Research Note

Prevalence and Antimicrobial Susceptibility of *Listeria monocytogenes* on Chicken Carcasses in Bandung, Indonesia

YONI DARMAWAN SUGIRI,¹ GRETA GÖLZ,² TONGKORN MEEYAM,³ MAXIMILIAN P. O. BAUMANN,⁴ JOSEF KLEER,² WARANGKHANA CHAISOWWONG,³ AND THOMAS ALTER²*¹

¹Veterinary Public Health Centre for Asia Pacific, Faculty of Veterinary Medicine, Chiang Mai University, Mae Hia, Muang, Chiang Mai 50100, Thailand; and Balai Pengujian dan Penyidikan Penyakit Hewan dan Kesmasvet (Animal Health and Veterinary Public Health Laboratory), West Java Livestock Services, Jl. Tangkuban Parahu KM. 22 Cikole Lembang, Kab. Bandung Barat 40391, Jawa Barat, Indonesia; ²Institute of Food Hygiene and ³International Animal Health, Department of Veterinary Medicine, Freie Universität Berlin, Königsweg 67, 14163 Berlin, Germany; ⁴Department of Veterinary Biosciences and Veterinary Public Health, Faculty of Veterinary Medicine, Chiang Mai University, Mae Hia, Muang, Chiang Mai 50100, Thailand

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**ABSTRACT**

This study was conducted to determine the prevalence and quantify the number of *Listeria monocytogenes* in fresh chicken carcasses sold in traditional markets and supermarkets in Bandung, West Java, Indonesia, and to determine the antimicrobial resistance patterns of the isolated *L. monocytogenes* strains. The overall prevalence of *L. monocytogenes* in chicken carcasses was 15.8% (29/184). When comparing samples from traditional markets and supermarkets, no significant difference in the *L. monocytogenes* prevalence was detectable (15.2 versus 16.3%). Of the samples, 97.3% had *L. monocytogenes* counts <100 CFU/g, 2.2% had *L. monocytogenes* counts between 101 and 1,000 CFU/g, and 0.5% had *L. monocytogenes* counts of 1,001 to 10,000 CFU/g. Of the isolates, 27.6% were resistant to at least one of the 10 antimicrobials tested, with the major resistant phenotypes to penicillin (17.2%), ampicillin (6.9%), and erythromycin (6.9%). All 29 isolates recovered in this study were grouped into the molecular serogroup IIb, comprising the serovars 1/2b, 3b, and 7.

*Listeria monocytogenes* has been largely studied in the last decades due to its importance as a foodborne human pathogen with high mortality rates on susceptible hosts. Most human *L. monocytogenes* cases are associated with the consumption of ready-to-eat food (5). Nonetheless, this pathogen can contaminate numerous food products, e.g., milk and dairy products, seafood, vegetables, and meat, including poultry and poultry products (1, 5). Some studies already investigated the prevalence of *L. monocytogenes* in chicken carcasses or fresh chicken meat, ranging from 5.7 to 70% (11, 20, 29). Contamination usually occurs during the slaughter and processing steps (by contamination during processing or by introduction of *L. monocytogenes*-carrying chicken into the slaughter premises). Few data are available on its prevalence in chicken farms. Aury et al. (1) demonstrated a *L. monocytogenes* prevalence of 31.7% at the broiler flock level. These authors identified several risk factors related to hygienic and management practices (e.g., pest control, disinfection method, litter storage, and separation of clean and dirty entrances) for *L. monocytogenes* contamination in these broiler flocks. The levels of contamination on raw chicken meat or carcasses are mostly <100 CFU/g, but levels of around 4 log CFU/g are occasionally reported (23). Although the heating process will eliminate *L. monocytogenes*, ready-to-eat poultry products can become recontaminated during production and processing.

