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Abstract

This study aimed at determining the genetic and virulence characteristics of the *Listeria monocytogenes* from smoked ducks. *L. monocytogenes* was isolated by plating, and the isolated colonies were PCR identified. All the obtained seven *L. monocytogenes* isolates possessed the virulence genes (*inl*A, *inl*B, *plc*B, and *hly*A) and a 385 bp *act*A amplicon. The *L. monocytogenes* isolates (SMFM2018 SD 1-1, SMFM 2018 SD 4-1, SMFM 2018 SD 4-2, SMFM 2018 SD 5-2, SMFM 2018 SD 5-3, SMFM 2018 SD 6-2, and SMFM 2018 SD 7-1) were inoculated in tryptic soy broth (TSB) containing 0.6 % yeast extract at 60 ℃, followed by cell counting on tryptic soy agar (TSA) containing 0.6 % yeast extract at 0, 2, 5, 8, and 10 min. We identified five heat resistant isolates compared to the standard strain (*L. monocytogenes* ATCC13932), among which three exhibited the serotype 1/2b *and* D-values of 5.41, 6.48, and 6.71, respectively at 60 ℃. The optical densities of the cultures were regulated to a 0.5 McFarland standard to assess resistance against nine antibiotics after an incubation at 30 ℃ for 24 h. All isolates were penicillin G resistant, possessing the virulence genes (*inl*A, *inl*B, *plc*B, and *hly*A) and the 385-bp *act*A amplicon, moreover, three isolates showed clindamycin resistance. In conclusion, this study allowed us to characterize *L. monocytogenes* isolates from smoked ducks, exhibiting clindamycin and penicillin G resistance, along with the 385-bp *act*A amplicon, representing higher invasion efficiency than the 268-bp *act*A, and the higher heat resistance serotype 1/2b.

Keywords: *Listeria monocytogenes*, antibiotic resistance, heat resistance, virulence, smoked ducks

Introduction

The smoked and sliced duck products are particularly convenient for the consumers as they could be consumed in general without any additional heating, and thus their consumption has increased in Korea (Lee et al., 2015). However, *Listeria monocytogenes* has been isolated from sliced smoked ducks in Korea. Hence, there is a possibility for listeriosis through smoked ducks. A previous study also suggested that smoked duck products are not microbiologically safe enough for consumption, if they are consumed without additional heating, although smoked, sliced duck could be consumed with additional heating (Kim et al., 2016). Thus, smoked duck-related listeriosis is a valid possibility.

L. monocytogenes is a gram-positive zoonotic pathogen causing listeriosis (Leclercq et al., 2019). According to Leclercq et al (2019), the 19 species consist *Listeria* genus, among which *L. ivanovii* and *L. monocytogenes* are considered pathogenic and only *L. monocytogenes* is considered as a foodborne pathogen, threatening human public health, especially in pregnant women, neonates, immunocompromised patients, and elderly. Although 13 *L. monocytogenes* strain serotypes have been identified, the human listeriosis cases occurred serotype 1/2a, 1/2b, and 4b (Kathariou et al., 2002). The pathogen could survive in foods, including high-salt-containing products at low temperatures. *L. monocytogenes* has been isolated from ready-to-eat (RTE) foods such as frankfurters and deli meat, which contain high fat approximately 30 % (Burall et al., 2012). *L. monocytogenes* was previously considered to most antibiotics to be susceptible (Wieczorek et al., 2012) but Oh et al (2018) suggested that recent studies have reported its resistance against amoxicillin, ampicillin, chloramphenicol, erythromycin, gentamicin, vancomycin, and, tetracycline. A previous study described the analysis of 400 dairy products, meat products, seafood, RTE foods, and fresh vegetables in Taipei identified *Listeria* isolates in these products resistant to chloramphenicol (3.70 %), tetracycline (1.96 %), and penicillin (7.58 %) (Wang et al., 2012).

However, studies on antibiotic-resistant *L. monocytogenes* are still limited in Korea (Oh et al., 2018).

L. monocytogenes reportedly possesses low thermotolerance, Linton et al (1992) reported that *L. monocytogenes* with increased heat resistance. Recently, the heat resistance of *Listeria* spp. has increased compared to other bacteria. Grutler et al (2011) suggested that inactivation of *Salmonella enteritidis* in 10 % salted egg yolk requires a minimum heating temperature of 63.3 ℃ for a minimum duration of 3.5 min, according to the current USDA's regulatory requirement. In 2014, the average pasteurization temperature (time) were 64.4 ℃ (4.4 min) for egg yolks for the thermal inactivation of *Listeria* species in the egg products, according to the survey of the egg industry.

