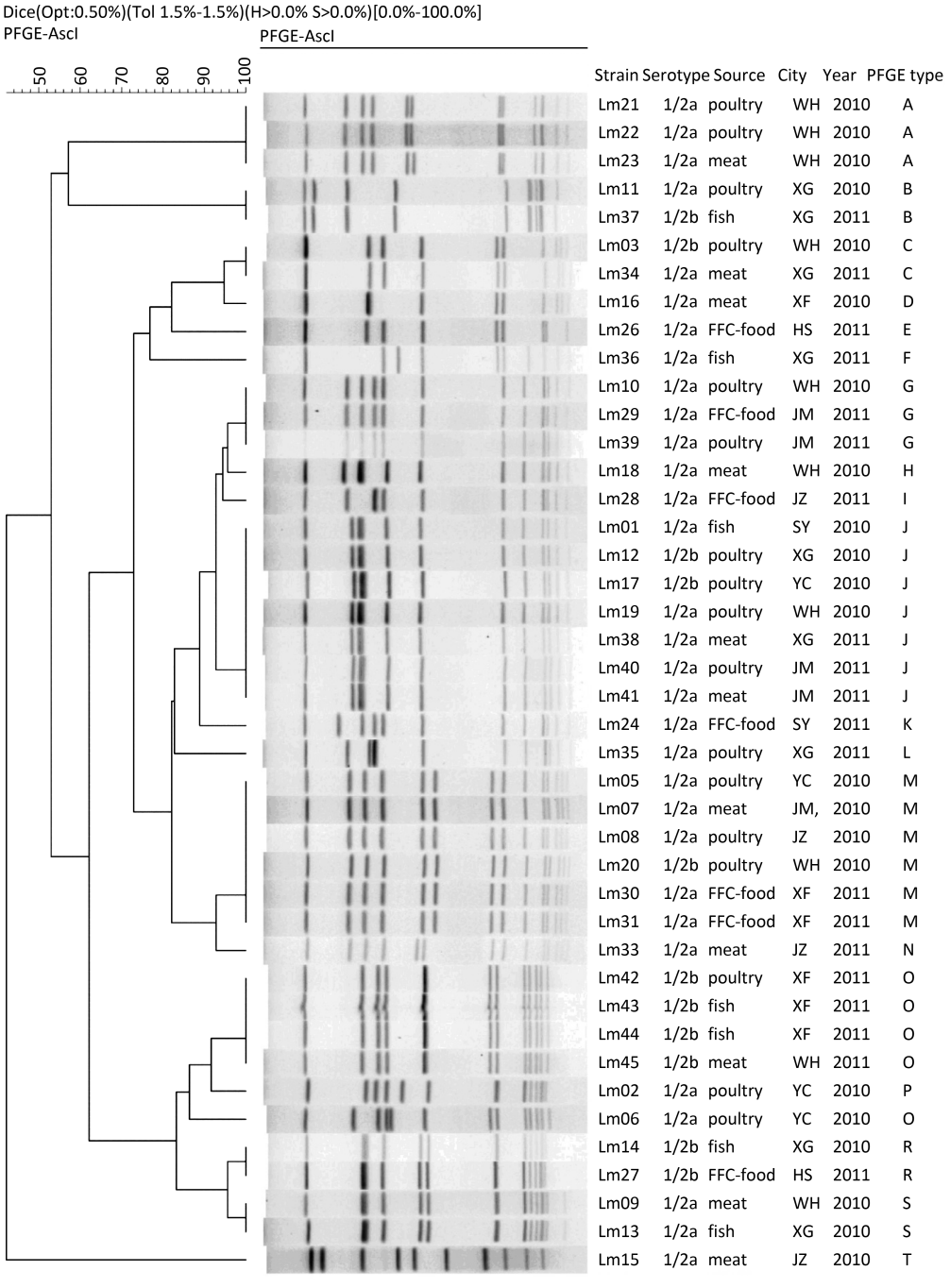

Figure 1. PFGE types of the 42 isolates of *L. monocytogenes*. WH: Wuhan; YC: Yichang; XF: Xiangfan; SY: Shiyan; JZ: Jingzhou; JM: Jingmen; XG: Xiaogan; HS: Huangshi.

