CORRESPONDING AUTHOR

Judul :

Phylogenetic Analysis and Antibiotics Resistance of Listeria MonocytogenesContaminating Chicken Meat in Surabaya, Indonesia

Penulis Utama dan Corresponding Author : Eduardus Bimo

Nama Jurnal : Veterinary Medicine International. Vol. 2020. Tahun 2020

Bukti:

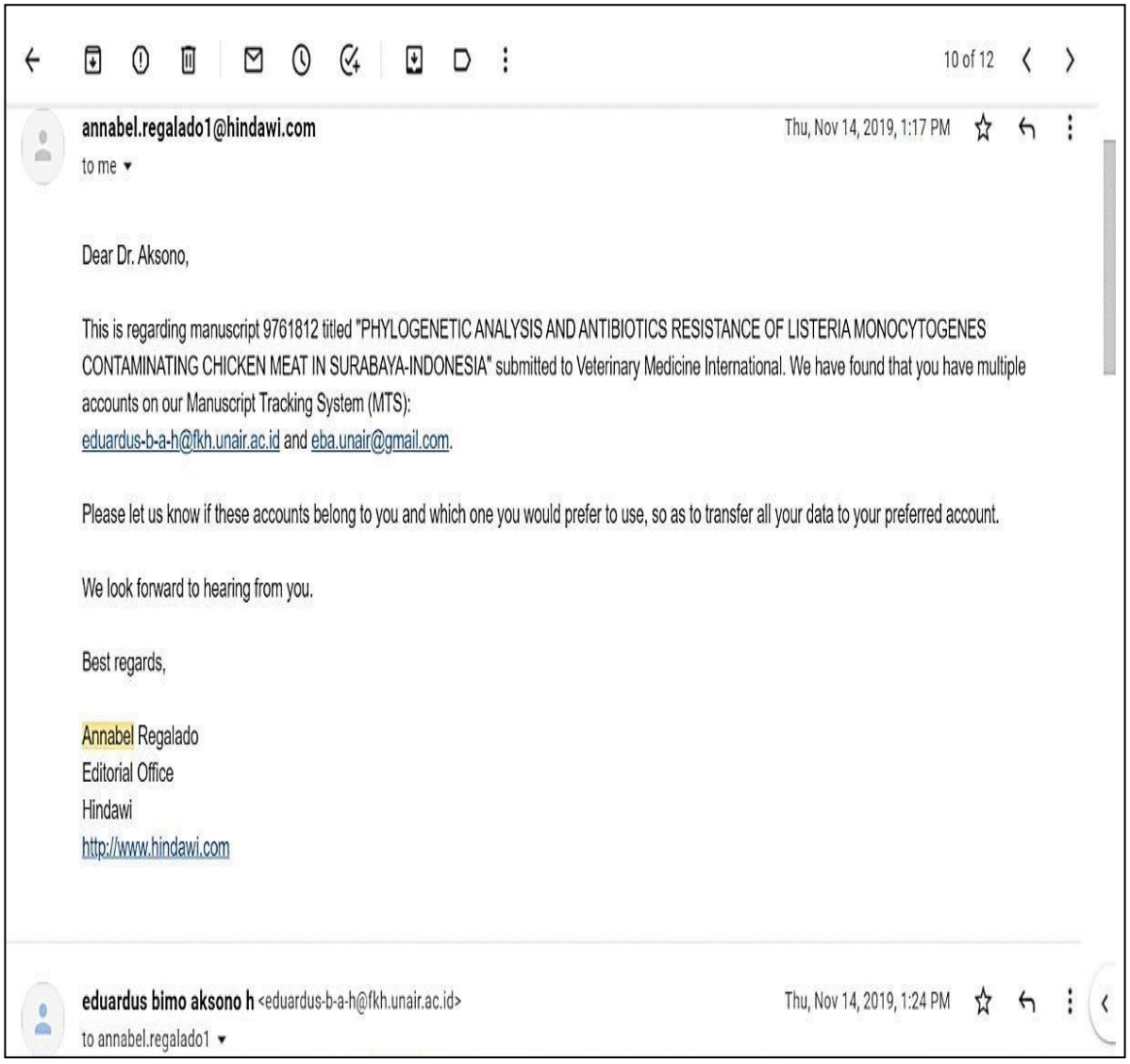
COVER LETTER FOR SUBMISSION OF NEW MANUSCRIPTS

Dear Reviewers and Editor,

I am enclosing herewith a manuscript entitled "**Phylogenetic Analysis and Antibiotics Resistance of *Listeria Monocytogenes* Contaminating Chicken Meat In Surabaya-Indonesia**" for possible evaluation and publication in your journal, after some revisions based on Reviewers comments and corrections

With the submission of this manuscript I would like to undertake that the above mentioned manuscript has not been published elsewhere, accepted for publication elsewhere or under editorial review for publication elsewhere; and that my Institute's **Airlangga University** representative is fully aware of this submission.

Best Regards
Eduardus Bimo
Airlangga University - Indonesia



10 of 12

annabel.regalado1@hindawi.com Thu, Nov 14, 2019, 1:17 PM

to me

Dear Dr. Aksono,

This is regarding manuscript 9761812 titled "PHYLOGENETIC ANALYSIS AND ANTIBIOTICS RESISTANCE OF LISTERIA MONOCYTOGENES CONTAMINATING CHICKEN MEAT IN SURABAYA-INDONESIA" submitted to Veterinary Medicine International. We have found that you have multiple accounts on our Manuscript Tracking System (MTS):
eduardus-b-a-h@fkh.unair.ac.id and eba.unair@gmail.com.

Please let us know if these accounts belong to you and which one you would prefer to use, so as to transfer all your data to your preferred account.

We look forward to hearing from you.

Best regards,

Annabel Regalado
Editorial Office
Hindawi
http://www.hindawi.com

eduardus bimo aksono h <eduardus-b-a-h@fkh.unair.ac.id> Thu, Nov 14, 2019, 1:24 PM

to annabel.regalado1

PHYLOGENETIC ANALYSIS AND ANTIBIOTICS RESISTANCE OF LISTERIA MONOCYTOGENES CONTAMINATING CHICKEN MEAT IN SURABAYA-INDONESIA

Eduardus Bimo Aksono[1], Katty Hendriana Priscilia Riwu[1], A. T. Soelih Estoepangestie[1], herinda pertiwi SA CA [2] + Show Affiliations

Article Type
Research Article

Journal
Veterinary Medicine International

Ortega-Pacheco Antonio

Submitted on 2019-11-14 (4 years ago)

Decision

Antonio Ortega-Pacheco 15.01.2020

Revision requested

Message for Author

major

— Reviewer Reports

1 submitted

Report

Reviewer 1 04.01.2020

The works fall perfectly within the journal scope. In my opinion, this manuscript should be accepted after major corrections according to the following comments: #1 Authors should explain more details about samples collection, particularly on the consideration of selecting places (supermarkets, mobile vendors, and traditional markets) of which samples were collected. It should be clearly explained how the authors chose chicken meat samples as the representatives of Surabaya and why did the samples from those places have been selected? #2 Authors should clearly explain the reason of using Ampicillin, Amoxylin sulbactam, Cefotaxime, Meropenem, Sulfamethonazole-trimetophrim for antibiotics resistance test. Consider the recent and up-to-date references list of antibiotics used for antibiotics resistance test. #3 The results are interesting but not well explained. Discussion is still needed to be strengthened. Authors should improve relevance of discussion and provide several previous similar researches to discuss the results more details and comprehensive. The relevance and up-to-date references should be cited to support the discussion and also introduction sections. Update and an increase of citations from the last 3-4 years would be welcome. The topic of this manuscript falls within the scope of Veterinary Medicine International

PHYLOGENETIC ANALYSIS AND ANTIBIOTICS RESISTANCE OF LISTERIA MONOCYTOGENES CONTAMINATING CHICKEN MEAT IN SURABAYA-INDONESIA

Eduardus Bimo Aksono[1], Katty Hendriana Priscilia Riwu[1], A. T. Soelih Estoepangestie[1], herinda pertiwi SA CA [2] + Show Affiliations

Article Type

Research Article

Journal

Veterinary Medicine International

Ortega-Pacheco Antonio

Submitted on 2020-01-19 (3 years ago)

Decision Antonio Ortega-Pacheco 31.01.2020

Revision requested

Message for Author

minor

— Response to Revision Request

Your Reply 07.04.2020

— Reviewer Reports 1 submitted

Report Reviewer 1 25.01.2020

Improvements are still needed for the manuscript according to the following comments. #1 Authors should explain more details about samples collection, particularly on the consideration of selecting places (supermarkets, mobile vendors, and traditional markets) of which samples were collected. They can explain it in materials and methods' section, since it should be clearly explained how the authors chose chicken meat samples as the representatives of Surabaya and why did the samples from those places have been selected? #2 Language used in the manuscript is still not satisfactory and require extensive revision. The topic of this manuscript falls within the scope of Veterinary Medicine International

PHYLOGENETIC ANALYSIS AND ANTIBIOTICS RESISTANCE OF LISTERIA MONOCYTOGENES CONTAMINATING CHICKEN MEAT IN SURABAYA-INDONESIA

PUBLISHED

ID 9761812

Eduardus Bimo Aksono[1], Katty Hendriana Priscilia Riwu[1], A. T. Soelih Estoepangestie[1], herinda pertiwi SA CA [2] + Show Affiliations

Article Type

Research Article

Journal

Veterinary Medicine International

Ortega-Pacheco Antonio

Submitted on 2020-02-04 (3 years ago)

> Abstract

> Author Declaration

> Files 2

Files 2

Editorial Comments

Decision

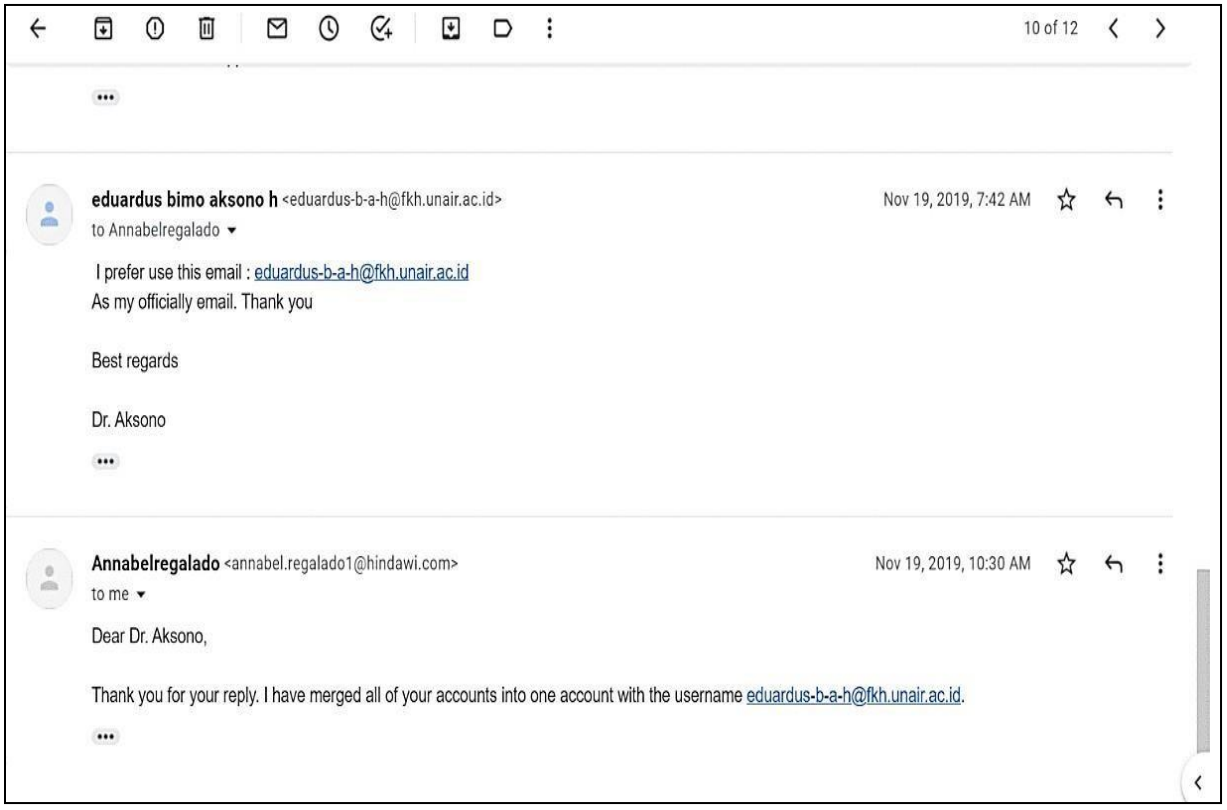
Publish

Antonio Ortega-Pacheco 05.02.2020

Response to Revision Request

Your Reply

07.04.2020



10 of 12

eduardus bimo aksono h <eduardus-b-a-h@fkh.unair.ac.id> Nov 19, 2019, 7:42 AM
to Annabelregalado

I prefer use this email : eduardus-b-a-h@fkh.unair.ac.id
As my officially email. Thank you

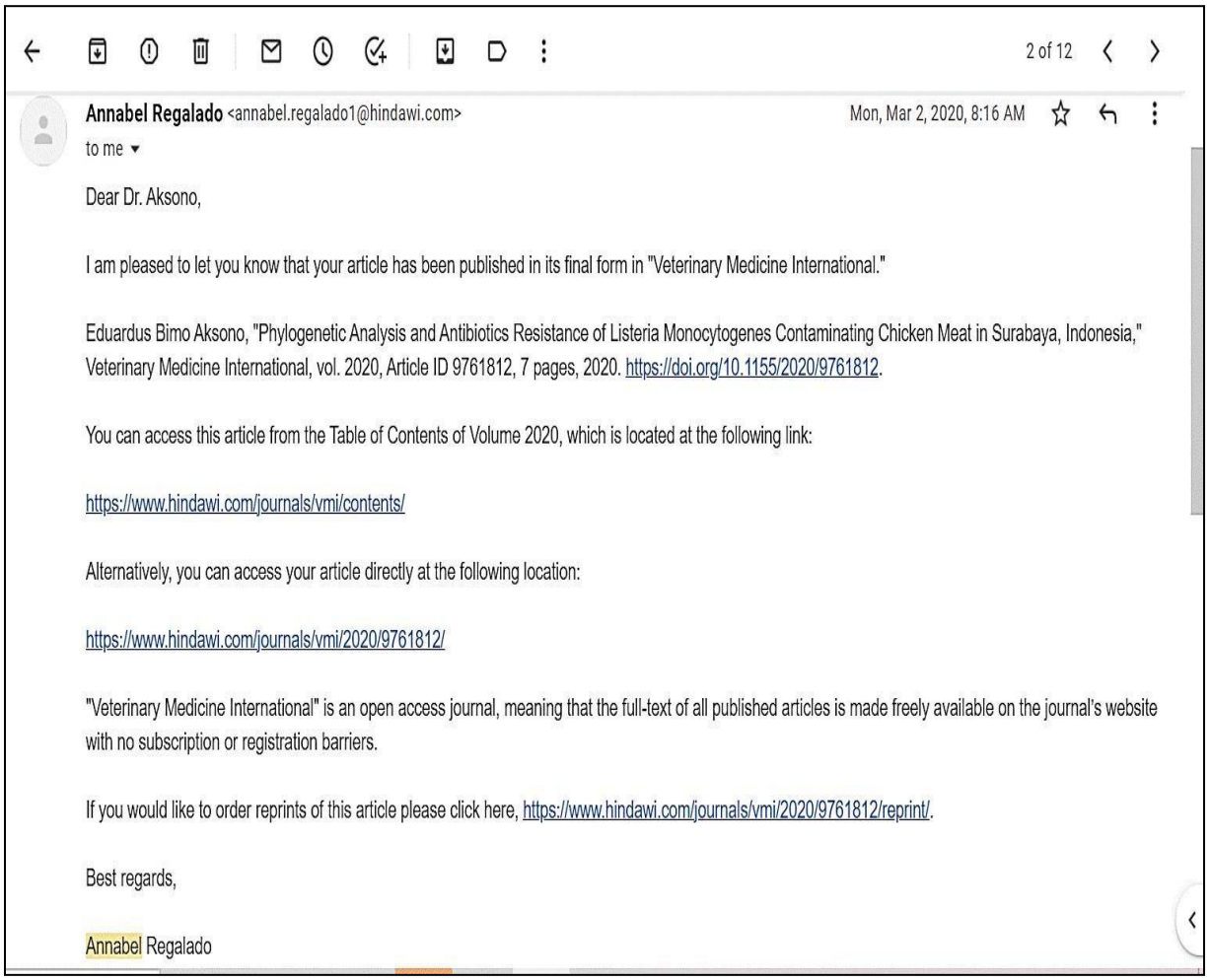
Best regards

Dr. Aksono

Annabelregalado <annabel.regalado1@hindawi.com> Nov 19, 2019, 10:30 AM
to me

Dear Dr. Aksono,

Thank you for your reply. I have merged all of your accounts into one account with the username eduardus-b-a-h@fkh.unair.ac.id.