A majority of the *L. monocytogenes* strains isolated from humans and food belong to serovars 1/2a, 1/2b, 1/2c, and 4b, with serovar 1/2a dominating in food (4, 19). In Indonesia broiler meat is considered an important source of animal protein for human nutrition, with an annual production of 1,540,000 metric tons (1,540,000,000 kg) in 2012 (27). To our knowledge, no data are available on the prevalence of *L. monocytogenes* in chicken flocks or chicken meat in Indonesia. In addition, neither information on the exposure of consumers to *L. monocytogenes* nor the listeriosis incidence in humans in this country is available.

To generate baseline data for *L. monocytogenes* contamination in chicken carcasses, this study was conducted to (i) identify the prevalence and to quantify *L. monocytogenes* in fresh broilers at Bandung City, West Java Province, Indonesia, to (ii) characterize the antimicrobial resistance patterns of the isolated *L. monocytogenes* strains, and to (iii) identify these isolates according to serogroup by molecular methods.

**MATERIALS AND METHODS**

Sample collection. A total of 184 raw chicken carcass samples were collected in Bandung, Indonesia, between November 2013 and 2014.
2012 and February 2013. The sample size was calculated by Win Episcope 2.0 (26), assuming 200,000 kg of fresh poultry products sold daily in Bandung (28), with an expected prevalence of 13.9% (8), 95% desired precision (confidence interval), and 5% accepted error. These samples consisted of 92 samples taken from 12 traditional markets and 92 samples from 12 supermarkets. Because no data are available on the proportion of traditional markets versus supermarkets in Bandung, that equal representation was applied. Sampling was distributed equally over the whole area of six cities of Bandung. Two traditional markets and two supermarkets within each area were randomly chosen for sample collection. Each market was visited once, and eight samples per market were taken by convenient sampling. After sampling, carcasses were transported to the laboratory aseptically in cool boxes. The samples were analyzed immediately. If the samples were not analyzed on the day of arrival at the laboratory, they were kept in a refrigerator (2 to 6°C) for not more than 24 h before analysis.

**Detection of L. monocytogenes.** The isolation and identification of L. monocytogenes was done according to ISO 11290-1:1996 (9), with modifications. Briefly, 10 g of each sample (chicken skin from neck and breast) were enriched in 90 ml of Half Fraser broth (Merck, Darmstadt, Germany) and incubated aerobically at 30°C for 24 h. The enriched sample (100 μl) was transferred to 10 ml of Fraser broth (Merck) and incubated aerobically at 37°C for 48 h. The enriched samples were streaked onto Chromocult Listeria selective agar and Palcam agar (both from Merck) and incubated aerobically at 37°C for 24 to 48 h. Presumptive L. monocytogenes colonies were subcultured on tryptone soya yeast extract agar (Merck) for confirmation by biochemical and Christie Atkins Munch-Peterson tests. All L. monocytogenes strains isolated in this study were stored in brain heart infusion broth (BD, Jakarta, Indonesia) with 20% glycerol (Merck) and kept at −70°C for further studies.

**Enumeration of L. monocytogenes.** The enumeration of L. monocytogenes was done according to ISO 11290-2:1998 (10), with modifications. Briefly, to revitalize stressed listeriae, the initial suspension (used for detection) of 10 g sample in 90 ml of Half Fraser broth was kept at room temperature for 1 h. From that suspension, a 100-μl aliquot was used to create a 10-fold dilution series. For plating, 100 μl of the individual dilution was spread onto Chromocult Listeria selective agar and Palcam agar and incubated aerobically at 37°C for 24 to 48 h. Presumptive L. monocytogenes colonies were counted, and three to five colonies per plate were subcultured on tryptone soya yeast extract agar and confirmed by biochemical and Christie Atkins Munch-Peterson tests.