Therefore, the objective of this study was to isolate *L. monocytogenes* from smoked ducks in Korea and to determine the serotypes, antibiotic susceptibility profiles, as well as the heat resistance of the isolates.

Materials and Methods

Isolation and enumeration of *L. monocytogenes*. Twelve smoked duck samples were collected from local supermarkets in Seoul, Korea. For qualitative analysis, smoked duck slices were aseptically removed from the packages, and 25 g slices were transferred aseptically into a sample bag (3M, St. Paul, MN, USA), with 50 mL Listeria enrichment broth (LEB; Becton Dickinson and Company, Sparks, MD, USA). The samples were homogenized for 1 min at high speed in a stomaker (BagMixer® 400, Interscience, Saint Nom, France), followed by incubation at 30 ℃ for 24 h. After, 1 mL of primary enrichment was cultured in Fraser broth (Becton Dickinson and Company, Sparks, MD, USA) containing Fraser broth supplement (Becton, Dickinson and Company) at 37 ℃ for 24 to 48 h. The tubes that turned

dark black were streaked on Palcam agar (Oxoid Ltd.) and inoculated at 30 ℃ for 24 to 48 h in order to isolate colonies with black circles.

Identification of *L. monocytogenes*. Single isolated colony on Palcam agar was aseptically transferred into 10 mL tryptic soy broth containing 0.6 % yeast extract (TSBYE), and cultured at 30 ℃ for 24 h. One-milliliter of the cultures were transferred into microtubes and centrifuged at 8,000×g for 3 min, and the resulting supernatants were discarded. The pellet was suspended with 0.1 mL of dH2O and then heated at 100 ℃ for 10 min to be used as the DNA template. After adding 0.5 μM primer *hlyA* (F: 5'CCTAACATATCCAGGTGCTCTC 3' R: 5'CTGATTGCGCCGAAGTTTAC 3', described in Table 1, following Burall et al., 2011) and 2 μL of DNA template to 20 μL of a phage hot start II DNA polymerase kit (Thermo Fisher, Waltham, MA, USA), withho Taq DNA polymerase (pH = 7.4 at 25 ℃; 50 % glycerol; 1 mM DTT; 20 mM Tris-HCl; 0.1 mM EDTA; 100 mM KCl; and 200 μg/mL BSA), 1× reaction buffer, and 200 μM dNTPs (dNTP; Promega Corporation, Madison, USA), the PCR reaction was performed. For the hlyA amplification, after an initial stage of heating at 98 ℃ for 30 s, the amplification conditions were performed that denaturation at 98 ℃ for 5 s, annealing at 60 ℃ for 5 s, and extension at 72 ℃ for 10 s(35 cycles). A final extension was performed at 72 ℃ for 1 min. The amplified products were followed by electrophoresis using a 1.5 % agarose gel. To identify *L. monocytogenes*, by 16S rRNA sequencing the *hlyA*-positive samples were further analyzed. The 16S rRNA sequencing were performed using the primers 27F (5'AGAGTTTGATCMTGGCTCAG3') and 1492R (5'TACGGYTACCTTGTTACGACTT 3') (Lane. 1991). The PCR reaction was conducted with 20 ng genomic DNA template in a 30-μl reaction mixture using EF-Taq (Solgent, Daejeon, Korea). The Taq polymerase was activated at 95 ℃ for 2 min, performed by 35 cycles of 95 ℃ for 1 min, 55 ℃ for 1 min, and 72 ℃ for 1 min, and a final stage of 72 ℃ for

10 min. Using a multiscreen filter plate (Millipore Corp., Bedford, MA, USA) the amplified products were purified and then added to Hi-Di formamide (Applied Biosystems, Foster City, CA, USA). The mixed products were stored at 95 ℃ for 5 min and on ice for 5 min, and then analyzed using an ABI Prism 3730XL DNA analyzer (Applied Biosystems).