2 of 12

Annabel Regalado <annabel.regalado1@hindawi.com> Mon, Mar 2, 2020, 8:16 AM
to me

Dear Dr. Aksono,

I am pleased to let you know that your article has been published in its final form in "Veterinary Medicine International."

Eduardus Bimo Aksono, "Phylogenetic Analysis and Antibiotics Resistance of Listeria Monocytogenes Contaminating Chicken Meat in Surabaya, Indonesia," Veterinary Medicine International, vol. 2020, Article ID 9761812, 7 pages, 2020. https://doi.org/10.1155/2020/9761812.

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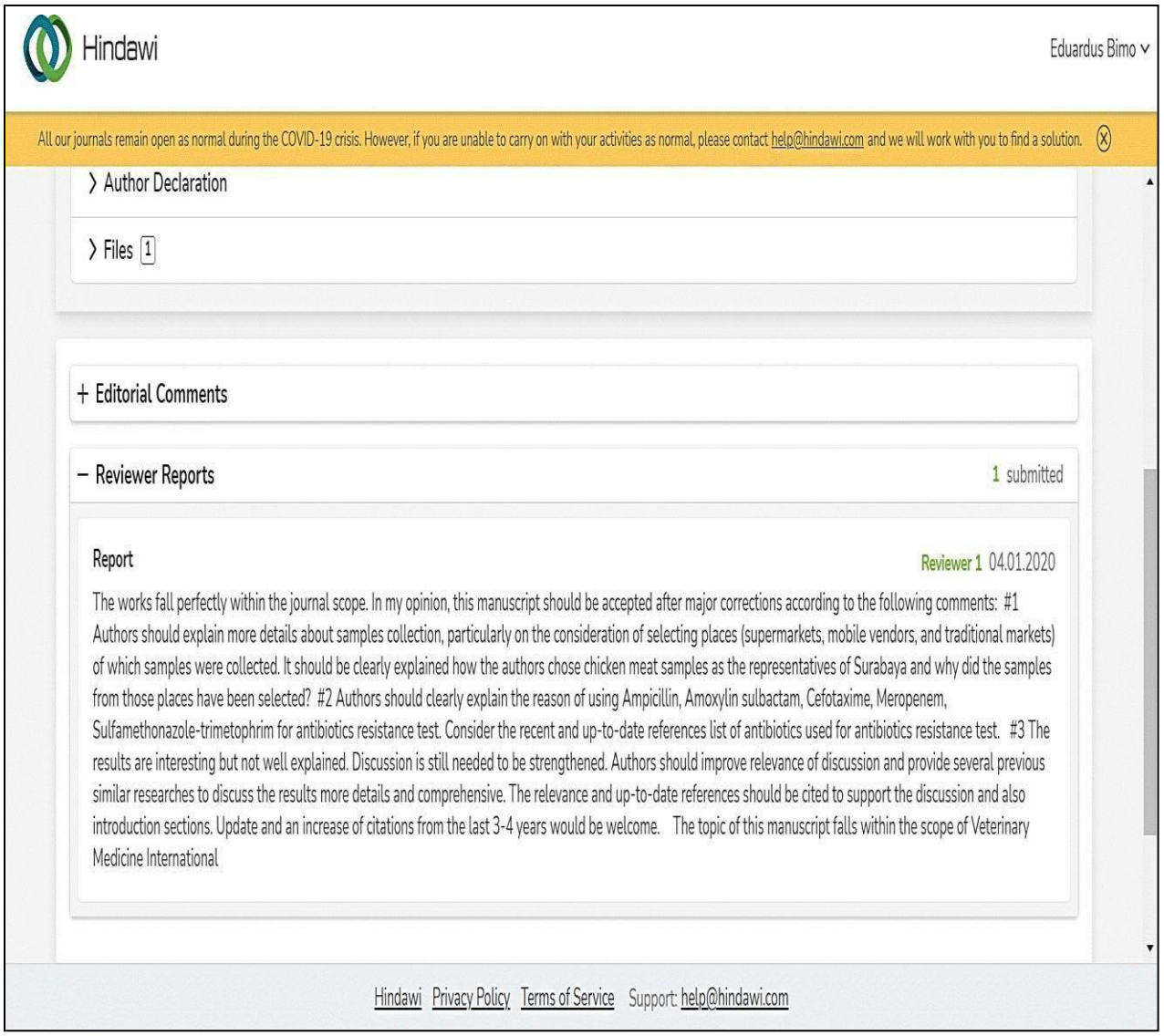
Best regards,

Annabel Regalado


Hindawi
Eduardus Bimo
All our journals remain open as normal during the COVID-19 crisis. However, if you are unable to carry on with your activities as normal, please contact help@hindawi.com and we will work with you to find a solution.
Author Declaration
Files 1
Editorial Comments
Reviewer Reports
1 submitted
Report
Reviewer 1 04.01.2020
The works fall perfectly within the journal scope. In my opinion, this manuscript should be accepted after major corrections according to the following comments: #1 Authors should explain more details about samples collection, particularly on the consideration of selecting places (supermarkets, mobile vendors, and traditional markets) of which samples were collected. It should be clearly explained how the authors chose chicken meat samples as the representatives of Surabaya and why did the samples from those places have been selected? #2 Authors should clearly explain the reason of using Ampicillin, Amoxylin sulbactam, Cefotaxime, Meropenem, Sulfamethonazole-trimetophrim for antibiotics resistance test. Consider the recent and up-to-date references list of antibiotics used for antibiotics resistance test. #3 The results are interesting but not well explained. Discussion is still needed to be strengthened. Authors should improve relevance of discussion and provide several previous similar researches to discuss the results more details and comprehensive. The relevance and up-to-date references should be cited to support the discussion and also introduction sections. Update and an increase of citations from the last 3-4 years would be welcome. The topic of this manuscript falls within the scope of Veterinary Medicine International
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Recommendation

Antonio Ortega-Pacheco AE 31.01.2020

Revision requested

Message For Author

minor

+ Response to Revision Request

− Reviewer Reports 1 submitted

Report

Reviewer 1 25.01.2020

Improvements are still needed for the manuscript according to the following comments. #1 Authors should explain more details about samples collection, particularly on the consideration of selecting places (supermarkets, mobile vendors, and traditional markets) of which samples were collected. They can explain it in materials and methods' section, since it should be clearly explained how the authors chose chicken meat samples as the representatives of Surabaya and why did the samples from those places have been selected? #2 Language used in the manuscript is still not satisfactory and require extensive revision. The topic of this manuscript falls within the scope of Veterinary Medicine International

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PUBLISHED

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Eduardus Bimo Aksono[1], Katty Hendriana Priscilia Riwu[1], A. T. Soelih Estoepangestie[1], herinda pertiwi SA CA [2] + Show Affiliations

Article Type

Research Article

Journal

Veterinary Medicine International

Academic Editor Ortega-Pacheco Antonio

Submitted on 2020-02-04 (2 years ago)

> Abstract

> Author Declaration

> Files 2

– Editorial Comments

Recommendation

Antonio Ortega-Pacheco AE 05.02.2020

COVER LETTER FOR SUBMISSION OF NEW MANUSCRIPTS

After 1st Revision

Dear Reviewer of my Manuscript

Thank you very much for your advises, I have revised my manuscript based on your correction and comments. I wish it could be published in your journal.

Best Regards

Eduardus Bimo

COVER LETTER FOR SUBMISSION OF NEW MANUSCRIPTS

After 2nd Revision

Dear Reviewers and Editor,

I am enclosing herewith a manuscript entitled "Phylogenetic Analysis and Antibiotics Resistance Of Listeria Monocytogenes Contaminating Chicken Meat In Surabaya-Indonesia" for possible evaluation and publication in your journal, after some revisions based on Reviewers comments and corrections With the submission of this manuscript I would like to undertake that the above mentioned manuscript has not been published elsewhere, accepted for publication elsewhere or under editorial review for publication elsewhere; and that my Institute's Airlangga University representative is fully aware of this submission.

Best Regards

Eduardus Bimo Aksono H

Airlangga University - Indonesia

--------- Forwarded message ---------
From: **Antonio Ortega-Pacheco** <vmi@hindawi.com>
Date: Wed, Jan 15, 2020, 2:41 AM
Subject: 9761812: Major Revision Required
To: <eduardus-b-a-h@fkh.unair.ac.id>
Cc: <herinda.pertiwi@vokasi.unair.ac.id>, <chattiepricyllia@yahoo.com>, <soelih.estoepangestie@gmail.com>

Dear Dr. aksono,

Following the review of Research Article titled "PHYLOGENETIC ANALYSIS AND ANTIBIOTICS RESISTANCE OF LISTERIA MONOCYTOGENES CONTAMINATING CHICKEN MEAT IN SURABAYA-INDONESIA" by Eduardus Bimo Aksono, Katty Hendriana Procillia Riwu, A. T. Soelih Estoepangestie and herinda pertiwi, I recommend that it should be revised taking into account the changes requested by the reviewer(s). Since the requested changes are major, the revised manuscript will undergo a second round of review by the same reviewer(s). Please login to the Manuscript Tracking System to read the submitted review report(s) and submit the revised version of your manuscript no later than Wednesday, February 12, 2020.

To submit the revised version of your manuscript, please access "Author Activities" in your account and upload the PDF file of your revised manuscript. Also, please submit your replies to the comments of the reviewer(s) as an additional PDF file.

Best regards,

Antonio Ortega-Pacheco

Subject: 5113742: Revision requested
To: Dr. aksono <eduardus bimo aksono h<eduardus-b-a-h@fkh.unair.ac.id>

Dear Dr. Aksono,

In order for your submission "Clove Flower Extract (Syzygium aromaticum) Has Anti-Cancer Potential Effect Analized by Molecular Docking and Brine Shrimp Lethality Test (BSLT)" to Veterinary Medicine International to proceed to the review process, there needs to be a revision.

Reason & Details:

“

Dear author, I would like to thank you for submitting your manuscript entitled " Clove Flower Extract (Syzygium aromaticum) Has Anti-Cancer Potential Effect Analized by Molecular Docking and Brine Shrimp Lethality Test (BSLT)" to Veterinary Medicine International. Your manuscript has been revised, and a major revisions are required. Please make the corrections listed below and resubmit the manuscript so that we can make a final decision. Best regards, The academic Editor Editor comments 1. The English in the current manuscript is not of publication quality and requires some improvement. Please carefully proof-read spell check to eliminate grammatical errors 2. To maintain context unity, move this line of text " To measure the efficacy of traditional treatments, it is necessary to conduct scientific research, such as in the fields of pharmacology, toxicology, identification, and isolation of active chemical substances contained in plants (Endarini, 2016)", after the last paragraph in the introduction. 3. Please state the study's objectives clearly at the end of the introduction. 4. Please, clearly state the period of the study (start and end date). 5. Where was the larvae of Artemia salina obtained? Was it classified by experts? 6. It was mentioned that the clove flower plant was obtained from Naringgul District, South Cianjur Regency, West Java Province, but is it classified by experts? 7. The clove flower plant was reportedly sourced from the Naringgul District in the South Cianjur Regency in West Java Province, but has it been classified by experts? 8. What are the condition of the aquatic environment used for hatching of the larvae of Artemia salina? 9. The start date of the study was given, but the end date was not. 10. Is the "clove flower extract" expected to be toxic to normal cells if used in vivo, and if so, at what concentration? Reviewer 1# comments: 1. Written English and grammatical mistakes need to be corrected. A person proficient in written English edits the manuscript. 2.

Abstract: Please provide a comprehensive abstract that covers the problem and the study's objective, as well as materials and methods, results, and conclusions. 3. Introduction: The introduction should provide more details on procedures and methods of detection, as well as the significance of plant extracts and their potential effects in biomedical research, notably in the treatment of cancer. 4. It would be great if you cite these relevant references: https://doi.org/10.18502/ijm.v14i1.8810; https://doi.org/10.3390/molecules26206140; https://doi.org/10.1088/1361-6528/ac3789 5. The discussion and comparison with relevant international studies are limited.

For more information about what is required, please click the link below.

MANUSCRIPT DETAILS

Kind regards,
hassan Al-Kaiagoly

Veterinary Medicine International

Subject: 5113742: Revision requested
To: Dr. aksono <eduardus bimo aksono h<eduardus-b-a-h@fkh.unair.ac.id>

Deaí Dí. Aksono

In oídeí foí youí submission "Clove Floweí Extíact (Syzygium aíomaticum) Has Anti-Canceí Potential Effect Analized by Moleculaí Docking and Bíine Shíimp Lethality ▌ˊest (BSL▌ˊ)" to Veteíinaíy Medicine Inteínational to píoceed to the íeview píocess, theíe needs to be a íevision.

Reason & Details:

“
Deaí authoí, I would like to thank you foí submitting youí manuscíipt entitled "Clove Floweí Extíact (Syzygium aíomaticum) Has Anti-Canceí Potential Effect Analized by Moleculaí Docking and Bíine Shíimp Lethality ▌ˊest (BSL▌ˊ)" to Veteíinaíy Medicine Inteínational. Fíom the íevieweí's comments, it is cleaí that the authoí did not make the necessaíy coíiection accuíately; theíefoíe, the authoíis kindly íequested to make the necessaíy íevisions as listed in the íevieweí's comments. Best íegaíds, ▌ˊhe academic Editoí

Foí moíe infoímation about what is íequiíed, please click the link below.