**Antimicrobial susceptibility test.** One L. monocytogenes isolate per sample (isolated by the detection method) was tested by the standard disc diffusion method described by Clinical and Laboratory Standards Institute (2) on Mueller Hinton agar (Merck) incubated at 37°C for 24 h. The antibiotic discs that were used in this study were ampicillin (10 μg), neomycin (10 μg), erythromycin (15 μg), penicillin (10 μg), ciprofloxacin (10 μg), streptomycin (10 μg), sulfamethoxazole-trimethoprim (23.75/1.25 μg), kanamycin (30 μg), tetracycline (30 μg), and gentamycin (10 μg; all from OXOID, Basingstoke, UK). *Staphylococcus aureus* ATCC 25923 was used as control strain (Clinical and Laboratory Standards Institute) (2).

**Molecular serotyping of L. monocytogenes isolates.** For DNA extraction, strains were recovered by streaking on 5% sheep blood agar (Oxoid) and Trypticase soya extract agar and grown overnight at 37°C. The colonies were scraped and suspended in 500 μl of sterile 1 x Tris-EDTA, mixed and boiled for 10 min. Molecular serogrouping was carried out by PCR reactions, using protocols and primers described by Dounith et al. and Kérouant et al. (4, 13).

**Data analysis.** Data analysis was done using “R” free statistical and data analysis software (21). Descriptive statistics were used to report the result of prevalence and enumeration analysis. The prevalences of L. monocytogenes between traditional markets and supermarkets were compared using a chi square test. To compare the quantitative load of traditional versus supermarket samples, data were log transformed, and calculation of statistical significance was performed with GraphPad Prism v5 (GraphPad Software, La Jolla, CA) using the unpaired t test with Welch's correction.

**RESULTS AND DISCUSSION**

The overall prevalence of L. monocytogenes in fresh chicken meat samples was 15.8% (29 of 184; 95% confidence interval: 10.49 to 21.02). This is in general agreement with regional data. For instance, Indrawattana et al. (8) detected a L. monocytogenes prevalence of 13.9% in fresh poultry meat in Thailand. Similar data are available from Pakistan (prevalence of 17.5%) (16) and Malaysia (prevalence of 20%) (7). In contrast, lower prevalences of 5.7% were observed in raw chicken meat in China (29) and in fresh and frozen chicken meat in Thailand (2.5%) (12).

When comparing samples taken from traditional markets versus supermarkets, L. monocytogenes was detected and isolated from 14 of 92 samples from traditional markets (15.2%; 95% confidence interval: 7.88 to 22.6), while 15 of 92 samples from supermarkets were positive for L. monocytogenes (16.3%; 95% confidence interval: 8.76 to 23.85). Even though the prevalence of L. monocytogenes from supermarkets was slightly higher than the prevalence from traditional markets, these differences were not significant (P = 0.839). In contrast, Goh et al. (7) detected a significantly higher prevalence of L. monocytogenes in chicken meat taken from supermarkets (25.71%) compared with traditional markets (14.29%). These authors speculate that the difference might be explained by longer holding times of chicken meat in supermarkets.

Of the samples, 97.3% had L. monocytogenes counts <100 CFU/g, 2.2% had L. monocytogenes counts between 101 and 1,000 CFU/g and 0.5% had L. monocytogenes counts of 1,001 to 10,000 CFU/g. The samples containing >100 CFU/g (n = 5) originated exclusively from traditional markets. This quantitative distribution is in agreement with an older study by Rorvik and Yndestad (24), where most of the positive samples contained <100 CFU/g, but counts of >1,000 CFU/g were detectable in a small portion of broiler carcass samples. Only limited quantitative data are available from this region: low L. monocytogenes loads in raw chicken meat and chicken offal were observed by Goh et al. and Kuan et al. in Malaysia with <3 to 93 most probable number/g (7, 14).