PCR analysis of virulence factors. Five virulence genes (*plcB, inlA, inlB, hlyA,* and *actA*) were detected with the primers using PCR analysis (Table 2). Among the isolated colonies from the Palcam agar plates. The isolated colonies on the plates were suspended in 50 μL of 0.05N NaOH (Samchun, Gyeonggi, Korea) added 0.25 % sodium dodecyl sulfate (SDS). The suspension was then suspended with 100 μl of sterile dH2O and inoculated at 99 ℃ for 15 min. For the PCR amplification, using Phire Hot Start II DNA Polymerase Kit (Thermo Fisher, Waltham, MA, US). This mixture (2 μL) was added 0.5 μM each primer, Taq DNA polymerase, and 1× reaction buffer (200 μM dNTP, 1.5 mM MgCl2). The PCR reaction was performed with initial denaturation at 98 ℃ for 30 s, followed by 98 ℃ for 5 s, 60 ℃ for 5 s, and 72 ℃ for 10 s(35 cycles), and a final extension at 72 ℃ for 1 min using Rotor-Gene Q (Qiagen). For the detection of the products, 20 μl of the PCR reactions were added 4 μl of loading star (Dyne Bio, Gyeonggi, Korea), followed by electrophoresis using a 1.5 % agarose gel.

Isolate serotyping. In order to identify the serotypes, both antigen-antibody agglutination assay (Denka Seiken, Tokyo, Japan) and multiplex PCR with five primers (*prs, lmo0737, lmo1118, ORF2819, ORF2110*) were performed. The isolates were cultured in 10 mL TSBYE and inoculated at 30 ℃ for 24 h. To increase the activity of the strain, 0.1 mL of the culture was inoculated into 10 mL fresh TSBYE and inoculated at 30 ℃ for 24 h. The cultures were streaked onto brain heart infusion agar (BHI agar; Becton Dickinson and Company, Sparks,

MD, USA) and inoculated at 30 ℃ for 24 h. One isolated colony was suspended in 0.3 mL of 0.2 % sodium chloride solution. The mixed suspension was boiled at 100 ℃ for 30 min and then centrifuged at 1,912×g for 20 min. The pellet that supernatant was removed, was added 0.1 mL of 0.2 % sodium chloride. One drop of OI/II, OV/VI antisera (Denka seiken, Tokyo, Japan) and saline solution were added to the slide glass, mixed with 5 μL of the antigen, and the agglutination was judged to occur within 1 min. When the OI / II antisera were positive, the OI and OIV antisera were used for the agglutination reaction. When the OV/VI serotype was positive, OVI, OVII, OVIII, and OIX antisera were used for the agglutination. To test the agglutination assay for the H antigen, the isolates were cultured in 10 mL TSBYE and inoculated at 30 ℃ for 24 h. One tenth milliliter of the culture was inoculated into 10 mL of fresh TSBYE and inoculated at 30 ℃ for 24 h. The cultures were plated on BHI broth semi-fluid medium with 0.2 % agar in triplicates. The cultures were mixed with the same volume of physiological saline with 1 % formalin and used as an antigen. The antigen (0.3 mL) was dispensed in a test tube, and 2 drops of H antisera (Denka Seiken, Tokyo, Japan) were dropped and placed in a water bath (Wisebath; Wisd Laboratory Instruments, Wertheim, Germany) at 50 ℃ for 1 h. To perform the serotyping multiplex PCR test, 1 colony of the isolates from the Palcam agar was suspended in 50 μl of 0.05N NaOH containing 0.25 % sodium dodecyl sulfate (SDS), heated at 100 ℃ for 15 min, then cooled at room temperature for 2 min. After cooling, the mixture was centrifuged at 14,000 rpm for 3 min. The supernatant was used as template DNA for PCR. The PCR was performed using the Qiagen multiplex PCR kit. Four microliters of primer set, and 19 μL of H2O, and 2 μL of template DNA were added into 25 μL of PCR premix with MgCl2, KCl, dNTPs, and Taq polymerase, and homogenization was initiated. For the *L. monocytogenes* gene of serovar amplification, the first stage was initiated at 94 ℃ for 15 sec, performed by the amplification conditions using denaturation at 94 ℃ for 30 s, annealing at 57 ℃ for 90 s, and extension at 72 ℃ for

60 sec (35 cycles), and then final 30 sec extension was performed at 60 ℃. The amplified products were subjected to electrophoresis on 2 % agarose.