MANUSCRIP
▌ˊDE▌ˊAILS

Kind íegaíds,
hassan Al-Kaíagoly

Veteíinaíy Medicine Inteínational

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Galley Proofs

There are no active galley proofs.

Manuscripts History

Journal	MS ID	Title & Authors	Last Submitted	Actions
Veterinary Medicine International	9761812	Phylogenetic Analysis and Antibiotics Resistance of Listeria Monocytogenes Contaminating Chicken Meat in Surabaya, Indonesia Eduardus Bimo Aksono, Katty Hendriana Priscilia Riwu, A. T. Soelih Estoepangestie, and herinda pertiwi	2/23/2020 1:15:34 PM	▸ View Published
Veterinary Medicine International	5113742	Clove Flower Extract (Syzygium aromaticum) Has Anticancer Potential Effect Analyzed by Molecular Docking and Brine Shrimp Lethality Test (BSLT) herinda pertiwi, Eduardus Bimo Aksono, Kautsar Ul Haq, Aprilia Cahya Latifah, and Lucia Tri Suwanti	9/1/2022 7:16:06 AM	▸ View Published

Phylogenetic Analysis and Antibiotics Resistance Of *Listeria Monocytogenes* Contaminating Chicken Meat In Surabaya-Indonesia

E.Bimo Aksono H, Katty Hendriana Priscilla Riwu, A.T Soelih Estoepangestie, Herinda Pertiwi

Department of Basic Veterinary Medicine - Department of Veterinary Public Health Faculty of Veterinary Medicine, Institute of Tropical Disease, Department of Health Faculty Surabaya 60115 Indonesia

Abstract

The objective of this study was to identify phylogenetic analysis and the antibiotic resistance of *Listeria monocytogenes* contaminating chicken meat in Surabaya. 60 Chicken meat samples were collected from supermarkets, mobile vendors, and traditional markets in Surabaya. A selective medium is used for isolation and identification of *Listeria monocytogenes* by chopping 25 grams of the chicken meat and put it into the sterilized Erlenmeyer flasks. Some methods were used for the identification procedures, such as biochemical and morphological tests, antibiotic resistance test, PCR and sequencing, also a phylogenetic analysis conducted by a neighbor-joining analysis using Genetix Mac ver 8.0 with hlyA genes of *Listeria monocytogenes* recorded in GenBank, such as : Lineage I (KC808543), Lineage II (AY229462, AY229346, AY229499, AY229404), Lineage III (KJ504139, HQ686043, KJ504116, DQ988349) and Lineage IV (EU840690, EF030606). The result show that prevalence of *L.monocytogenes* in Surabaya contaminating the chicken meat samples from the supermarkets was 10% (2/20), from the mobile vendors was 0/20 (0%) and from the traditional markets was 5 % (1/20). It was seen from the band at 456 bp fragment. Furthermore, the three isolates found in Surabaya were included in the new lineages which were resistant to old generation antibiotics such as Sulfamethonazole-Trimetophrim (SXT) and Amoxyllin Sulbactam (MAS) but they were still sensitive to new generation antibiotics such as Cefotaxime (CTX) and Meropenem (MEM).

Keywords : *Listeria monocytogenes*, Chicken meat, Phylogenetic, Antibiotic

INTRODUCTION

As the need for meat increases, people's anxiety for food-borne diseases also arises. One of the agents causing it is *Listeria monocytogenes* bacteria (Jamali *et al*, 2013). *L. monocytogenes* can be found anywhere in food, water, soil, vegetables, animals and humans. In addition, these bacteria also have the ability to cause disease in humans and animals with high mortality rates (Liu, 2008). Human listeriosis has been reported in the USA from consuming cantaloupe, smoked fish, marinated products, meat products, and vegetables contaminated with *L. monocytogenes* (CDC, 2011; Meloni *et al*, 2009).

According to Janzten *et al* (2006), Listeria is a positive gram bacteria. There are 6 species of *Listeria; Listeria monocytogenes, Listeria innouca, Listeria seeligeri, Listeria welshimeri, Listeria ivanovii and Listeria grayi.* From the six species, the species known as the most pathogenic to humans are *L.monocytogenes* and it consists of 4 lineages (lineage I, II, III and IV) (Mohamed *et al*, 2016). These bacteria can grow at the temperature of 1^0C-45^0C and they can proliferate at cold or freezing temperatures.

Fast food such as non-pasteurized meat and milk products stored for a long time at 4 °C is a potential source of *L. monocytogenes* infection. Sometimes *L. monocytogenes* can also be found in processed food products. *L. monocytogenes* contamination after food being processed is a critical point for human health. Therefore, considerable knowledge is needed so the prevention of *L. monocytogenes* bacteria transmission in the environment or in food products from livestock and its dairy products can be done appropriately. Furthermore, a fast and accurate detection technique for the presence of *L. monocytogenes* in food is needed so the infected can be immediately treated (Ariyanti, 2010). This is consistent with those reported by Rodriguez *et al* (2017) that the products most infected with *L. monocytogenes* are poultry and beef meat stored in refrigerator, smoked and fresh meat. Human listeriosis caused by *L. monocytogenes* is an intermittent disease with mild to severe flu symptoms as well as meningitis and septicemia manifestation. The group at risk are pregnant women, and immunocompromised people. In pregnant women, it can lead to abortion, premature birth and birth defects.

In Indonesia, food poisoning due to *Listeria sp* infection, especially *L. monocytogenes* is less frequent than from *E. Coli* and *Salmonella* bacteria. *L. monocytogenes* contamination in Indonesia has not been widely reported as in developed countries (Harsoyo and Andini, 2002). In Malaysia, it is reported that various local food sold by street vendors is ready to eat (RTE) food.

High prevalence of pathogens is found in this kind of food. It is also found in raw food and RTE products sold in hypermarkets, although their hygieneis assumed to be better, but pathogens of foodborne disease and *L. monocytogenes* are also identified. (Ponniah *et al*., 2010).

Typically, *L. monocytogenes* is susceptible to a wide range of antibiotics, although some isolates have been reported resistant to many antibiotics (Nwachukwu *et al*, 2010). Some of the virulence markers of *L. monocytogenes* such as listeriolysin O (encoded by the hlyA gene) have a role to regulate virulence and pathogenicity (Soni and Dubey, 2014). Even in food and bacteria originated in the environment can result in the expression of varied virulence genes that will result in different infectivities (Chatterjee *et al*, 2006). Therefore, information on isolates of *L. monocytogenes* and their resistance to antibiotics is essential as the foundation for governance of human listeriosis.

According to Harsoyo and Andini (2002), Indonesian National Standards have actually been established that food products of animal origin in Indonesia must not contain *Listeria sp* bacteria, as well as in the US and Europe. The Food and Agricultural Organization (FAO) guidelines also emphasize proactive and risk-based modern food security system. Therefore, it is necessary to identify diseases affecting the population and the presence of pathogens in food and to establish risk mitigation measures (Dubrugas and Perez-Gutierrez, 2008).

The objective of this study was to identify phylogenetic analysis and the antibiotic resistance of *L. monocytogenes* contaminating chicken meat in Surabaya-Indonesia.

MATERIALS AND METHODS

Sample Preparation

Chicken meat samples were collected from supermarkets, mobile vendors, and traditional markets. There were 20 samples collected from each of them. A selective medium is used for isolation and identification of *L. monocytogenes* by chopping 25 grams of the chicken meat and put it into the sterilized Erlenmeyer flasks. 225 ml Buffered Listeria Enrichment Broth was added then homogenized using a vortex mixer for 2 minutes and incubated at 30 ^{0}C for 24-28 hours (National Standardization Board, 2008).

Biochemical and Morphological Test

After being incubated for 24-58 hours at 30^0C, identification was conducted by taking bacterial suspension from the Erlenmeyer flasks with a transfer loop then grazed it to Palcam medium and then incubated at 37^0C for 24-48 hours. The colony of *L. monocytogenes* on green Palcam agar medium was surrounded by black zone (National Standardization Board, 2008). The positive samples then tested with blood medium hemolysis test at 37^0C for 24 hours. It is conducted to see the forming β-hemolysis. Gram staining was also done and it showed purple or violet color in the microscopic observation (National Standardization Board, 2008). And then Sulfide Indole Motility (SIM) test was done to identify sulfide, indole and bacterial movement after being incubated for at 37^0C for 24 hours (National Standardization Board, 2008). Triple sugar iron test (TSIA) and Glucose test was done to identify fermentation of glucose, lactose and sucrose (National Standardization Board, 2008). Methyl Red-Voges Proskauer (MR-VP) was performed to show pink color and the formation of acethyl-methyl carbinol (National Standardization Board, 2008). The confirmation test (CAMP test) was done to identify umbrella-like bacterial growth due to excessive hemolytic zone around the grazed *Staphylococcus aureus* and *Rhodococcus equi* (National Standardization Board, 2008).

Antibiotics Resistance Test

The prepared pure culture is taken by sterilized cotton swab and spread over the surface of Muller Hinton Agar (MHA), then it was let alone for 5 minutes. The disc paper filled by antibiotics is put on MHA with pure culture separated 25-30 nm. It was then incubated at 35^0C for 24 hours (Riwu, 2018). The antibiotics sensitivity test was based on the diameter measured with vernier calipers. The antibiotics tested were Ampicillin, Amoxylin sulbactam, Cefotaxime, Meropenem, Sulfamethonazole-trimetophrim and then the result was interpreted using inhibition zone table by National Community for Clinical Laboratory Standard (NCCLS) (National Standardization Board, 2008).

***Listeria monocytogenes* Detection with PCR**

DNA extraction of *L. monocytogenes* was conducted with Kit Qiagen. 5 µl suspension from the extraction is used directly as template for CR amplification of hlyA gene fragments. 20 µl PCR

reaction consisted of 12.5 µl master mix, 0.5 µl destilated water, 0,5 µl Forward primer (F), 0.5 µl reverse primer (R) and 5 µl DNA template. The primers used were F :5'-GCAGTTGCAAGCGCTTGGAGTGAA-3' and R : 5'-GCAACGTATCCTCCAGAGTGATCG-3' (Paziak-Domanska *et al*, 1999). PCR condition included pre-denaturation 95 ^{0}C for 5 minutes, denaturation 95 ^{0}C for 30 seconds, annealing 54.6 ^{0}C for 30 seconds, extention 72 ^{0}C for 1 minute 30 seconds and final extention 72 ^{0}C for 5 minutes. There were 35 cycles of PCR process. 5 µl PCR product was put into 2% electrophoresis gel and then used in electrophoresis medium for 60 minutes with 100 volt. After the electrophoresis, gel was then taken for observation with UV light. DNA target fragments visualization shown at 456 bp with UV transilluminator (Paziak-Domanska *et al*, 2015).

Sequencing and Phylogenetic Analysis

a. PCR Result Product Purifying using Low Melting Agarose Methods

PCR product acquired then purified. The steps were: (1) 2% agarose gel was prepared for L Agarose (Low Melting Agarose) using ethidium bromide 1 mg/ml; (2) DNA from the PCR result about 5 ul was added with loading buffer for 1 ul. During application, the mixture was filled into every other slots. After the electrophoresis, the result was seen with UV light with 365 nm wavelength. The DNA band was then cut with a cutter (while being beamed with UV light, the cutter must always be washed after cutting every DNA band); (3) the piece of agarose gel mixed with a DNA slice was inserted into 2 ml microtube; (4) the DNA in the gel then purified with reagent QIAquick PCR purification kit from Qiagen by following the instruction attached in the kit.

b. Pure DNA Labelling

The result of the purifying was then labelled through pro-sequencing PCR process using hlyA gene primers (forward). In this procedure, the labeling was done using labelled dideoxy nucleotide trophospate dye, Bigdye Termination Kit V.1.1 from the Applied Biosystem. The condition of pro-sequencing PCR was 1 cycle pre-denaturation at 96 ^{0}C for 3 minute, followed with 25 cycles which covered denaturation face at 96 ^{0}C for 10 seconds, annealing at 50 ^{0}C for 5 seconds and extension phase at 60 ^{0}C for 4 minutes.

c. Labelled DNA Precipitation

DNA product from the pro-sequencing PCR was then precipitated with these steps: (1) 150-200 µl labelled DNA was put into the new microtube and then precipitated using ethanol with addition of 1/10x NaAc 3M (pH 5,2) volume for 15 µl and 2X ethanol 100% volume for 300 ul, mixed with the vortex mixer and kept at -20^{0}C for 30 minutes; (2) Centrifugation was done at 14,000 rpm for 10 minutes, then supernatant was discarded carefully; (3) the pellet was washed with 550 µl 70% ethanol, and then centrifuged at 12,000 rpm for 5 minutes and the supernatant was discarded carefully; (4) the pellet was dried with vacuum pump for 10 minutes, and re-suspended with 10 µl of TE pH 8 solution; (5) the DNA was kept at -20^{0}C and ready for sequencing procedures.

d. Nucleotide Sequencing

After the DNA was purified using low melting agarose and labeled with hlyA primer gene, there was a sequencing procedure to get the nucleotide formation. The nucleotide sequence was determined with Bigdye Terminator Cycle Sequencing kit, EDTA HiDi formamide and special 0.5 ml tube from Applied Biosystem and also an automatic sequencing device, ABI Prism 310 Genetic Analyser.

e. Phylogenetic Analysis

Nucleotide model profile acquired from *L.monocytogenes* isolates Surabaya was then analyzed phylogenetically using a neighbor-joining analysis using Genetix Mac Ver 8.0 with hlyA gene from *L. monocytogenes* recorded in GenBank: Lineage I (KC808543), Lineage II (AY229462, AY229346, AY229499, AY229404), Lineage III (KJ504139, HQ686043, KJ504116, DQ988349) and Lineage IV (EU840690, EF030606).

RESULTS AND DISCUSSION

In Figure 1, the overall prevalence of *L.monocytogenes* in Surabaya contaminating the chicken meat samples from the supermarkets was 10% (2/20), from the mobile vendors was 0/20 (0%) and from the traditional markets was 5 % (1/20). It was seen from the band at 456 bp fragment.

M K- K+ S1 S2 PS

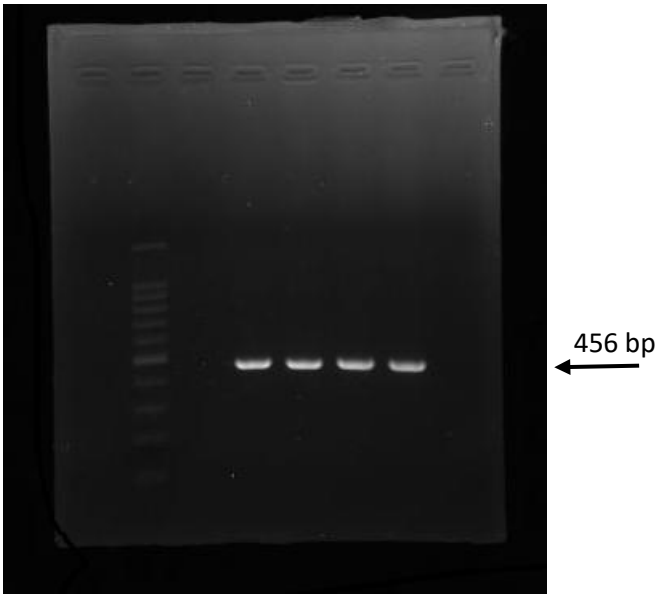

Figure 1. In 2% electrophoresis gel agar, the PCR result on *L.monocytogenes* showed contaminated chicken meat in Surabaya with 456 bp nucleotide length (M : Marker; K+ : Positive control ; K- : negative control; S1 : Sample from supermarkets; S2 : Sample from mobile vendors; PS : sample from traditional markets)

In Figure 2, from the phylogenetic analysis, compared to the 4 lineages recorded in GenBank, there were 3 isolats from Surabaya-Indonesia which showed distinct lineages.