Drug-resistance of the 42 isolats of *L.* monocytogenes was evaluated by using the standard E-test method, and antibiotic resistance frequencies were shown in Table 1. All strains were sensitive to ampicillin, gentamicin, amikacin, sulfamethoxazole, vancomycin, tetracycline, doxycycline, and imipennem. Except one strain with intermediate sensitivity to erythromycin, all the stains were sensitive to erythromycin. No isolate was resistant to streptomycin except three stains with intermediate sensitivity, and for chloramphenicol, the isolates showed the same sensitivity features. A total of 20 strains were resistant to sulfadiazine, with a drug-resistant rate of 47.6%. To ciprofloxacin, approximately 21.43% of the strains were resistant, 16.67% intermediately sensitive and 61.9% sensitive. Furthermore, 11.9% (5/42) isolates were resistant to both sulfadiazine and ciprofloxacin, which were defined as mutliresistant strains.

The emergence of multiresistant strains of *L. monocytogenes* in food represents a potential threat to the Public Health. In this study, all the isolates were sensitive to ampicillin, gentamicin, amikacin, sulfamethoxazole, vancomycin, tetracycline, doxycycline, and imipennem. Therefore, single or combination use of these antibiotics is the option of the treatment for listeriosis, and sulfadiazine and ciprofloxacin are not recommended for the treatment of listeriosis. This result is consistent with that of previous studies[8].

Among the 15 strains resistant to sulfadiazine, 14 strains belonged to serotype 1/2a and the other 1 strain to serotype 1/2b. The resistance rate of serotype 1/2a *L. monocytogenes* to sulfadiazine was 45.16%, but the rate of serotype 1/2b isolates was 9.09%. Ciprofloxacin-resistant isolates were all five serotype 1/2a stains. The results mentioned above showed that the serotype 1/2a *L. monocytogenes* was more prone to drug resistance which were inconsistent with the views of Hansen et al.[8], who believed that there was no difference in the susceptibility pattern between the serogroups.

A few strains shared the same PFGE types, but diverged in antibiotics-resistant characteristics. As seen in Table 2, strains from PFGE-type J, M, and O could be divided into three, three and two groups, respectively, based on their drug-resistant patterns. PFGE-type M was shared by 6 isolates of *L. monocytogenes*, in which Lm08, Lm20, and Lm31 were resistant to sulfadiazine and ciprofloxacin, Lm05 and Lm07 were only resistant to sulfadiazine, and Lm30 was only resistant to ciprofloxacin. Findings from our study also showed that *L. monocytogenes* belonging to the same PFGE type were from different sources. A total of 25 subtypes would be differentiated among the 42 isolates of *L. monocytogenes*, by combining the PFGE types and the drug-resistant pattern.

L. monocytogenes isolates may acquire resistance by the acquisition of mobile genetic components such as plasmids and conjugative transposons[5]. It is apparent that the genome sequence between the drug-resistant strain and the drug-sensitive strain is different. The influence of antibiotic-resistant gene insertion on the PFGE types

Table 1. Antibiotics Susceptibility of 42 *L. monocytogenes* Strains

Drug	Number of Stains with Drug-resistance			Drug-resistant Rate (%)
	Sensitive	Intermediate	Resistant	
Ampicillin	42	0	0	0.00
Gentamicin	42	0	0	0.00
Amikacin	42	0	0	0.00
Erythromycin	41	1	0	0.00
Sulfamethoxazole	42	0	0	0.00
Sulfadiazine	22	0	20	48.39
Ciprofloxacin	26	7	9	22.58
Vaneomycin	42	0	0	0.00
Tetracycline	42	0	0	0.00
Doxycycline	42	0	0	0.00
Streptomycin	39	3	0	0.00
Chloramphenicol	39	3	0	0.00
Imipenem	42	0	0	0.00

Table2. Drug-resistant Pattern of the 20 PFGE-types of *L. monocytogenes* Isolates