Antibiotic susceptibility examination. To evaluate whether *L. monocytogenes* isolates have antibiotic resistance, an antibiotic susceptibility examination was performed according to the Clinical Laboratory Standards Institute procedure to evaluate antibiotic resistance (CLSI, 2014). A single isolated colony of *L. monocytogenes* on Palcam agar was transferred into 10 mL of TSBYE and inoculated at 30 ℃ for 24 h. After incubation, using 0.5 McFarland opacity standard (Biomerieux, Marcy l'Etotile, France) the cultures were adjusted in phosphate-buffered saline (PBS; pH=7.4, 8.0 g of NaCl, 0.2 g of KCl, 1.5 g of Na2HPO4, 0.2 g of KH2PO4, 1 of distilled water). The sterilized cotton swab containing the suspension was swabbed on the surface of a Muller-Hinton agar (MHA; Becton Dickinson and Company) plate, which was dried. Antibiotic discs (Oxoid, UK) were then placed on a MHA plate using disc dispenser and inoculated at 30 °C for 24 h. Nine antibiotic discs [ciprofloxacin (5 μg), chloramphenicol (30 μg), clindamycin (10 μg), doxycycline (30 μg), erythromycin (15 μg), minocycline (30 μg), penicillin G (10 units), rifampicin (5 μg), and tetracycline (30 μg)] were examined in this study. After the inoculation, the diameters of the inhibition zones were measured. Antibiotic susceptibility profiles of the isolates were classified as either resisant, intermediate, or sensitive, according to the M45 guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2014).

Heat resistance examination. To test the heat resistance, the isolates on Palcam agar were inoculated onto TSBYE and cultured at 30 ℃ for 24 h. One tenth milliliter of the cultures were inoculated into 10 mL fresh TSBYE at 30 ℃ for 24 h. After the incubation, seven cultures were centrifuged at 1,912 ×g and 4 ℃ for 15 min, and then the pellets were resuspended and diluted with PBS in order to obtain an inoculum concentration of 6.5 to 7.5

log CFU/mL. One tenth milliliter of the *L. monocytogenes* suspensions were transferred into 10 mL of fresh TSBYE and heated in a water bath at 60 ℃ for 10 min. In order to quantify the survival, 1 mL of the samples were spread-plated on TSA containing 0.6 % yeast extract (TSAYE) at 0, 2, 5, 8, and 10 min. The TSAYE were inoculated at 30 ℃ for 48 h, and then the colonies were counted manually. D-values (in minutes) were calculated from survivor curves as described in the Laboratory Manual for Food Canners and Processors, volume 1, 1968.

Statistical analysis. The data from the antibiotic susceptibility tests and heat resistance were analyzed with the general linear model of the SAS® program version 9.3 (SAS Institute Inc., Cary). The least square means among sampling times were compared using a t-test at $\alpha = 0.05$.

Results

***L. monocytogenes* prevalence and contaminant level.** Five samples among the total of twelve (41.7 %) showed positive results in the qualitative analysis. These isolates harbored the *hly*A gene (Figure 1) and were eventually identified as *L. monocytogenes* through our 16s rRNA analysis (data not shown).

***L. monocytogenes* virulence characterization.** Five virulence genes (*inl*A, *inl*B, *act*A, *plc*B, and *hly*A) were observed in the *L. monocytogenes* isolates. All strains contained these aforementioned five genes. Interestingly, all seven isolates of this study harbored the 385 bp amplicon of *act*A (Figure 2A-C), a gene which is essential for F-actin assembly and cellular movements, with a length reportedly related to pathogenicity (Wiedmann et al., 1997). A previous study described that the invasion efficiency of *L. monocytogenes* carrying the 385

bp *act*A amplicon was approximately 1 to 2.5 times higher than that of *L. monocytogenes* carrying the 268 bp version of *act*A in healthy cells (Ha et al., 2018). Thus, these results indicate that the isolates from smoked duck products exhibited high pathogenicity.

***L. monocytogene*s isolate serotypes.** The *L. monocytogenes* serotypes were determined using both agglutination assay and multiplex PCR (Oh et al., 2018), and a common serotype from both assays were used as the serotype in Table 3. The serotypes of the isolates were 1/2a (3 cases out of 7, 42.9 %), 1/2b (3 cases out of 7, 42.9 %), and 3b (1 case out of 7, 13.3 %), except for one isolates, which are generally regarded as the most common serotypes of foodborne diseases (Table 3). A previous study suggested that serotype 1/2a has an increased persistence in food and seems to transfer more plasmids, which confer resistance to toxic metals and other compounds (Wu et al., 2015). The serotype 1/2b (42.9 %) was present more often in non-pregnant individuals with insignificant occurrence of severe underlying diseases (McLauchlin. 1990). Finally, serotype 3b, which is not a highly prevalent serotype, exhibited a very high adhesion and invasion efficiency in Caco-2 cells (Jaradat and Bhunia, 2003).