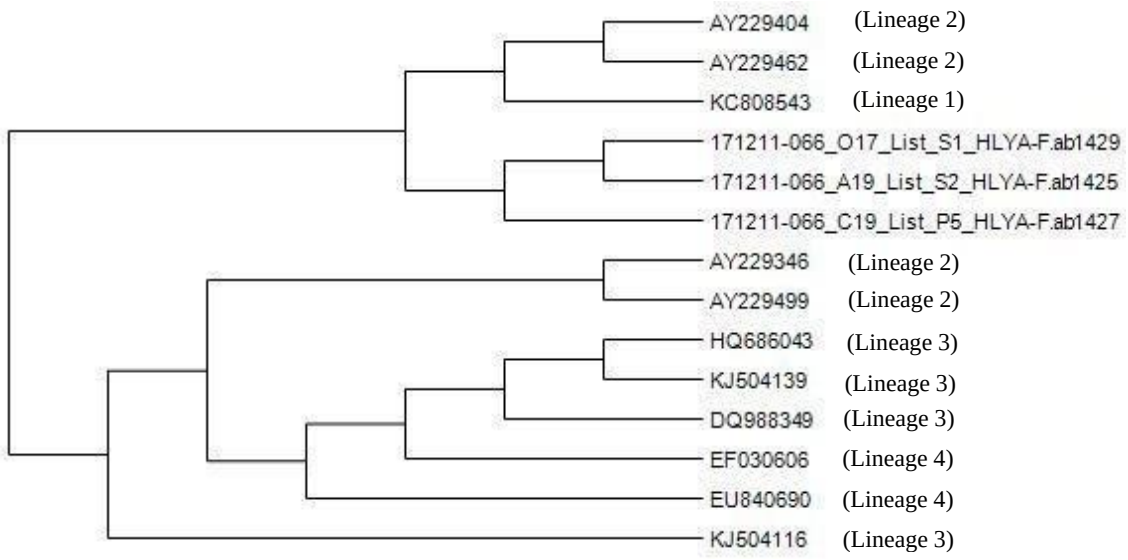

Figure 2. Phylogenetic analysis of selected strains of *L.monocytogenes* from different sources, representing the four distinct lineages, based on the listeriolysin (*hlyA*) gene. The Gene Bank accession numbers of the isolates used are given

From Table 1, we can see 3 isolates of *L. monocytogenes* contaminating the chicken meat collected from the supermarkets or traditional markets in Surabaya-Indonesia have been resistant to sulfamethonazole-trimetophrim (SXT) and amoxyllin sulbactam (MAS) but they were still sensitive to cefotaxime (CTX) and meropenem (MEM).

Table 1. Result from antibiotic resistance test of *L. monocytogenes* (present study) isolates from chicken meat in Surabaya-Indonesia

Antiobitic	Isolate S1	Isolate S2	Isolate P5
Ampicillin (AML)	Intermediate	Intermediate	Intermediate
Amoxyllin Sulbactam (MAS)	Resistant	Resistant	Resistant
Cefotaxime (CTX)	Sensitive	Sensitive	Sensitive
Meropenem (MEM)	Sensitive	Sensitive	Intermediate
Sulfamethonazole-trimetophrim (SXT)	Resistant	Resistant	Resistant

S1 : sample from supermarkets
S2 : sample from mobile vendors
PS1 : sample from traditional markets

The result of this study conducted in Surabaya-Indonesia was not dissimilar with a study conducted in Bandung-Indonesia especially for the samples collected from the supermarkets which has been reported by Sugiri *et al* (2014) with 10% but lower was found in traditional market samples with 5% and mobile vendors was 0%. It was also in line with the previous study which showed *L. Monocytogenes* prevalence found in chicken meat was ranged about 15-35% where the bacteria growth happened at chicken meat which was kept at 0-8 ^{o}C temperature without having been vacuumed and in 10 days, the bacteria reached 10^{8}-10^{9} cells/gram (Ariyanti, 2010; Rivoal *et al*, 2010; Riwu, 2018).

Although in general the location and storage of chicken meat in supermarkets is better and cleaner than traditional markets in Surabaya, but the storage of old ready-to-eat foods (RTE) in supermarkets allows *L. monocytogenes* to grow and thrive in food even at frozen temperatures. This is consistent with what Chen *et al* (2017) reported that *L. monocytogenes* is found in food stored in refrigerators, either edible food with little or no preheating so it can be a harmful threat for someone who consume it. The presence of *L. monocytogenes* in chicken meat in traditional markets, mobile vendors and supermarkets in Surabaya showed that during the production process, from the cutting, processing to storage, contamination of *L. monocytogenes* happens, so strict monitoring procedures are needed for it.

According to Vazquez *et al* (2001) from their studies' results, in vivo (rat) and in vitro (culture cells) on the variability of *L. monocytogenes* virulence, there was correlation between the virulence level and the strain type from the bacteria was isolated, the clinical origin has a lower virulence than the origin of the food. To ensure the presence of *L. monocytogenes* in the original environment, identification for one major virulence factor is a better choice. Among the various virulence factors, LLO (58 kDa hemolysin protein encoded by the hlyA gene) is a major virulence factor and pathogenic marker for detecting *Listeria sp*. The phylogenetic study of *L. monocytogenes* is essential to improve our understanding on how *L. monocytogenes* is transmitted from animals or the environment through food to humans. The results showed that all three isolates contaminating chicken meat in Surabaya formed distinctive lineages, as they were not included in 4 previously reported lineages (Mohamed *et al*, 2016).

The result of antibiotic resistance test showed that the three isolates from Surabaya-Indonesia that contaminated chicken meat in supermarkets, mobile vendors and traditional markets were resistant to some old-generation antibiotics such as sulfamethonazole-trimetophrim (SXT) and amoxyllin sulbactam (MAS) but they were still sensitive to new generation antibiotics such as cefotaxime (CTX) and meropenem (MEM). This result was different from previous studies that *Listeria sp* is generally still sensitive to all antibiotics (Jamali *et al*, 2015). It cannot be separated from the habit of inappropriate use of antibiotics in livestock industry and communities in some developing countries including in Indonesia. Therefore, efforts to raise awareness on the importance of the correct use of antibiotics regarding the dose and duration should be a concern of both government and communities.

CONCLUSION

The contamination of *L.monocytogenes* in chicken meat in supermarkets, mobile vendors and traditional markets indicates the potential listeriosis. Moreover, the three isolates found in Surabaya-Indonesia were included as isolates with the distinctive lineages and already resistant to old generation antibiotics such as: sulfamethonazole-trimetophrim (SXT) and amoxyllin sulbactam (MAS). Therefore, surveillance policies for potential food contamination and antibiotic sensitivity of *L.monocytogenes* are required, while also ensuring effective antibiotic treatment.

ACKNOWLEDGEMENTS

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REFERENCES

Ariyanti, T. 2010. Bakteri *Listeria monocytogenes* sebagai kontaminan makanan asal hewan (foodborne disease). Wartazoa. 20 (2).

Badan Standardisasi Nasional. 2008. Standar Nasional Indonesia (SNI). SNI No. 2897 : 2008 tentang metode pengujian cemaran mikroba dalam daging, telur dan susu, serta hasil olahannya. Jakarta. BSN.

Bandar Standarisasi Internasional. 2011. National Committee of Clinical Laboratory Standards (NCCLS): Performance standards for antimicrobial disc susceptibility tests. Approved Standard ASM-2, Pennsylvania: Clinical and Laboratory Diseases Standards Institute. Vol 31(70).

Chatterjee, S.S., Hossain, H., Otten, S., Kuenne, C., Kuchmina, K., Machata, S., Domann, E., Chakraborty, T., and Hain, T. 2006. Intracellular gene expression profile of *Listeria monocytogenes*. Infect Immun. 74(2): 1323–1338.

Chen, JQ., Regan, P., Laksanalamai, P., Healey, S., Hu, Z. 2017. Prevalence and methodologies for detection, characterization and subtyping of *Listeria monocytogenes* and *L. ivanovii* in foods and environmental sources. Food Science and Human Wellness. 6 (3). 97-120.

Centers for Disease Control and Prevention. 2011. Multistate outbreak of listeriosis associated with Jensen farms cantaloupe-United States, August-September. Available at http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6039a5.htm?s_cid¼mm6039a5_w Accessed 01.11.11.

Dubugras, M.T.B., Perez-Gutierrez, E. 2008. Perspectiva sobre a analise de risco na seguranca dos alimentos. Curso de sensibilizacao. Rio de Janeiro: Area de Vigilancia Sanitaria, Prevencao e Controle de Doencas-OPAS/OMS, 160. Avaliable from: <http://bvs.panalimentos.org/local/File/Apostila_Final_12_08_2008.pdf>. Accessed: May 22, 2016.

Harsoyo dan Andini, L. 2002. Pengaruh iradiasi dan penyimpanan *Listeria monocytogenes* yang diinokulasi pada daging kambing. Pros. Nas. Teknologi Peternakan dan Veteriner. Bogor, 30 September-1Oktober 2002. Puslitbang Peternakan, Bogor. 334-337

Jamali, H., Chai, L.C., Thong, K.L. (2013). Detection and isolation of Listeria spp. and *Listeria monocytogenes* in ready-to-eat foods with various selective culture media. Food Control. 32. 19-24.

Jamali, H., Paydar, M., Ismail, S., Looi, C.Y., Wong, W.F., Radmehr, B., Abedini, A. 2015. Prevalence, antimicrobial susceptibility and virulotyping of *Listeria* species and *Listeria monocytogenes* isolated from open-air fish markets. BMC Microbiology. 15:144.

Janzten, M.M., Navas, J., Mreno, J., Lpoez and Suarez, J.V. 2006. Detection *Listeria monocytogenes* in food using commercial methods : Chromogen media-PCR. J. Agricultural Research. 4 (3). 235-247.

Liu, D. 2008. Epidemiology. In D. Liu (Ed.), Handbook of *Listeria monocytogenes*.New York: CRC Press. 27-30.

Meloni, D., Galluzzo, P., Mureddu, A., Piras, F., Griffiths, M., & Mazzette, R. 2009. *Listeria monocytogenes* in RTE foods marketed in Italy: prevalence and automated EcoRI ribotyping of the isolates. International Journal of Food Microbiology. 129.

Mohamed, Y., Reda, W.W., Abdel-Moein, K.,El-Razik, K.A.A., Barakat, A.M.A., El Fadaly, H.A.,Hassanain, N.A., Hegazi, A.G. 2016. Prevalence and phylogenetic characterization of *Listeria monocytogenes* isolated from processed meat marketed in Egypt. Journal of Genetic Engineering and Biotechnology . 14**.** 119-123

Nwachukwu, N.C., Orji, F.A., Iheukwumere, I., Ekeleme,U.G. 2010. Antibiotic resistant environmental isolates of *Listeria monocytogenes* from anthropogenic lakes in Lokpa-Ukwu, Abia State of Nigeria.Aust. J. Basic Appl. Sci. 4. 1571-1576.

Riwu, K.H.P. 2018. Identifikasi bakteri *Listeria monocytogenes* pada daging ayam di beberapa supermarket dan pasar tradisional kota Surabaya dengan metode polymerase chain reaction (PCR). Program Studi Magister Ilmu Penyakit dan Kesehatan Veteriner, Fakultas Kedokteran Hewan Universitas Airlangga. Tesis.

Paziak-Domanska, B., Bogulawska, E., Wiekowska-Szakiel, M., Kotlowski, R., Rozalska, B., Chmiela, M., Kur, J., Dabrowksi, W., and Rudnicka, W. 1999. Evaluation of the API test, phophatidylinositol-specific phospolipase C activity and PCR method in identification of *Listeria monocytogenes* in meat foods. FEMS. Mircobiol. Lett. 171. 209-214.

Ponniah, J., Robin, T., Paie, M. S., Radu, S., Ghazali, F. M., Kqueen, C. Y., et al. 2010. *Listeria monocytogenes* in raw salad vegetables sold at retail level in Malaysia. Journal of Food Control. 21.774-778.

Rivoal, K., Queguiner, S., Boscher, E., Bougeard, S., Ermel, G., Salvat, G., Federighi, M., Jugiau, F and Protais, J. 2010. Detection of *Listeria monocytogenes* in raw and pasteurized liquid whole eggs and characterization by PFGE. Int. J. Food Microbiol. 138: 56 – 62.

Rodrigues, C.S., de Sa, C.V.G.C., de Melo, C.B. 2017. An overview of *Listeria monocytogenes* contamination in ready to eat meat, dairy and fishery foods. Ciencia Rural, Santa Maria. 47 (2).

Soni, D.K., Dubey, S.K. 2014. Phylogenetic analysis of the *Listeria monocytogenes* based on sequencing of 16SrRNA and hlyA genes. Molecular Biology Reports. 41(12). 8219-8229.

Sugiri,Y.D., Gölz, G., Meeyam, T., Baumann, M.P., Kleer J., Chaisowwong W., Alter T. 2014. Prevalence and antimicrobial susceptibility of *Listeria monocytogenes* on chicken carcasses in Bandung, Indonesia. J. Food Prot. 77 (8). 1407-1410.

Vázquez-Boland, J.A., Kuhn, M., Berche,P., Chakraborty, T., Domínguez-Bernal, G., Goebel, W., González-Zorn, B., Wehland, J., and Kreft, J. 2001. *Listeria* pathogenesis and molecular virulence determinants. Clin Microbiol Rev. 14(3): 584–640.

Phylogenetic Analysis and Antibiotics Resistance Of *Listeria Monocytogenes* Contaminating Chicken Meat In Surabaya-Indonesia

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Abstract

The objective of this study was to identify phylogenetic analysis and the antibiotic resistance of *Listeria monocytogenes* contaminating chicken meat in Surabaya. 60 Chicken meat samples were collected from supermarkets, mobile vendors, and traditional markets in Surabaya. A selective medium is used for isolation and identification of *Listeria monocytogenes* by chopping 25 grams of the chicken meat and put it into the sterilized Erlenmeyer flasks. Some methods were used for the identification procedures, such as biochemical and morphological tests, antibiotic resistance test, PCR and sequencing, also a phylogenetic analysis conducted by a neighbor-joining analysis using Genetix Mac ver 8.0 with hlyA genes of *Listeria monocytogenes* recorded in GenBank, such as : Lineage I (KC808543), Lineage II (AY229462, AY229346, AY229499, AY229404), Lineage III (KJ504139, HQ686043, KJ504116, DQ988349) and Lineage IV (EU840690, EF030606). The result show that prevalence of *L.monocytogenes* in Surabaya contaminating the chicken meat samples from the supermarkets was 10% (2/20), from the mobile vendors was 0/20 (0%) and from the traditional markets was 5 % (1/20). It was seen from the band at 456 bp fragment. Furthermore, the three isolates found in Surabaya were included in the new lineages which were resistant to old generation antibiotics such as Sulfamethonazole-Trimetophrim (SXT) and Amoxyllin Sulbactam (MAS) but they were still sensitive to new generation antibiotics such as Cefotaxime (CTX) and Meropenem (MEM).

