

Antimicrobial susceptibilities of *Listeria monocytogenes* isolated from the imported and the domestic foods in Japan

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Abstract: *In vitro* antimicrobial susceptibility of *Listeria monocytogenes* isolated from the imported and the domestic foods in Japan was determined by plate dilution method. Eleven isolates from domestic meat, meat products, liver, seafood and environment, and 16 isolates from imported meat and meat products were examined their susceptibilities against ampicillin, chloramphenicol, enrofloxacin, erythromycin, gentamicin, kanamycin, penicillin and tetracycline. All of the isolates except the one isolate from domestic scallop were susceptible to all the antibiotics tested. Only 1 isolate showed resistance to kanamycin and gentamicin. The minimum inhibitory concentration (MIC) for 50% of the strains and the MIC for 90% of the strains were comparable between the imported and the domestic food origins. These results suggest there were less differences of antimicrobial susceptibility between the two origins of *Listeria* isolates.

Keywords: *Listeria monocytogenes*, Antibiotic Susceptibility

1. Introduction

Listeriosis, a severe-infectious disease caused by *Listeria monocytogenes*, is known to transmit to human via contaminated foods. In contrast to the situation in the North America or EU countries, the outbreaks of listeriosis have occurred in every few years, there is only one outbreak officially recognized in Japan to the date (Makino, 2006). As the sporadic cases, however, the incidence rates in Japan were estimated as 0.65 cases per million in 1996-2002 (Okutani, 2004a) and 1.0-1.6 per million in 2008-2011 (Yamane, 2012). These numbers are comparable to those in European countries (EC). The reason that the frequency of outbreak in Japan is very low compared with those of the European countries and United States remains uncertain. *L. monocytogenes* is isolated from many types of foods in Japan, and their levels of contamination are similar to those in the other countries (Okutani, 2004b). Self-sufficiency rate in food is about 40% in Japan, and many types of food are imported. Non-cooked meat products like raw ham and the dairy products like fresh cheese from Europe and the other countries are popular in Japan, and the amount of import of these foods is continuously increased. However, these foods are known to be often

contaminated by *L. monocytogenes*.

Antimicrobial drugs such as ampicillin or gentamicin are used commonly for the treatment of listeriosis, although, there are many reports about antibiotics resistant strains of *L. monocytogenes* from Europe, America and the other countries. In this study, we determined the antimicrobial susceptibility of *L. monocytogenes* isolated from imported and domestic foods in Japan.

2. Materials and Methods

2.1. Bacterial Strains and Media

Eleven isolates from domestic meat, meat products, liver and fishery products, and 16 isolates of *L. monocytogenes* from imported meat and meat products were used for examination of their antibiotic susceptibilities (Table 1).

These were isolated in Japan from 2000 to 2008. The raw ham and salami samples were imported from Spain, and the chicken meat samples are imported from Brasil. *L. monocytogenes* EGD (serotype 1/2a) and *Enterococcus faecalis* ATCC29212 (provided from Riken Bioresource Center, Japan) were used as control.

All of the isolates were stocked in Brain Heart Infusion

(BHI) broth (Difco) with 20% glycerol at -80°C until used. Bacterial cells were grown in BHI broth at 37°C for 24h, and diluted to 1/10 with saline for antibiotic resistance tests. Muller-Hinton agar (Difco) was used for the plate dilution method.

2.2. Antibiotics Susceptibility Test

All strains were tested their susceptibilities against 8 types of antibiotics; ampicillin (ABPC; Wako Chemicals, Tokyo, Japan), chloramphenicol (CM; Wako), enrofloxacin (ERFX; Sigma, St. Louis, USA), erythromycin (EM; Sigma), gentamicin (GM; Wako), kanamycin (KM; Wako), penicillin (PN; Wako) and tetracycline (TC; Wako) by standard plate dilution method as in the previous study (Okada, 2011). All antibiotics were used in two-fold serial dilution at concentrations from 0.25 to 128µg/ml. The inoculation of the bacterial cells on Muller-Hinton agar was performed using

MicroPlanter MIT-P (SAKUMA, Tokyo, Japan) according to manufacturer's instruction. All plates were incubated at 37°C. The formation of colonies was observed at 48 h after inoculation, and the minimum inhibitory concentration (MIC), the MIC for 50% of the strains (MIC₅₀), and the MIC for 90% of the strains (MIC₉₀) were determined for each antibiotic. The breakpoints for the susceptibility of *L. monocytogenes* to ABPC and PN were followed by the Clinical and Laboratory Standards Institute (CLSI) guideline, M31-A3 (CLSI, 2008). For the antibiotics which had bimodal MIC distributions, the microbiological breakpoints were applied as well as the report of the Japanese Veterinary Antimicrobial Resistance Monitoring System (Takahashi, 2006). The microbiological breakpoint is defined as the intermediate MIC between the 2 peak distributions. For the antibiotics which had monomodal MIC distributions, the breakpoints were not determined.

Table 1. *L. monocytogenes* strains used in this study.