Antibiotic susceptibility. *L. monocytogenes* is sensitive to many of antibiotics. However, the emergence of resistant strains has been recently reported (Wu et al., 2015). All seven isolates of this study were resistant to penicillin G, and three of them were also resistant to clindamycin (Table 4). Several previous studies reported the low prevalence of penicillin G-resistant *L. monocytogenes*. Duck and goose-derived, beef and broiler meat-derived, and beef, pork, or poultry-derived penicillin G-resistant *L. monocytogenes* isolates have been reported in 31.6, 0 to 5, and 0 % of the cases, respectively, in different studies (Jamali et al., 2014). Therefore, the result from our study suggests that the previously reported *L. monocytogenes* penicillin G-resistance has obviously increased. A previous study suggested that the

234 resistance of gram-positive bacteria, including *L. monocytogenes*, to β-lactam antibiotics (e.g.,
235 penicillin) might be associated with a reduced antibiotic affinity of the penicillin-binding
236 proteins (PBPs) (Malouin et al., 1986). In addition, Thedieck et al (2006) suggested that *L.*
237 *monocytogenes* activates the antimicrobial peptide sensor (APS) system when it detects a
238 treat to antibiotics and that active efflux systems might contribute to the adaptation of Listeria
239 to these environmental challenges. The use of β-lactam antibiotics (e.g., penicillin) shows a
240 global increase. Penicillin, a β-lactam antibiotic, has been reported as the first-line listeriosis
241 treatment in immunocompromised patients (Hof, 2003). These results suggest that the
242 resistance against β-lactam antibiotics has increased, and thus, the use of these antibiotics
243 should be reduced. Table 4 shows that certain *L. monocytogenes* isolates were resistant to
244 clindamycin (3 cases out of 7, 42.9 %). The high prevalence of the clindamycin-resistant *L.*
245 *monocytogenes* isolates is consistent with a previous report (Wieczorek et al., 2012). Another
246 study also showed that 35 % of the *L. monocytogenes* isolates from poultry, pork, and beef
247 were resistant to clindamycin (Gómez et al., 2014). Clindamycin binds to the ribosome (50S
248 subunit) of bacteria and inhibits synthesis of protein (Depardieu et al., 2007). The results of
249 this study suggest the emergence of multi-resistant strains in smoked ducks, representing a
250 potential threat to human health.
251
252 **Heat resistance.** In this study, five isolates showed thermal resistance, compared to the
253 standard strain *L. monocytogenes* ATCC13932, after 60 ℃ of heat exposure for 10 min, and
254 three of these isolates decreased to less than 1.7 log CFU/mL (63.6 % survival) ($p<0.05$), and
255 they all represented serotype 1/2b (Figure 3). This result suggests that the *L. monocytogenes*
256 serotype 1/2b exhibits high heat resistance at 60 ℃, compared to other serotypes. Such
257 thermal tolerance at 60 ℃ has also been observed in the case of the serotype 1/2b among
258 13 *L. monocytogenes* serotypes *(*Shen et al., 2014). The *L. monocytogenes* D-value at 60 ℃

was reportedly 3.10 min (Dogruyol et al., 2020). However, the D-values at 60 ℃ of three isolates (SMFM2018 SD 5-3, SMFM2018 SD 6-2, and SMFM2018 SD 7-1) in our study were 5.41, 6.48, and 6.71, respectively (p<0.05), as summarized in Table 5. Therefore, compared to the other studies, these *L. monocytogenes* isolates exhibited higher heat resistance

Discussion

In conclusion, this study showed a high smoked duck product-derived *L. monocytogenes* prevalence in Korea. In addition, all seven *L. monocytogenes* isolates, presented in this study, were resistant to penicillin G and certain strains were even resistant to clindamycin as well. These strains possessed the longer, 385-bp amplicon of the *act*A gene. We found that three of all the isolates were heat resistant and they identified as serotype 1/2b. Thus, 1/2b might exhibit better heat resistance than other serotypes. These results indicated that the risk of *L. monocytogenes* contamination in smoked ducks could be considered high due to antibiotic resistance, invasion efficiency, and the heat resistance of the 1/2b serotype.