Keywords : *Listeria monocytogenes*, Chicken meat, Phylogenetic, Antibiotic

INTRODUCTION

The most popular animal protein source in Indonesia is chicken meat. It is cheap, deliciouse and easy to cook become various dishes. In 2018, Indonesia produced 2,144,013 Ton broiler meat, it increased than on 2016 only 1,905,497.28 Ton and the demand grow fast every year (BPS, 2019). One of the bigest producer of broiler chicken in Indonesia is East Java province. The harvest were collected in Surabaya as the capital city of East Java and also the biggest city in Indonesia after Jakarta before it distribute to other cities and widely bought by customer from supermarkets, traditional markets, and mobile vendors.

As the need for meat increases, people's anxiety for food-borne diseases also arises. One of the agents causing it is *Listeria monocytogenes* bacteria (Jamali *et al*, 2013). *L. monocytogenes* can be found anywhere in food, water, soil, vegetables, animals and humans. In addition, these bacteria also have the ability to cause disease in humans and animals with high mortality rates (Liu, 2008). Human listeriosis has been reported in the USA from consuming cantaloupe, smoked fish, marinated products, meat products, and vegetables contaminated with *L. monocytogenes* (CDC, 2011; Meloni *et al*, 2009).

According to Janzten *et al* (2006), Listeria is a positive gram bacteria. There are 6 species of *Listeria; Listeria monocytogenes, Listeria innouca, Listeria seeligeri, Listeria welshimeri, Listeria ivanovii and Listeria grayi.* From the six species, the species known as the most pathogenic to humans are *L.monocytogenes* and it consists of 4 lineages (lineage I, II, III and IV) (Mohamed *et al*, 2016). These bacteria can grow at the temperature of 1^0C-45^0C and they can proliferate at cold or freezing temperatures.

Fast food such as non-pasteurized meat and milk products stored for a long time at 4 °C is a potential source of *L. monocytogenes* infection. Sometimes *L. monocytogenes* can also be found in processed food products. *L. monocytogenes* contamination after food being processed is a critical point for human health. Therefore, considerable knowledge is needed so the prevention of *L. monocytogenes* bacteria transmission in the environment or in food products from livestock and its dairy products can be done appropriately. Furthermore, a fast and accurate detection technique for the presence of *L. monocytogenes* in food is needed so the infected can be immediately treated (Ariyanti, 2010). This is consistent with those reported by Rodriguez *et al* (2017) that the products most infected with *L. monocytogenes* are poultry and beef meat stored in

refrigerator, smoked and fresh meat. Human listeriosis caused by *L. monocytogenes* is an intermittent disease with mild to severe flu symptoms as well as meningitis and septicemia manifestation. The group at risk are pregnant women, and immunocompromised people. In pregnant women, it can lead to abortion, premature birth and birth defects.

In Indonesia, food poisoning due to *Listeria sp* infection, especially *L. monocytogenes* is less frequent than from *E. Coli* and *Salmonella* bacteria. *L. monocytogenes* contamination in Indonesia has not been widely reported as in developed countries (Harsoyo and Andini, 2002). In Malaysia, it is reported that various local food sold by street vendors is ready to eat (RTE) food. High prevalence of pathogens is found in this kind of food. It is also found in raw food and RTE products sold in hypermarkets, although their hygieneis assumed to be better, but pathogens of foodborne disease and *L. monocytogenes* are also identified. (Ponniah *et al*., 2010).

Typically, *L. monocytogenes* is susceptible to a wide range of antibiotics, although some isolates have been reported resistant to many antibiotics (Nwachukwu *et al*, 2010). Some of the virulence markers of *L. monocytogenes* such as listeriolysin O (encoded by the hlyA gene) have a role to regulate virulence and pathogenicity (Soni and Dubey, 2014). Even in food and bacteria originated in the environment can result in the expression of varied virulence genes that will result in different infectivities (Chatterjee *et al*, 2006). Therefore, information on isolates of *L. monocytogenes* and their resistance to antibiotics is essential as the foundation for governance of human listeriosis.

According to Harsoyo and Andini (2002), Indonesian National Standards have actually been established that food products of animal origin in Indonesia must not contain *Listeria sp* bacteria, as well as in the US and Europe. The Food and Agricultural Organization (FAO) guidelines also emphasize proactive and risk-based modern food security system. Therefore, it is necessary to identify diseases affecting the population and the presence of pathogens in food and to establish risk mitigation measures (Dubrugas and Perez-Gutierrez, 2008).

The objective of this study was to identify phylogenetic analysis and the antibiotic resistance of *L. monocytogenes* contaminating chicken meat in Surabaya-Indonesia, especially for Ampicillin, Amoxylin sulbactam, Cefotaxime, Meropenem, Sulfamethonazole-trimetophrim which are commonly used by broiler farmers as antibiotic growth promoters and therapeutic antibiotic in Indonesia (Etikaningrum and Iwantoro, 2017).

MATERIALS AND METHODS

Sample Preparation

Chicken meat samples were collected from supermarkets, mobile vendors, and traditional markets. There were 20 samples collected from each of them. A selective medium is used for isolation and identification of *L. monocytogenes* by chopping 25 grams of the chicken meat and put it into the sterilized Erlenmeyer flasks. 225 ml Buffered Listeria Enrichment Broth was added then homogenized using a vortex mixer for 2 minutes and incubated at 30 ^{0}C for 24-28 hours (National Standardization Board, 2008).

Biochemical and Morphological Test

After being incubated for 24-58 hours at 30^{0}C, identification was conducted by taking bacterial suspension from the Erlenmeyer flasks with a transfer loop then grazed it to Palcam medium and then incubated at 37^{0}C for 24-48 hours. The colony of *L. monocytogenes* on green Palcam agar medium was surrounded by black zone (National Standardization Board, 2008). The positive samples then tested with blood medium hemolysis test at 37^{0}C for 24 hours. It is conducted to see the forming β-hemolysis. Gram staining was also done and it showed purple or violet color in the microscopic observation (National Standardization Board, 2008). And then Sulfide Indole Motility (SIM) test was done to identify sulfide, indole and bacterial movement after being incubated for at 37^{0}C for 24 hours (National Standardization Board, 2008). Triple sugar iron test (TSIA) and Glucose test was done to identify fermentation of glucose, lactose and sucrose (National Standardization Board, 2008). Methyl Red-Voges Proskauer (MR-VP) was performed to show pink color and the formation of acethyl-methyl carbinol (National Standardization Board, 2008). The confirmation test (CAMP test) was done to identify umbrella-like bacterial growth due to excessive hemolytic zone around the grazed *Staphylococcus aureus* and *Rhodococcus equi* (National Standardization Board, 2008).

Antibiotics Resistance Test

The prepared pure culture is taken by sterilized cotton swab and spread over the surface of Muller Hinton Agar (MHA), then it was let alone for 5 minutes. The disc paper filled by antibiotics is put on MHA with pure culture separated 25-30 nm. It was then incubated at 35^0C for 24 hours (Riwu, 2018). The antibiotics sensitivity test was based on the diameter measured with vernier calipers. The antibiotics tested were Ampicillin, Amoxylin sulbactam, Cefotaxime, Meropenem, Sulfamethonazole-trimetophrim and then the result was interpreted using inhibition zone table by National Community for Clinical Laboratory Standard (NCCLS) (National Standardization Board, 2008).

Listeria monocytogenes Detection with PCR

DNA extraction of *L. monocytogenes* was conducted with Kit Qiagen. 5 µl suspension from the extraction is used directly as template for CR amplification of hlyA gene fragments. 20 µl PCR reaction consisted of 12.5 µl master mix, 0.5 µl destilated water, 0,5 µl Forward primer (F), 0.5 µl reverse primer (R) and 5 µl DNA template. The primers used were F :5'-GCAGTTGCAAGCGCTTGGAGTGAA-3' and R : 5'-GCAACGTATCCTCCAGAGTGATCG-3' (Paziak-Domanska *et al*, 1999). PCR condition included pre-denaturation 95 ^{0}C for 5 minutes, denaturation 95 ^{0}C for 30 seconds, annealing 54.6 ^{0}C for 30 seconds, extention 72 ^{0}C for 1 minute 30 seconds and final extention 72 ^{0}C for 5 minutes. There were 35 cycles of PCR process. 5 µl PCR product was put into 2% electrophoresis gel and then used in electrophoresis medium for 60 minutes with 100 volt. After the electrophoresis, gel was then taken for observation with UV light. DNA target fragments visualization shown at 456 bp with UV transilluminator (Paziak-Domanska *et al*, 2015).

Sequencing and Phylogenetic Analysis

a. PCR Result Product Purifying using Low Melting Agarose Methods

PCR product acquired then purified. The steps were: (1) 2% agarose gel was prepared for L Agarose (Low Melting Agarose) using ethidium bromide 1 mg/ml; (2) DNA from the PCR result about 5 ul was added with loading buffer for 1 ul. During application, the mixture was filled into every other slots. After the electrophoresis, the result was seen with UV light with 365 nm wavelength. The DNA band was then cut with a cutter (while being beamed with UV light, the cutter must always be washed after cutting every DNA band); (3) the

piece of agarose gel mixed with a DNA slice was inserted into 2 ml microtube; (4) the DNA in the gel then purified with reagent QIAquick PCR purification kit from Qiagen by following the instruction attached in the kit.

b. Pure DNA Labelling

The result of the purifying was then labelled through pro-sequencing PCR process using hlyA gene primers (forward). In this procedure, the labeling was done using labelled dideoxy nucleotide trophospate dye, Bigdye Termination Kit V.1.1 from the Applied Biosystem. The condition of pro-sequencing PCR was 1 cycle pre-denaturation at 96 ^{0}C for 3 minute, followed with 25 cycles which covered denaturation face at 96 ^{0}C for 10 seconds, annealing at 50 ^{0}C for 5 seconds and extension phase at 60 ^{0}C for 4 minutes.

c. Labelled DNA Precipitation

DNA product from the pro-sequencing PCR was then precipitated with these steps: (1) 150-200 µl labelled DNA was put into the new microtube and then precipitated using ethanol with addition of 1/10x NaAc 3M (pH 5,2) volume for 15 µl and 2X ethanol 100% volume for 300 ul, mixed with the vortex mixer and kept at -20^{0}C for 30 minutes; (2) Centrifugation was done at 14,000 rpm for 10 minutes, then supernatant was discarded carefully; (3) the pellet was washed with 550 µl 70% ethanol, and then centrifuged at 12,000 rpm for 5 minutes and the supernatant was discarded carefully; (4) the pellet was dried with vacuum pump for 10 minutes, and re-suspended with 10 µl of TE pH 8 solution; (5) the DNA was kept at -20^{0}C and ready for sequencing procedures.

d. Nucleotide Sequencing

After the DNA was purified using low melting agarose and labeled with hlyA primer gene, there was a sequencing procedure to get the nucleotide formation. The nucleotide sequence was determined with Bigdye Terminator Cycle Sequencing kit, EDTA HiDi formamide and special 0.5 ml tube from Applied Biosystem and also an automatic sequencing device, ABI Prism 310 Genetic Analyser.

e. Phylogenetic Analysis

Nucleotide model profile acquired from *L.monocytogenes* isolates Surabaya was then analyzed phylogenetically using a neighbor-joining analysis using Genetix Mac Ver 8.0 with hlyA gene from *L. monocytogenes* recorded in GenBank: Lineage I (KC808543), Lineage II (AY229462, AY229346, AY229499, AY229404), Lineage III (KJ504139, HQ686043, KJ504116, DQ988349) and Lineage IV (EU840690, EF030606).

RESULTS AND DISCUSSION

In Figure 1, the overall prevalence of *L.monocytogenes* in Surabaya contaminating the chicken meat samples from the supermarkets was 10% (2/20), from the mobile vendors was 0/20 (0%) and from the traditional markets was 5 % (1/20). It was seen from the band at 456 bp fragment.

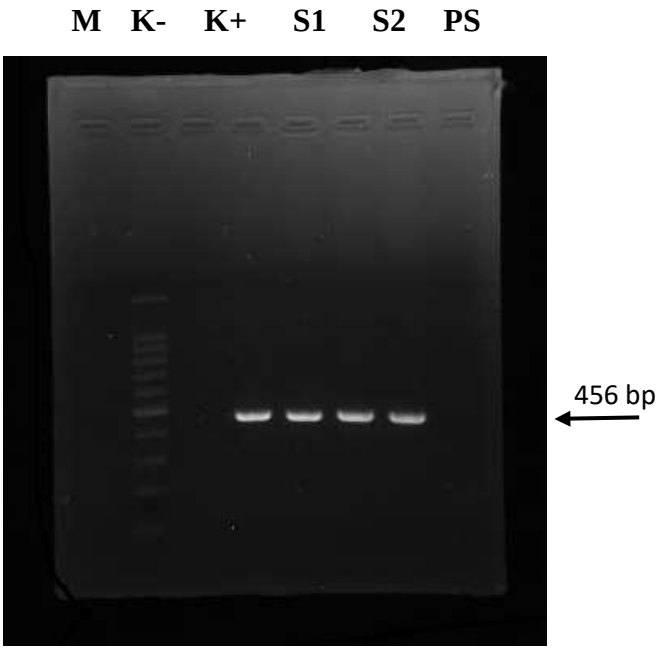

Figure 1. In 2% electrophoresis gel agar, the PCR result on *L.monocytogenes* showed contaminated chicken meat in Surabaya with 456 bp nucleotide length (M : Marker; K+ : Positive control ; K- : negative control; S1 : Sample from supermarkets; S2 : Sample from mobile vendors; PS : sample from traditional markets)

In Figure 2, from the phylogenetic analysis, compared to the 4 lineages recorded in GenBank, there were 3 isolats from Surabaya-Indonesia which showed distinct lineages.