When comparing the quantitative load of L. monocytogenes-positive samples taken from traditional markets...
versus supermarkets, the mean load of samples taken from
traditional markets (1.286 log CFU/g) was significantly
higher than the mean load of samples taken from
supermarkets (0.773 log CFU/g; P < 0.05). Chicken
carcasses from traditional markets originated from small-
scale slaughterhouses that usually apply substandard
hygienic practices during the slaughter process. In contrast,
all supermarkets received poultry carcasses and fresh
poultry meat from registered medium- to high-scale poultry
slaughterhouses that apply good hygienic and manufactur-
ing practices. Unhygienic conditions during slaughter and
processing can lead to higher L. monocytogenes loads, as
already described by Cox et al. (3).

Most L. monocytogenes strains in our study displayed
sensitivity to the antimicrobial substances tested. Nonethe-
less, 17.2% were resistant to penicillin, 6.9% to ampicillin,
6.9% to erythromycin, and 3.4% to a combination of
ampicillin and penicillin (Table 1). This is in agreement
with previous studies that reported a substantial share
of penicillin-resistant or nonsusceptible strains (22, 25).
Resistances against ampicillin and erythromycin have also
been reported by Rodaz-Suarez et al. (22). A Chinese study
on L. monocytogenes strains originating from different retail
foods demonstrated a high resistance to oxacillin (46.2%),
followed by tetracycline (12.8%), erythromycin (10.3%),
trimethoprim-sulfamethoxazole (7.7%), chloramphenicol
(5.1%), and vancomycin (2.6%). All isolates were sensitive
displayed intermediate resistance to gentamycin, ampi-
cillin, and ciprofloxacin (29).

In all 29 isolated L. monocytogenes strains, prs, prfA,
and ORF2819 were detectable by PCR. In none of the 29
isolates was lmo0737, lmo1118, ORF2110, and flaA
detectable. When using the molecular serogrouping pro-
pessed by Doumith et al. and Kérouantou et al. (4, 13), all L.
monocytogenes isolates from this study were grouped into
the molecular serogroup IIb, comprising serotypes 1/2b, 3b,
and 7, with serotypes 1/2b and 3b belonging to lineage I. In
contrast, in most studies that investigated poultry meat,
serotype 1/2a dominated (17, 20), but other serotypes were
detectable as well, such as 1/2b, 1/2c, and 4b (6, 8, 15, 18).

Similar to our data, Zhang et al. (30) demonstrated that
serogroups 1/2b and 3b dominated (50%) in L. monocytogenes
strains originating from conventional retail chicken,
followed by serogroups 4b, 4d, and 4e.

In conclusion, the results of this study demonstrate a
L. monocytogenes prevalence of 15.8% on fresh chicken
carcasses at retail level in Bandung, Indonesia. Raw chicken
carcasses might act as a source of L. monocytogenes cross-
contamination during processing or preparation of other
foods. Such prevalence data on L. monocytogenes in other
types of food (including ready-to-eat food) are needed for
estimating the exposure of consumers to L. monocytogenes
in Indonesia, especially in Bandung.

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**TABLE 1. Antimicrobial resistance patterns of L. monocytogenes isolates (n = 29)**

<table>
<thead>
<tr>
<th>Antimicrobial agent (µg)</th>
<th>Resistant (%)</th>
<th>Intermediate (%)</th>
<th>Susceptible (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin (10)</td>
<td>6.9</td>
<td>0</td>
<td>93.1</td>
</tr>
<tr>
<td>Neomycin (10)</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Erythromycin (15)</td>
<td>6.9</td>
<td>3.4</td>
<td>90.1</td>
</tr>
<tr>
<td>Penicillin (10)</td>
<td>17.2</td>
<td>0</td>
<td>75.9</td>
</tr>
<tr>
<td>Ciprofloxacin (10)</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Streptomycin (10)</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Sulfamethoxazole-trimethoprim (25)</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Kanamycin (30)</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Tetracycline (30)</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Gentamycin (10)</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Ampicillin (10) + penicillin (10)</td>
<td>3.4</td>
<td>0</td>
<td>96.6</td>
</tr>
</tbody>
</table>