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478 **Table 1.** Primers used for the identification of the *Listeria monocytogenes* isolates

Primer	Size (bp)	Sequence (5′ to 3′)	Reference
*hly*A	350	F : CCTAACATATCCAGGTGCTCTC R : CTGATTGCGCCGAAGTTTAC	Burall et al., 2011

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480 **Table 2.** Primers used for the PCR amplification of the virulence genes from the *Listeria*

481 *monocytogenes* isolates

Primer	Size (bp)	Sequence (5′ to 3′)	Reference
inlA	255	F : CCTAGCAGGTCTAACCGCAC R : TCGCTAATTTGGTTATGCCC	Mathakiya et al., 2009
inlB	146	F : AAAGCACGATTTCATGGGAG R : ACATAGCCTTGTTTGGTCGG	Corantin *et al*., 2005
actA	268 (385)	F : GACGAAAATCCCGAAGTGAA R : CTAGCGAAGGTGCTGTTTCC	Jaradat et al., 2002
plcB	261	F : GGGAAATTTGACACAGCGTT R : ATTTTCGAAGGTAGTCCGCTTT	Corantin *et al*., 2005
hlyA	174	F : GCATCTGCATTCAATAAAGA R : TGTCACTGCATCTCCGTGGT	Wesley et al., 2002

482 **Table 3.** Serotypes and information of the *Listeria monocytogenes* isolates from smoked
483 ducks

Strains	Agglutination assay	Multiplex PCR	Final serotype
SMFM2018 SD 1-1	1/2a	1/2a, 3a	1/2a
SMFM2018 SD 4-1	1/2a	1/2a, 3a	1/2a
SMFM2018 SD 4-2	1/2a	1/2a, 3a	1/2a
SMFM2018 SD 5-2	1/2b	1/2b, 3b, 7	1/2b
SMFM2018 SD 5-3	1/2b	1/2b, 3b, 7	1/2b
SMFM2018 SD 6-2	1/2b	1/2b, 3b, 7	1/2b
SMFM2018 SD 7-1	3b	1/2b, 3b, 7	3b

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485 **Table 4.** Antibiotic sensitivity of *Listeria monocytogenes* isolates from smoked ducks (n = 7)

Antibiotics	*L. monocytogenes* isolates							Sensitivity of the isolates n (%)[1)]		
	SMFM 2018 SD 1-1	SMFM20 18 SD 4-1	SMFM20 18 SD 4-2	SMFM20 18 SD 5-2	SMFM20 18 SD 5-3	SMFM20 18 SD 6-2	SMFM20 18 SD 7-1	Susceptible	Intermediate	Resistant
Ciprofloxacin	S	S	S	S	S	S	S	7 (100)		
Clindamycin	R	I	R	I	I	R	I		4(57.1)	3(42.9)
Chloramphenicol	S	S	S	S	S	S	S	7 (100)		
Doxycycline	S	S	S	S	S	S	S	7 (100)		
Erythromycin	S	S	S	S	S	S	S	7 (100)		
Minocycline	S	S	S	S	S	S	S	7 (100)		
Penicillin G	R	R	R	R	R	R	R			7 (100)

Rimfampicin	S	S	S	S	S	S	S	7 (100)		
Tetracycline	S	S	S	S	S	S	S	7 (100)		
Susceptible	7 (77.8 %)	7 (77.8 %)	7 (77.8 %)	7 (77.8 %)	7 (77.8 %)	7 (77.8 %)	7 (77.8 %)			
Intermediate	0	1 (11.2 %)	0	1 (11.2 %)	1 (11.2 %)	0	1 (11.2 %)			
Resistant	2 (22.3 %)	1 (11.2 %)	2 (22.3 %)	1 (11.2 %)	1 (11.2 %)	2 (22.3 %)	1 (11.2 %)			

1) According to the CLSI guidelines using the breakpoints of *Staphylococcus* species resistance due to the lack of resistance criteria for *Listeria* susceptibility testing in the CLSI guidelines (Vasconcelos Byrne et al., 2016)

489 **Table 5.** D-values (mean± SD) of *Listeria monocytogenes* from smoked duck in TSBYE

490 at 60 °C

L. monocytogenes strains	D_{60} (min)	Serotype
ATCC13932	2.47±0.2	4b
SMFM2018 SD 1-1	3.44±0.1	1/2a
SMFM2018 SD 4-1	3.68±0.6	1/2a
SMFM2018 SD 4-2	2.67±0.2	1/2a
SMFM2018 SD 5-2	5.41±0.6*	1/2b
SMFM2018 SD 5-3	6.48±2.2*	1/2b
SMFM201803 SD 6-2	6.71±0.4*	1/2b
SMFM2018 SD 7-1	3.07±0.9	3b

491 * Statically significant, compared to the standard strain by pairwise t-test ($p < 0.05$)

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