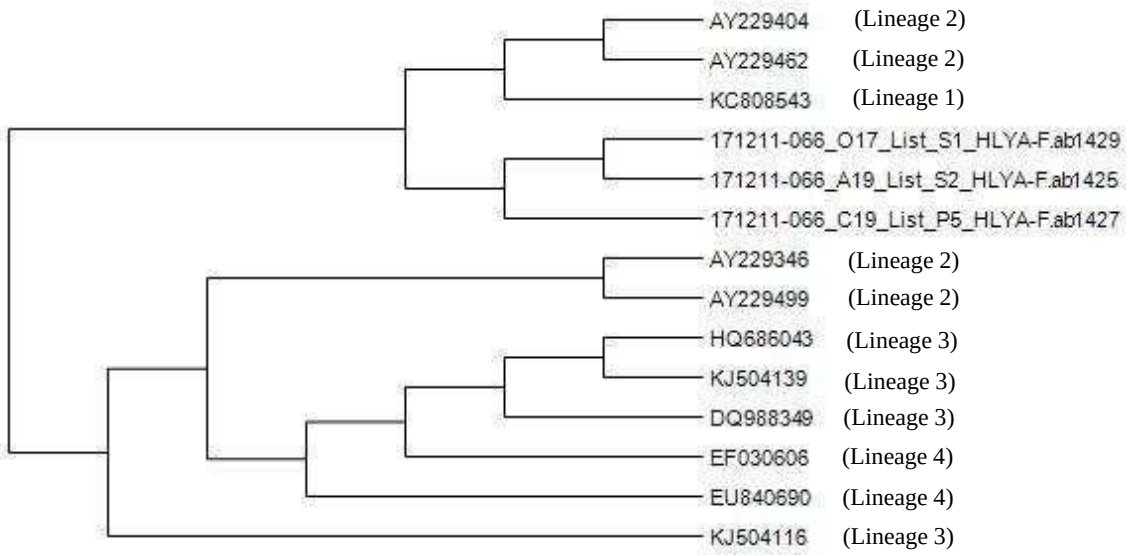

Figure 2. Phylogenetic analysis of selected strains of *L.monocytogenes* from different sources, representing the four distinct lineages, based on the listeriolysin (*hlyA*) gene. The Gene Bank accession numbers of the isolates used are given

From Table 1, we can see 3 isolates of *L. monocytogenes* contaminating the chicken meat collected from the supermarkets or traditional markets in Surabaya-Indonesia have been resistant to sulfamethonazole-trimetophrim (SXT) and amoxyllin sulbactam (MAS) but they were still sensitive to cefotaxime (CTX) and meropenem (MEM).

Table 1. Result from antibiotic resistance test of *L. monocytogenes* (present study) isolates from chicken meat in Surabaya-Indonesia

Antiobitic	Isolate S1	Isolate S2	Isolate P5
Ampicillin (AML)	Intermediate	Intermediate	Intermediate
Amoxyllin Sulbactam (MAS)	Resistant	Resistant	Resistant
Cefotaxime (CTX)	Sensitive	Sensitive	Sensitive
Meropenem (MEM)	Sensitive	Sensitive	Intermediate
Sulfamethonazole-trimetophrim (SXT)	Resistant	Resistant	Resistant

S1 : sample from supermarkets
S2 : sample from mobile vendors
PS1 : sample from traditional markets

This study was not linear with a research conducted in Bandung-Indonesia especially for the samples collected from the supermarkets which has been reported by Sugiri *et al* (2014) with 10% but lower was found in traditional market samples with 5% and mobile vendors was 0%. It was also in line with the previous study which showed *L. Monocytogenes* prevalence found in chicken meat was ranged about 15-35% where the bacteria growth happened at chicken meat which was kept at 0-8 ^{o}C temperature without having been vacuumed and in 10 days, the bacteria reached 10^{8}-10^{9} cells/gram (Ariyanti, 2010; Rivoal *et al*, 2010; Riwu, 2018)

Ampicillin, Amoxyllin Sulbactam, Cefotaxime, Meropenem, Sulfamethonazole-trimetophrim are the most widely used antibiotic drugs in broiler farms in Indonesia as feed additive, growth promoter, increase production and increase the efficiency of feed use (Etikaningrum and Iwantoro, 2017). This is associated with a high prevalence of respiratory infections, paratyphoid fever, pharyngitis, tonsillitis, varicella, typhoid fever and tuberculosis during the 2008-2009 period in human case due to antibiotic resistantce reaction (Pradipta et al, 2015)

The presence of antibiotics residues in chicken meat beyond maximum permissible limits is a matter of serious concern. Heat treatments can decrease the risk of some antibiotics groups but do not guarantee the complete degradation of these antibiotic residues present in meat. Some of the developed countries, including Denmark, Norway, Sweden, and the European Union have restricted the application of antibiotics for growth-promoting purposes. Training farmers to monitor withdrawal periods, banning the use of antibiotics as preventive treatment, and adopting the veterinary feed and drugs regulation are important parameters to mitigate the emergence of antibiotic resistance in bacteria related to broiler production (Muaz, *et al.*, 2018).

Personal hygiene of chicken meat seller in mobile vendors and traditional markets in Surabaya is very poor because none of them wear masks and gloves. Rafikah et al (2018) explain that all personnel who work in the food processing should maintain hygene, for example they must wear clean clothes and equipment, apron, cap, masks and gloves to minimize bacterial contamination on food product.

Although in general the location and storage of chicken meat in supermarkets is better and cleaner than traditional markets in Surabaya, but the storage of old ready-to-eat foods (RTE) in supermarkets allows *L. monocytogenes* to grow and thrive in food even at frozen

temperatures. This is similar with Chen *et al* (2017) that reported *L. monocytogenes* is found in food stored in refrigerators, either edible food with little or no preheating so it can be a harmful threat for someone who consume it. The presence of *L. monocytogenes* in chicken meat in traditional markets, mobile vendors and supermarkets in Surabaya showed that during the production process, from the cutting, processing to storage, contamination of *L. monocytogenes* happens, so strict monitoring procedures are needed for it.

Antunes *et al.* (2002) and Srinivasan, *et al.* (2005) showed that *L. monocytogenes* isolates from poultry meat and dairy product exhibited resistance to one or more antibiotics, indicating animal product as a potential carrier for antibiotic-resistant foodborne diseases. *Listeria monocytogenes* is naturally susceptible to β-lactams group antibiotic such as penicillin amoxcyllin, meropenem and ampicillin, or combined with an aminoglycoside (gentamicin) as standard therapy for human listeriosis (Sanlibaba, 2018; Al-Nabulsi, 2015). β-lactams against *L. monocitogenes* by inhibit the synthesis of bacterial cell wall peptidoglycan (Miller *et al.*, 2014). For patients who allergic to lactams antibiotic, sulfamethoxazole – trimethoprim can be used (Swaminathan and Gerner-Smodth, 2007), however Srinivasan *et al.* (2005) and Yucel (2005) has reported that this antibiotic resistant with *L. monocytogenes* isolated from meat and dairy farm product.

Resistant microbes strains that emerged owing to antibiotic misdose can tolerate the effect of antibiotics at inhibitory concentration levels (CDC, 2010). Consumption of meat contaminated with such resistant strains after improper processing or mismanagement enhances the chances of their transmission in humans (Van Den Bogaard *et al.*, 2000). *L. monocytogene* may transfer resistant genes to human natural microbes through mutation, plasmid mediation (self-tranferable plasmids and mobilizable plasmids), conjugative transposons, and efflux pumps (Hershberger *et al.*, 2005; Gould, 2008; Charpentier *et al.*, 1999, Godreuil *et al.*, 2003). The presence of such resistant strains can reduce the effectiveness of antibiotics used to treat infected individuals (Chastre, 2008)

Vazquez *et al* (2001) proved that there was correlation between the virulence level and the strain type from the bacteria was isolated, the clinical origin has a lower virulence than the origin of the food. To ensure the presence of *L. monocytogenes* in the original environment, identification for one major virulence factor is a better choice. Among the various virulence

factors, LLO (58 kDa hemolysin protein encoded by the hlyA gene) is a major virulence factor and pathogenic marker for detecting *Listeria sp*. The phylogenetic study of *L. monocytogenes* is essential to improve our understanding on how *L. monocytogenes* is transmitted from animals or the environment through food to humans. The results showed that all three isolates contaminating chicken meat in Surabaya formed distinctive lineages, as they were not included in 4 previously reported lineages (Mohamed *et al*, 2016).

In the present study, The result of antibiotic resistance test showed that the three isolates from Surabaya-Indonesia that contaminated chicken meat in supermarkets, mobile vendors and traditional markets were resistant to some old-generation antibiotics such as sulfamethonazole-trimetophrim (SXT) and amoxyllin sulbactam (MAS) but they were still sensitive to new generation antibiotics such as cefotaxime (CTX) and meropenem (MEM).

Meropenem in monotherapy has been found actively against interacelullar *L. monocitegenes* infection on meningitis case in the experimenlat studi , it was 10-fold more potent than ampicillin and ertapenem (Lemaire, 2005). Meropenem was bacteriostatic effect after 5 hours treatment and start bactericidal effect after 24 hours (Carryn *et al.,* 2003), it coud intiate bacterial severe cell damages (Lemaire 2005).

Cefotaxime is a bactericidal derived from cephalosporin has a broad-spectrum activity against gram-positive microorganisms and exceptional activity against most gram-negative microorganisms by interfering with synthesis of their cell walls. It is widely considered to be the antibiotic of choice for the management of neonatal meningitis and sepsis caused by gram-negative bacteria including *L. monocytogenes* (Pacifici and Marchini, 2017). Cefotaxime has received wide acceptance as a first-line antibiotic for many infections in neonates, infants and children. cefotaxime caused a significant enhancement of IL-2 production by cells and increased the secretion of TNF α by peripheral blood mononuclear cells, it is also suggested that may modify the host immune response. Cefotaxime is a safe and effective antibiotic in treating meningitis bacterial infections which is 80% of these microorganism are resistant with ampicillin (Pacifici and Marchini, 2017)

This result was different from previous studies that *Listeria sp* is generally still sensitive to all antibiotics (Jamali *et al*, 2015). It cannot be separated from the habit of inappropriate use of antibiotics in livestock industry and communities in some developing countries including in Indonesia. Therefore, efforts to raise awareness on the importance of the correct use of

antibiotics regarding the dose and duration should be a concern of both government and communities.

CONCLUSION

The contamination of *L.monocytogenes* in chicken meat in supermarkets, mobile vendors and traditional markets indicates the potential listeriosis. Moreover, the three isolates found in Surabaya-Indonesia were included as isolates with the distinctive lineages and already resistant to old generation antibiotics such as: sulfamethonazole-trimetophrim (SXT) and amoxyllin sulbactam (MAS). Therefore, surveillance policies for potential food contamination and antibiotic sensitivity of *L.monocytogenes* are required, while also ensuring effective antibiotic treatment.

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REFERENCES

Al-Nabulsi, A.A., Osaili, T.M., Shaker, R.R. 2015. Effects of osmotic pressure, acid, or cold stresses on antibiotic susceptibility of Listeria monocytogenes. Food Microbiology, 46: 154–160.

Ariyanti, T. 2010. Bacteria Listeria monocytogenes as food contaminants from animal origin (foodborne disease). Wartazoa. 20 (2): 94-102.

Antunes P, Reu C, Sousa JC, Pestana N, and Peixe L. 2002. Incidence and susceptibility to antimicrobial agents of Listeria spp and Listeria monocytogenes isolated from poultry carcasses in Porto, Portugal. J Food Prot 2002;65:1888–1893.

Lemaire, S., Bambeke, F.V., Tulkens, P., Mingeot-Leclercq, M.P. 2005. Activity of Three B-Lactams (Ertapenem, Meropenem, and Ampicillin) against intraphagocytic *Listeria monocytogenes* and *Staphylococcus aureus.* J. Antimicrobial Chemoterapy 2005: 1-5

Carryn, S., Van Bambeke, F., Mingeot-Leclercq, M. P. et al. 2003. Activity of b-lactams (ampicillin, meropenem), gentamicin, azithromycin and moxifloxacin against intracellular Listeria monocytogenes in a 24 h THP-1 human macrophage model. Journal of Antimicrobial Chemotherapy 51, 1051–2.

Charpentier E, Gerbaud G, Jacquet C, Rocourt J, and Courvalin P. 1995.Incidence of antibiotic resistance in Listeria spp. J Infect Dis.172:277–281.

Chastre, J. 2008. Evolving problems with resistant pathogens. Clin. Microbiol. Infect. 14:3–14.

Chatterjee, SS, Hossain, H., Otten, S., Kuenne, C., Kuchmina, K., Machata, S., Domann, E., Chakraborty, T., and Hain, T. 2006. Intracellular gene expression profile of Listeria monocytogenes. Infect Immun. 74 (2): 1323–1338.

Chen, JQ., Regan, P., Laksanalamai, P., Healey, S., Hu, Z. 2017. Prevalence and methodologies for detection, characterization and subtyping of Listeria monocytogenes and L. ivanovii in foods and environmental sources. Food Science and Human Wellness. 6 (3). 97-120.

CDC -Centers for Disease Control and Prevention (CDC). 2010. Get smart: know when antibiotics work. CDC, Atlanta.

CDC - Centers for Disease Control and Prevention. 2011. Multistate outbreak of listeriosis associated with Jensen farms cantaloupe. United States of America

Etikaningrum and Iwantoro, S. 2017. Study of antibiotic residues in poultry products in Indonesia. Journal of Animal Husbandry Production and Technology. Vol. 09 (1). 29-33

Godreuil S, Galimand M, Gerbaud G, Jacquet C, and Courvalin P. 2003. Efflux pump lde is associated with fluroquinolone resistance in Listeria monocytogenes. Antimicrob Agents Chemother;47:704–708.

Gould, I. 2008. The epidemiology of antibiotic resistance. Int. J. Antimicrob. Agents 32:S2–S9.

Harsoyo and Andini, L. 2002. Effect of irradiation and storage of Listeria monocytogenes inoculated in goat meat. Pros. Nas. Animal Husbandry and Veterinary Technology. Bogor, 30 September-1 October 2002. Center for Animal Husbandry, Bogor. 334-337

Hershberger, E., S. F. Oprea, S. M. Donabedian, M. Perri, P. Bozigar, P. Bartlett, and M. J. Zervos. 2005. Epidemiology of antimicrobial resistance in enterococci of animal origin. J. Antimicrob. Chemother. 55:127–130

Jamali, H., Chai, L.C., Thong, K.L. 2013. Detection and isolation of Listeria spp. and Listeria monocytogenes in ready-to-eat foods with various selective culture media. Food Control. 32: 19-24.

Jamali, H., Paydar, M., Ismail, S., Looi, CY, Wong, WF, Radmehr, B., Abedini, A. 2015. Prevalence, antimicrobial susceptibility and virulotyping of Listeria species and Listeria monocytogenes isolated from open- water fish markets. BMC Microbiology. 15: 144.

Janzten, M.M., Navas, J., Mreno, J., Lpoez and Suarez, J.V. 2006. Detection of Listeria monocytogenes in food using commercial methods: Chromogen media-PCR. J. Agricultural Research. 4 (3): 235-247.

Liu, D. 2008. Epidemiology. In D. Liu (Ed.), Handbook of Listeria monocytogenes. New York: CRC Press: 27-30.

Meloni, D., Galluzzo, P., Mureddu, A., Piras, F., Griffiths, M., & Mazzette, R. 2009. Listeria monocytogenes in RTE foods marketed in Italy: prevalence and automated EcoRI ribotyping of the isolates. International Journal of Food Microbiology. 129(2): 166-172

Miller, W.R., Munita, J.M., Arias, C.A. 2014. Mechanisms of antibiotic resistance in enterococci. Expert Review of AntiInfective Herapy, 12(10): 1221–1236

Mohamed, Y., Reda, W.W., Abdel-Moein, K., El-Razik, K.A.A., Barakat, A.M.A., El Fadaly, H.A., Hassanain, N.A., Hegazi, A.G. 2016. Prevalence and phylogenetic characterization of Listeria monocytogenes isolated from processed meat marketed in Egypt. Journal of Genetic Engineering and Biotechnology. 14: 119-123

Muaz, K., Riaz, M., Akhtar, S., Park, S., Ismail, A. Antibiotic Residues in Chicken Meat: Global Prevalence, Threats, and Decontamination Strategies: a Review. J. Food Protection, 8(14): 619 - 627

National Standardization Board. 2008. Indonesian National Standard (SNI). SNI No. 2897: 2008 concerning methods of testing microbial contamination in meat, eggs and milk, and their processed products. Jakarta. BSN.