Origin	Type of foods	Number of isolates	Isolated year	Serotype
Domestic foods	Beef liver	1	2000	4b
	Pork liver	2	2000	4b
	Beef meat	3	2000	1/2b, 4b
	Pork meat	1	2000	1/2b
	Chicken sasami	1	2000	1/2b
	Pork cotlet	1	2008	1/2a
	Scallop	1	2008	4b
	Environment	1	2006	1/2b
	Total	11		
Imported foods	Raw ham	2	2007	1/2a, 1/2c
	Salami	4	2007	1/2a, 1/2b, 1/2c, 3b
	Chicken meat	10	2006-2008	1/2a, 1/2c, 3a, 4b
	Total	16		

3. Results

3.1. Antimicrobial Susceptibility of *L. monocytogenes* from Imported and Domestic Foods

MIC against ABPC and PN of the all isolates in this study were 2 µg/mL or below (Table 2).

Table 2. MIC distributions, MIC₅₀ and MIC₉₀ of *L. monocytogenes* isolates.

Antibiotic	Origin ^a	Number of strains with MIC (µg/ml) of										(µg/ml)	
		0.25	0.5	1	2	4	8	16	32	64	MIC ₅₀	MIC ₉₀	
ABPC	D			7	4							1	2
	I			14	2							1	2
CP	D						1	10				16	16
	I						9	7				8	16
EM	D		9	2								0.5	1
	I		14	2								0.5	1
ERFX	D		4	6	1							1	1
	I		4	12								1	1
GM	D		6	4		1 ^b						0.5	1
	I	6	7	3								0.5	1
KM	D				7	3			1 ^b			2	4
	I			2	13	1						2	2
PN	D	10	1									0.25	0.25
	I	16										0.25	0.25
TE	D			1	10							2	2
	I			4	10	2						2	4

^a D: domestic, I: imported

^b Resistance

CLSI defined that the MIC of ≤ 2 $\mu\text{g}/\text{mL}$ is susceptible in *Listeria* spp., and the MIC of >2 $\mu\text{g}/\text{mL}$ should be described as non-susceptible (CLSI). In accordance with this guideline, all isolates were determined as susceptible to ABPC and PN. The MIC distributions of CP (8-16 $\mu\text{g}/\text{mL}$), EM (0.5-1 $\mu\text{g}/\text{mL}$), ERFX (0.5-1 $\mu\text{g}/\text{mL}$), and TE (1-4 $\mu\text{g}/\text{mL}$) were monomodal, suggesting that all the isolates were susceptible to these antibiotics (Table 2). The MIC distributions of GM and KM were bimodal, and the microbiological breakpoint was determined as 2 and 8 $\mu\text{g}/\text{mL}$, respectively (Table 2). One isolate from a domestic scallop in 2008 was resistant to GM and KM and their MICs were 4 and 16 $\mu\text{g}/\text{mL}$, respectively. In all antibiotics, there were no differences of MIC₅₀ and MIC₉₀ between the imported and the domestic foods.

4. Discussion

From the result in this study, *L. monocytogenes* strains isolated from the imported and the domestic foods in Japan were mainly susceptible to the major antibiotics. In our previous study about the resistance against 16 types of antibiotic in 101 isolates from patients and 100 isolates from foods and environment, we found that only 1 isolate from patient was resistant to oxytetracyclin and tetracyclin (Okada, 2011). This result supports that the listerial resistant rates to antibiotics in Japan are at low level basically. However, 1 isolate from scallop was found to be resistant against GM and KM in this study. GM is often used for the treatment of human listeriosis, therefore, the emergence of the resistant isolates can be threat for public health. In another study in Japan, one isolate from the feces of patient cow was resistant to GM (Hara, 1983). GM-resistant isolates were isolated from several countries; from 60.9 % (14/23) of dairy products in Spain (Rota, 1996), 42.9% (3/7) of milk in India (Bhilegaonkar, 1997), 2% (1/52) of cancer patients in USA (Safdar, 2003), 4.6% (2/43) of foods in Poland (Majczyna, 2006). Zamora *et al.* described that 15% (3 of 20) of clinical isolates were resistant to GM in Costa Rica (Zamora, 2006). In Mexico, Rodas-Suarez *et al.* researched fish and seawater and found the 2.3% (1 of 68) of isolates were GM-resistant (Rodas-Suarez, 2006). In Canada, 31.8% (14/44) of isolates from the feces of dairy cow and isolates from the feces of wildlife animals were resistant to GM (Lyautey, 2007). These indicate that the GM-resistant isolates were mainly found from dairy products, clinical specimen and seafood. It suggests the possibility that the frequency and/or the amount of GM used in medical practices and at dairy farms might be related with the appearance of GM-resistant isolates. Only one isolate from seafood was examined, therefore, further research which subject to seafood and its products should be done.

The result in this study shows that the continuous survey about the antibiotic susceptibility in *L. monocytogenes* isolates is important to secure the trend of antibiotics resistance for the essential treatment of listeriosis.

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