PFGE Type	Strains	Drug-resistant Pattern					
		SDI	CIP	ERY	STR	CHL	Others
A	Lm21, Lm22, Lm23	R	S	S	S	S	S
B	Lm11, Lm37	R	S	S	S	S	S
C	Lm03, Lm34	S	S or I	S	S	S	S
D	Lm16	S	R	S	S	S	S
E	Lm26	R	I	S	S	S	S
F	Lm36	S	I	S	S	S	S
G	Lm10, Lm29, Lm39	S	S or I	S	S	S	S
H	Lm18	S	R	S	S	S	S
I	Lm28	R	S	S	S	S	S
J	Lm01, Lm17, Lm12, Lm40	S	S or I	S	S	S	S
J	Lm19	S	R	S	S	S	S
J	Lm38, Lm41	R	S or I	S	S	S	S
K	Lm24	S	S	S	S	S	S
L	Lm35	S	S	S	S	S	S
M	Lm05, Lm07, Lm08, Lm20	R	S	S	S	S or I	S
M	Lm30	S	R	S	S	S	S
M	Lm31	R	R	S	S	S	S
N	Lm33	R	S	S	S	S	S
O	Lm43	R	S	I	S	I	S
O	Lm42, Lm44, Lm45	S	S	S	S or I	S	S
P	Lm02	R	R	S	S	S	S
Q	Lm06	R	S	S	S	S	S
R	Lm14, Lm27	S	S	S	S or I	S	S
S	Lm09, Lm13	S	S	S	S	S	S
T	Lm15	R	R	S	S	S	S

Note. S: Sensitive; I: Intermediately sensitive; R: Resistant; SDI: sulfadiazine; CIP: ciprofloxacin; ERY: erythromycin; STR: streptomycin; CHL: chloramphenicol; Others: ampicillin, gentamicin, amikacin, sulfamethoxazole, vaneomycin, tetracycline, doxycycline, and imipenem.

is neglected. This may be explained as that, in the PFGE method, the sites recognized and cut by restriction enzyme are scarce and therefore only large restriction fragments are separated. However, the length of antibiotic-resistant gene is limited and the gene sequence does not possess any enzyme recognition site and thus, the change of the moving rate for large nucleic fragments in gel electrophoresis could not be observed.

PFGE is generally recognized as the most discriminatory subtyping method for *L. monocytogenes*[9]. It was showed in this study that the combined use of the PFGE and the drug-resistant analysis is more discriminatory for subtyping in the epidemiological investigation of *L. monocytogenes.*

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REFERENCES

1. Aznar R, Alarcon B. PCR detection of *Listeria monocytogenes*: a study of multiple factors affecting sensitivity. J Appl Microbiol, 2003; 95, 958-66.
2. De Valk H, Jacquet C, Goulet V, et al. Surveillance of *Listeria* infectious in Europe. Euro Surveillance, 2005; 10, 251-5.
3. Autio T, Keto-Timonen R, Lunden J, et al. Characterisation of Persistent and Sporadic *Listeria monocytogenes* Strains by Pulsed-Field Gel Electrophoresis (PFGE) and Amplified Fragment Length Polymorphism (AFLP). Syst Appl Microbiol, 2003; 26, 539-45.
4. Brosch R, Brett M, Catimel B, et al. Genomic fingerprinting of 80 strains from the WHO multicenter international typing study of *Listeria monocytogenes* via pulsed-field gel electrophoresis (PFGE). Int J Food Microbiol, 1996; 32, 343-55.
5. Harakeh S, Saleh I, Zouhairi O, et al. Antimicrobial resistance of *Listeria monocytogenes* isolated from dairy-based food products. Sci Total Environ, 2011; 407, 4022-7.
6. Doumith M, Buchrieser C, Glaser P, et al. Differentiation of the major *Listeria monocytogenes* serovars by multiplex PCR. J Clin Microbiol, 2004; 42, 3819-22.
7. Graves LM, Swaminathan B. PulseNet standardized protocol for subtyping *Listeria monocytogenes* by macrorestriction and pulsed-field gel electrophoresis. Int J Food Microbiol, 2001; 65, 55-62.
8. Hansen JM, Greener-Smidt P, Bruun B. Antibiotic susceptibility of *Listeria monocytogenes* in Denmark 1958-2001. Acta pathologica, Microbiologica Et Immunologica Scandinavica, 2005; 113, 31-6.
9. Graves LM, Swaminathan B. PulseNet standardized protocol for subtyping *Listeria monocytogenes* by macrorestriction and pulsed-field gel electrophoresis. Int J Food Microbiol, 2001; 65, 55-62.