Pradipta IS., Ronasih, E., Kartikawati, AD., Hartanto, H., Amelia, R., Febrina, E., and Abdulah, E. 2015. Three years of antibacterial consumption in Indonesian Community Health Centers: The application of anatomical therapeutic chemical / defined daily doses and drug utilization 90% method to monitor antibacterial use. J Family Community Med. Vol. 22 (2): 101-105.

Nwachukwu, N.C., Orji, F.A., Iheukwumere, I., Ekeleme, U.G. 2010. Antibiotic resistant environmental isolates of Listeria monocytogenes from anthropogenic lakes in Lokpa-Ukwu, Abia State of Nigeria.Aust. J. Basic Appl. Sci 4: 1571-1576.

Pacifici, G.M., Marchini, G. Clinical Pharmacology of Cefotaxime in Neonates and Infants: Effects and Pharmacokinetics. Int J Pediatr 2017; 5(11): 6111-6138

Paziak-Domanska, B., Bogulawska, E., Wiekowska-Szakiel, M., Kotlowski, R., Rozalska, B., Chmiela, M., Kur, J., Dabrowksi, W., and Rudnicka, W. 1999 Evaluation of the API test, phathylinlinositol-specific phospholipase C activity and PCR method in identification of Listeria monocytogenes in meat foods. FEMS. Mircobiol. Lett. 171: 209-214.

Ponniah, J., Robin, T., Paie, M. S., Radu, S., Ghazali, F. M., Kqueen, C. Y., et al. 2010. Listeria monocytogenes in raw salads vegetables sold at retail level in Malaysia. Journal of Food Control. 21: 774-778.

Rafikah, N., Irmawaty., Kiramang, K. 2018. The level of contamination of Escherichia coli bacteria in chicken meat sold in Makassar traditional markets. Proceedings of the Indonesian National Megabiodiversity Seminar. April 9, 2018.

Rivoal, K., Queguiner, S., Boscher, E., Bougeard, S., Ermel, G., Salvat, G., Federighi, M., Jugiau, F and Protais, J. 2010. Detection of Listeria monocytogenes in raw and pasteurized liquid whole eggs and characterization by PFGE. Int. J. Food Microbiol. 138: 56 - 62.

Riwu, K.H.P. 2018. Identification of Listeria monocytogenes bacteria in chicken meat in several supermarkets and traditional markets in the city of Surabaya by using the polymerase chain reaction (PCR) method. Thesis. Master of Science Disease and Veterinary Health Study Program, Faculty of Veterinary Medicine, Airlangga University.

Rodrigues, C.S., de Sa, C.V.G.C., de Melo, C.B. 2017. An overview of Listeria monocytogenes contamination in ready to eat meat, dairy and fishery foods. Ciencia Rural, Santa Maria. 47 (2): 1-8

Sanlibaba, P., Cakmak, G.A., Tezel, B.U. 2018 Prevalence and Antibiotic Resistantce of *Listeria monocytogenes* isolated from Ready-to-Eat Food in Turkey. Journal od Food Quality 2019: 1-11

Soni, D.K., Dubey, S.K. 2014. Phylogenetic analysis of the Listeria monocytogenes based on sequencing of 16SrRNA and hlyA genes. Molecular Biology Reports. 41 (12). 8219-8229.

Srinivasan V, Nam HM, Nguyen LT, Tamilselvam B, Murinda SE, and Oliver SP. 2005. Prevalence of antimicrobial resistance genes in Listeria monocytogenes isolated from dairy farms. Foodborne Pathog Dis 2005;2:201–210.

Sugiri, Y.D., Gölz, G., Meeyam, T., Baumann, M.P., Kleer J., Chaisowwong W., Alter T. 2014. Prevalence and antimicrobial susceptibility of Listeria monocytogenes on chicken carcasses in Bandung, Indonesia. J. Food Prot. 77 (8). 1407-1410.

Swaminathan B and Gerner-Smidt P. 2007. The epidemiology of human listeriosis. Microbes Infect ;9:1236–1243

Van Den Bogaard, A. E., and E. E. Stobberingh. 2000. Epidemiology of resistance to antibiotics: links between animals and humans. Int. J. Antimicrob. Agents 14:327–335

Vazquez-Boland, JA, Kuhn, M., Berche, P., Chakraborty, T., Domínguez-Bernal, G., Goebel, W., González-Zorn, B., Wehland, J., and Kreft, J. 2001. Listeria pathogenesis and molecular virulence determinants. Clin Microbiol Rev. 14 (3): 584–640.

Yucel N, Citak S, and Onder M. 2005. Prevalence of antibiotic resistance of Listeria species in meat products in Ankara, Turkey. Food Microbiol;22:241–245.

Phylogenetic Analysis and Antibiotics Resistance Of *Listeria Monocytogenes* Contaminating Chicken Meat In Surabaya-Indonesia

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Abstract

The objective of this study was to identify phylogenetic analysis and the antibiotic resistance of *Listeria monocytogenes* contaminating chicken meat in Surabaya. 60 Chicken meat samples were collected from supermarkets, mobile vendors, and traditional markets in Surabaya. A selective medium is used for isolation and identification of *Listeria monocytogenes* by chopping 25 grams of the chicken meat and put it into the sterilized Erlenmeyer flasks. Some methods were used for the identification procedures, such as biochemical and morphological tests, antibiotic resistance test, PCR and sequencing, also a phylogenetic analysis conducted by a neighbor-joining analysis using Genetix Mac ver 8.0 with hlyA genes of *Listeria monocytogenes* recorded in GenBank, such as : Lineage I (KC808543), Lineage II (AY229462, AY229346, AY229499, AY229404), Lineage III (KJ504139, HQ686043, KJ504116, DQ988349) and Lineage IV (EU840690, EF030606). The result show that prevalence of *L.monocytogenes* in Surabaya contaminating the chicken meat samples from the supermarkets was 10% (2/20), from the mobile vendors was 0/20 (0%) and from the traditional markets was 5 % (1/20). It was seen from the band at 456 bp fragment. Furthermore, three isolates found in Surabaya were included in the new lineages which were resistant to old generation antibiotics such as Sulfamethonazole-Trimetophrim (SXT) and Amoxyllin Sulbactam (MAS) but they were still sensitive to new generation antibiotics such as Cefotaxime (CTX) and Meropenem (MEM).

Keywords : *Listeria monocytogenes*, Chicken meat, Phylogenetic, Antibiotic

INTRODUCTION

The most popular animal protein source in Indonesia is chicken meat. It is cheap, deliciouse and easy to cook become various dishes. In 2018, Indonesia produced 2,144,013 Ton broiler meat, it increased than on 2016 only 1,905,497.28 Ton and the demand grow fast every year (BPS, 2019). One of the bigest producer of broiler chicken in Indonesia is East Java province. The harvest were collected in Surabaya as the capital city of East Java and also the biggest city in Indonesia after Jakarta before it distribute to other cities and widely bought by customer from supermarkets, traditional markets, and mobile vendors.

As the increase of chicken meat demand, people's anxiety for food-borne diseases also arises. One of the agents causing it is *Listeria monocytogenes* (Jamali *et al*, 2013). This bacteria can be found anywhere in food, water, soil, vegetables, animals and humans. In addition, it also has high potential to infect humans and animals with high mortality rates (Liu, 2008). Human listeriosis has been reported in the USA caused by consuming cantaloupe, smoked fish, marinated products, meat products, and vegetables which is contaminated with *L. monocytogenes* (CDC, 2011; Meloni *et al*, 2009).

According to Janzten *et al* (2006), Listeria genus is categorized as positive gram bacteria, that contain 6 species of *Listeria; Listeria monocytogenes, Listeria innouca, Listeria seeligeri, Listeria welshimeri, Listeria ivanovii and Listeria grayi.* One of them is known as the most pathogenic to humans is *L.monocytogenes* which consists of 4 lineages (lineage I, II, III and IV) (Mohamed *et al*, 2016). This bacteria can grow at the temperature of 1^0C-45^0C and they can proliferate at cold or freezing temperatures.

Fast food such as non-pasteurized meat and milk products stored for a long time at 4 °C is a potential source of *L. monocytogenes* infection. Sometimes *L. monocytogenes* can also be found in processed food products. *L. monocytogenes* contamination after food being processed is a critical point for human health. Therefore, considerable knowledge is needed so the prevention of *L. monocytogenes* bacteria transmission in the environment or in food products from livestock and its dairy products can be done appropriately. Furthermore, a fast and accurate detection technique for the presence of *L. monocytogenes* in food is needed so the infected can be immediately treated (Ariyanti, 2010). Rodriguez *et al* (2017) also reported that the products most infected with *L. monocytogenes* are poultry and beef meat stored in refrigerator, smoked and

fresh meat. Human listeriosis caused by *L. monocytogenes* is an intermittent disease with mild to severe flu symptoms as well as meningitis and septicemia manifestation. The group at risk are pregnant women, and immunocompromised people. In pregnant women, it can lead to abortion, premature birth and birth defects.

In Indonesia, food poisoning due to *Listeria sp* infection, especially *L. monocytogenes* is less frequent than from *E. Coli* and *Salmonella* bacteria. *L. monocytogenes* contamination in Indonesia has not been widely reported as in developed countries (Harsoyo and Andini, 2002). In Malaysia, it is reported that various local food sold by street vendors is ready to eat (RTE) food. High prevalence of pathogens is found in this kind of food. It is also found in raw food and RTE products sold in hypermarkets, although their hygieneis assumed to be better, but pathogens of foodborne disease and *L. monocytogenes* are also identified. (Ponniah *et al*., 2010).

Typically, *L. monocytogenes* is susceptible to a wide range of antibiotics, although some isolates have been reported resistant to many antibiotics (Nwachukwu *et al*, 2010). Some of the virulence markers of *L. monocytogenes* such as listeriolysin O (encoded by the hlyA gene) have a role to regulate virulence and pathogenicity (Soni and Dubey, 2014). Even in food, bacteria originated from environment can result in the expression of varied virulence genes that will result in different infection level (Chatterjee *et al*, 2006).

According to Harsoyo and Andini (2002), Indonesian National Standards have actually been established that food products of animal origin in Indonesia should not contain *Listeria sp* bacteria, as well as in the US and Europe. The Food and Agricultural Organization (FAO) guidelines also emphasize proactive and risk-based modern food security system. Therefore, it is necessary to identify diseases affecting the population and the presence of pathogens in food and to establish risk mitigation measures (Dubrugas and Perez-Gutierrez, 2008).

The objective of this study was to identify phylogenetic analysis and the antibiotic resistance of *L. monocytogenes* contaminating chicken meat in Surabaya-Indonesia, especially for Ampicillin, Amoxylin sulbactam, Cefotaxime, Meropenem, Sulfamethonazole-trimetophrim which are commonly used by broiler farmers as antibiotic growth promoters and therapeutic antibiotic in Indonesia (Etikaningrum and Iwantoro, 2017).

MATERIALS AND METHODS

Study Design, Study Area and Sampling.

a cross-sectional prospective study was carried out in Surabaya metropolitan city. 60 raw chicken meat samples was collected from supermarkets, traditional markets, and mobile vendors as the main meat suppliers for Surabaya people, which was contained 20 sample from each place. Simple random sampling technique was employed for the all samples were collected aseptically from randomly selected supermarkets, traditional markets, and mobile vendors in Surabaya. The meats were placed in sterile leakproof container in cold chain box. The samples were transported to the Tropical Disease Diagnosis Center (TDDC) Institute Tropical Disease Airlangga University.

Biochemical and Morphological Test

A selective medium is used for isolation and identification of *L. monocytogenes*. 25 grams the chicken meat was put into the sterilized Erlenmeyer flasks, and added 225 ml Buffered Listeria Enrichment then homogenized using a vortex mixer for 2 minutes and incubated at 30 ^{0}C for 24-28 hours (National Standardization Board, 2008). After incubation, identification was conducted by taking bacterial suspension from the Erlenmeyer flasks with a transfer loop then grazed it to Palcam medium and then incubated at 37^{0}C for 24-48 hours. The colony of *L. monocytogenes* on green Palcam agar medium was surrounded by black zone (National Standardization Board, 2008). The positive samples undergone blood medium hemolysis test at 37^{0}C for 24 hours. It was conducted to see the forming β-hemolysis. Gram staining processing showed purple or violet color in the microscopic observation (National Standardization Board, 2008). And then Sulfide Indole Motility (SIM) test was done to identify sulfide, indole and bacterial movement after being incubated for at 37^{0}C for 24 hours (National Standardization Board, 2008). Triple sugar iron test (TSIA) and Glucose test was conducted to identify fermentation of glucose, lactose and sucrose (National Standardization Board, 2008). Methyl Red-Voges Proskauer (MR-VP) was performed to show pink color and the formation of acethyl-methyl carbinol (National Standardization Board, 2008). The confirmation test (CAMP test) was used to identify umbrella-like bacterial growth due to excessive hemolytic zone around the grazed *Staphylococcus aureus* and *Rhodococcus equi* (National Standardization Board, 2008).

Antibiotics Resistance Test

The prepared pure culture was taken by sterilized cotton swab and spread over the surface of Muller Hinton Agar (MHA), and waited for 5 minutes. Paper disc filled contained antibiotics was put on MHA with pure culture separated 25-30 nm, then incubated at 35^0C for 24 hours (Riwu, 2018). The antibiotics sensitivity test was measured base on the inhibition diameter used vernier calipers. The antibiotics tested were Ampicillin, Amoxylin sulbactam, Cefotaxime, Meropenem, Sulfamethonazole-trimetophrim and then the result was interpreted using inhibition zone table by National Community for Clinical Laboratory Standard (NCCLS) (National Standardization Board, 2008).

***Listeria monocytogenes* Detection with PCR**

DNA extraction of *L. monocytogenes* was conducted with Kit Qiagen. 5 µl suspension from the extraction was used directly as template for CR amplification of hlyA gene fragments. 20 µl PCR reaction consisted of 12.5 µl master mix, 0.5 µl destilated water, 0,5 µl Forward primer (F), 0.5 µl reverse primer (R) and 5 µl DNA template. The primers used were F :5'-GCAGTTGCAAGCGCTTGGAGTGAA-3' and R : 5'-GCAACGTATCCTCCAGAGTGATCG-3' (Paziak-Domanska *et al*, 1999). PCR condition included pre-denaturation 95 ^{0}C for 5 minutes, denaturation 95 ^{0}C for 30 seconds, annealing 54.6 ^{0}C for 30 seconds, extention 72 ^{0}C for 1 minute 30 seconds and final extention 72 ^{0}C for 5 minutes. There were 35 cycles of PCR process. 5 µl PCR product was put into 2% electrophoresis gel and then used in electrophoresis medium for 60 minutes with 100 volt. After the electrophoresis, gel was then taken for observation with UV light. DNA target fragments visualization shown at 456 bp with UV transilluminator (Paziak-Domanska *et al*, 2015).

Sequencing and Phylogenetic Analysis

a. PCR Result Product Purifying using Low Melting Agarose Methods

PCR product acquired then purified. The steps were: (1) 2% agarose gel was prepared for L Agarose (Low Melting Agarose) using ethidium bromide 1 mg/ml; (2) DNA from the PCR

result about 5 ul was added with loading buffer for 1 ul. During application, the mixture was filled into every other slots. After the electrophoresis, the result was seen with UV light with 365 nm wavelength. The DNA band was then cut with a cutter (while being beamed with UV light, the cutter must always be washed after cutting every DNA band); (3) the piece of agarose gel mixed with a DNA slice was inserted into 2 ml microtube; (4) the DNA in the gel then purified with reagent QIAquick PCR purification kit from Qiagen by following the instruction attached in the kit.

b. Pure DNA Labelling

The result of the purifying was then labelled through pro-sequencing PCR process using hlyA gene primers (forward). In this procedure, the labeling was done using labelled dideoxy nucleotide trophospate dye, Bigdye Termination Kit V.1.1 from the Applied Biosystem. The condition of pro-sequencing PCR was 1 cycle pre-denaturation at 96 ^{0}C for 3 minute, followed with 25 cycles which covered denaturation face at 96 ^{0}C for 10 seconds, annealing at 50 ^{0}C for 5 seconds and extension phase at 60 ^{0}C for 4 minutes.

c. Labelled DNA Precipitation

DNA product from the pro-sequencing PCR was then precipitated with these steps: (1) 150-200 µl labelled DNA was put into the new microtube and then precipitated using ethanol with addition of 1/10x NaAc 3M (pH 5,2) volume for 15 µl and 2X ethanol 100% volume for 300 ul, mixed with the vortex mixer and kept at -20^{0}C for 30 minutes; (2) Centrifugation was done at 14,000 rpm for 10 minutes, then supernatant was discarded carefully; (3) the pellet was washed with 550 µl 70% ethanol, and then centrifuged at 12,000 rpm for 5 minutes and the supernatant was discarded carefully; (4) the pellet was dried with vacuum pump for 10 minutes, and re-suspended with 10 µl of TE pH 8 solution; (5) the DNA was kept at -20^{0}C and ready for sequencing procedures.

d. Nucleotide Sequencing

After the DNA was purified using low melting agarose and labeled with hlyA primer gene, there was a sequencing procedure to get the nucleotide formation. The nucleotide sequence was determined with Bigdye Terminator Cycle Sequencing kit, EDTA HiDi formamide

and special 0.5 ml tube from Applied Biosystem and also an automatic sequencing device, ABI Prism 310 Genetic Analyser.

e. Phylogenetic Analysis

Nucleotide model profile acquired from *L.monocytogenes* isolates Surabaya was then analyzed phylogenetically using a neighbor-joining analysis using Genetix Mac Ver 8.0 with hlyA gene from *L. monocytogenes* recorded in GenBank: Lineage I (KC808543), Lineage II (AY229462, AY229346, AY229499, AY229404), Lineage III (KJ504139, HQ686043, KJ504116, DQ988349) and Lineage IV (EU840690, EF030606).

RESULTS AND DISCUSSION

In Figure 1, the overall prevalence of *L.monocytogenes* in Surabaya contaminating the chicken meat samples from the supermarkets was 10% (2/20), from the mobile vendors was 0/20 (0%) and from the traditional markets was 5 % (1/20). It was seen from the band at 456 bp fragment.

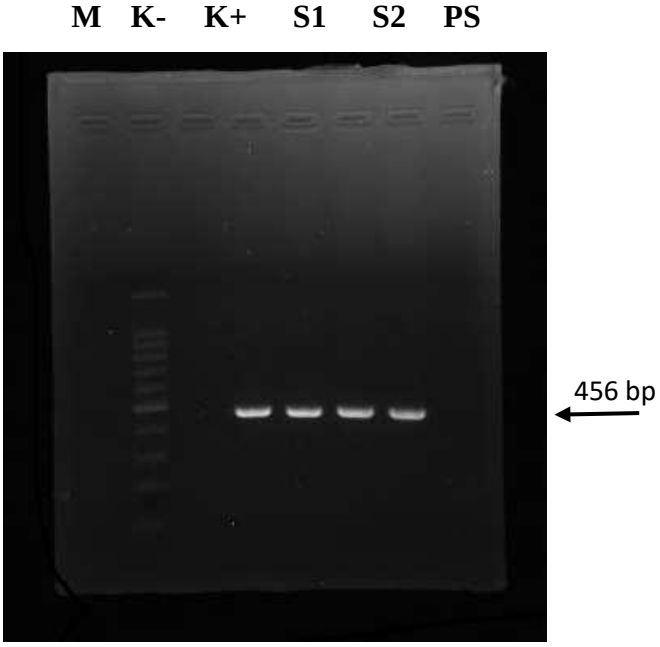

Figure 1. In 2% electrophoresis gel agar, the PCR result on *L.monocytogenes* showed contaminated chicken meat in Surabaya with 456 bp nucleotide length (M : Marker;

K+ : Positive control ; K- : negative control; S1 : Sample from supermarkets; S2 : Sample from mobile vendors; PS : sample from traditional markets)

In Figure 2, from the phylogenetic analysis, compared to the 4 lineages recorded in GenBank, there were 3 isolats from Surabaya-Indonesia which showed distinct lineages.

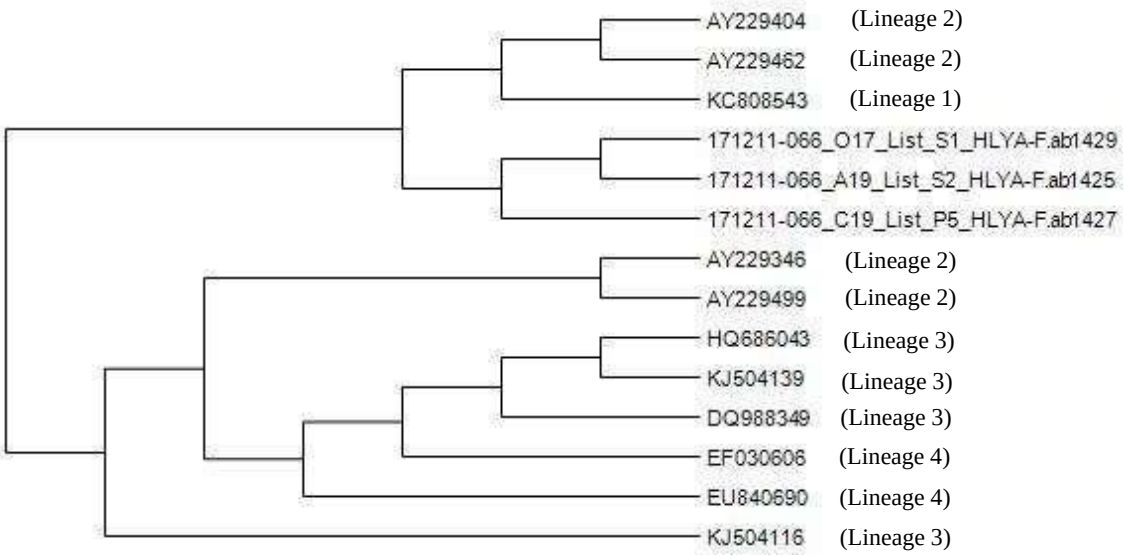

Figure 2. Phylogenetic analysis of selected strains of *L.monocytogenes* from different sources, representing the four distinct lineages, based on the listeriolysin (*hlyA*) gene. The Gene Bank accession numbers of the isolates used are given

From Table 1, we can see 3 isolates of *L. monocytogenes* contaminating the chicken meat collected from the supermarkets or traditional markets in Surabaya-Indonesia have been resistant to sulfamethonazole-trimetophrim (SXT) and amoxyllin sulbactam (MAS) but they were still sensitive to cefotaxime (CTX) and meropenem (MEM).

Table 1. Result from antibiotic resistance test of *L. monocytogenes* (present study) isolates from chicken meat in Surabaya-Indonesia

Antiobitic	Isolate S1	Isolate S2	Isolate P5
Ampicillin (AML)	Intermediate	Intermediate	Intermediate
Amoxyllin Sulbactam (MAS)	Resistant	Resistant	Resistant

Cefotaxime (CTX)	Sensitive	Sensitive	Sensitive
Meropenem (MEM)	Sensitive	Sensitive	Intermediate
Sulfamethonazole-trimetophrim (SXT)	Resistant	Resistant	Resistant

S1 : sample from supermarkets
S2 : sample from mobile vendors
PS1 : sample from traditional markets

This study was not linear with Sugiri *et al* (2014) that showed that *L. Monocytogenes* contamination founde in supermarket about 10%, traditional markets about and mobile vendors was 0% in Bandung City Indonesia. However It was also in line with the previous study which reported that *L. Monocytogenes* prevalence found in chicken meat was ranged about 15-35% where the bacteria growth well in chicken meat which was kept at 0-8 ^{o}C temperature without having been vacuumed and in 10 days the bacteria reached 10^{8}-10^{9} cells/gram (Ariyanti, 2010; Rivoal *et al*, 2010; Riwu, 2018)

Ampicillin, Amoxyllin Sulbactam, Cefotaxime, Meropenem, Sulfamethonazole-trimetophrim are the most widely used antibiotic drugs in broiler farms in Indonesia as feed additive, growth promoter, increase production and increase the efficiency of feed use (Etikaningrum and Iwantoro, 2017). This is associated with a high prevalence of respiratory infections, paratyphoid fever, pharyngitis, tonsillitis, varicella, typhoid fever and tuberculosis during the 2008-2009 period in human case due to antibiotic resistantce reaction (Pradipta et al, 2015)

The presence of antibiotics residues in chicken meat beyond maximum permissible limits is a matter of serious concern. Heat treatments can decrease the risk of some antibiotics groups but do not guarantee the complete degradation of these antibiotic residues present in meat. Some of the developed countries, including Denmark, Norway, Sweden, and the European Union have restricted the application of antibiotics for growth-promoting purposes. Training farmers to monitor withdrawal periods, banning the use of antibiotics as preventive treatment, and adopting the veterinary feed and drugs regulation are important parameters to mitigate the emergence of antibiotic resistance in bacteria related to broiler production (Muaz, *et al.,* 2018).

Personal hygiene of chicken meat seller in mobile vendors and traditional markets in Surabaya is very poor because none of them wear masks and gloves. Rafikah et al (2018) explain that all personnel who work in the food processing should maintain hygene, for example

they must wear clean clothes and equipment, apron, cap, masks and gloves to minimize bacterial contamination on food product.

Although meat storage in supermarkets is better and cleaner than traditional markets in Surabaya, the condition of ready-to-eat foods (RTE) storage and etalase in supermarkets allows *L. monocytogenes* to grow and thrive in food even at frozen temperatures. Chen *et al* (2017) reported that *L. monocytogenes* was found in the food stored refrigerators, either edible food with little or no preheating process, so it can be a harmful threat for consumer. The presence of *L. monocytogenes* in chicken meat in traditional markets, mobile vendors and supermarkets in Surabaya showed that during the production process, from the cutting to storage, *L. monocytogenes* contamination occured , therefore strict monitoring and preventive procedures are needed minimize it.

Antunes *et al.* (2002) and Srinivasan, *et al.* (2005) explained that *L. monocytogenes* isolates from poultry meat and dairy product exhibited resistance to one or more antibiotics, it indicating animal products are potential carrier for antibiotic-resistant foodborne diseases. *Listeria monocytogenes* is naturally susceptible to β-lactams group antibiotic such as penicillin amoxcyllin, meropenem and ampicillin, or combined with an aminoglycoside (gentamicin) as standard therapy for human listeriosis (Sanlibaba, 2018; Al-Nabulsi, 2015). β-lactams against *L. monocitogenes* by inhibit the synthesis of bacterial cell wall peptidoglycan (Miller *et al.*, 2014). For patients who allergic to lactams antibiotic, sulfamethoxazole – trimethoprim can be used (Swaminathan and Gerner-Smodth, 2007), however Srinivasan *et al.* (2005) and Yucel (2005) has reported that this antibiotic resistant with *L. monocytogenes* isolated from meat and dairy farm product.

Resistant microbes strains that emerged owing to antibiotic misdose can tolerate the effect of antibiotics at inhibitory concentration levels (CDC, 2010). Consumption of meat contaminated with such resistant strains after improper processing or mismanagement enhances the chances of their transmission in humans (Van Den Bogaard *et al.*, 2000). *L. monocytogene* may transfer resistant genes to human natural microbes through mutation, plasmid mediation (self-tranferable plasmids and mobilizable plasmids), conjugative transposons, and efflux pumps (Hershberger *et al.*, 2005; Gould, 2008; Charpentier *et al.*, 1999, Godreuil *et al.*, 2003). The presence of such resistant strains can reduce the effectiveness of antibiotics used to treat infected individuals (Chastre, 2008)

Vazquez *et al* (2001) proved that there was correlation between the virulence level and the strain type from the bacteria was isolated, the clinical origin has a lower virulence than the origin of the food. To ensure the presence of *L. monocytogenes* in the original environment, identification for one major virulence factor is a better choice. Among the various virulence factors, LLO (58 kDa hemolysin protein encoded by the hlyA gene) is a major virulence factor and pathogenic marker for detecting *Listeria sp*. The phylogenetic study of *L. monocytogenes* is essential to improve our understanding on how *L. monocytogenes* is transmitted from animals or the environment through food to humans. The results showed that all three isolates contaminating chicken meat in Surabaya formed distinctive lineages, as they were not included in 4 previously reported lineages (Mohamed *et al*, 2016).

In the present study, The result of antibiotic resistance test showed that the three isolates from Surabaya-Indonesia that contaminated chicken meat in supermarkets, mobile vendors and traditional markets were resistant to some old-generation antibiotics such as sulfamethonazole-trimetophrim (SXT) and amoxyllin sulbactam (MAS) but they were still sensitive to new generation antibiotics such as cefotaxime (CTX) and meropenem (MEM).

Meropenem in monotherapy has been found actively against interacelullar *L. monocitegenes* infection on meningitis case in the experimenlat studi , it was 10-fold more potent than ampicillin and ertapenem (Lemaire, 2005). Meropenem was bacteriostatic effect after 5 hours treatment and start bactericidal effect after 24 hours (Carryn *et al.,* 2003), it coud intiate bacterial severe cell damages (Lemaire 2005).

Cefotaxime is a bactericidal derived from cephalosporin has a broad-spectrum activity against gram-positive microorganisms and exceptional activity against most gram-negative microorganisms by interfering with synthesis of their cell walls. It is widely considered to be the antibiotic of choice for the management of neonatal meningitis and sepsis caused by gram-negative bacteria including *L. monocytogenes* (Pacifici and Marchini, 2017). Cefotaxime has received wide acceptance as a first-line antibiotic for many infections in neonates, infants and children. cefotaxime caused a significant enhancement of IL-2 production by cells and increased the secretion of TNF α by peripheral blood mononuclear cells, it is also suggested that may modify the host immune response. Cefotaxime is a safe and effective antibiotic in treating meningitis bacterial infections which is 80% of these microorganism are resistant with ampicillin (Pacifici and Marchini, 2017)

This result was different from previous studies that *Listeria sp* is generally still sensitive to all antibiotics (Jamali *et al*, 2015). It cannot be separated from the habit of inappropriate use of antibiotics in livestock industry and communities in some developing countries including in Indonesia. Therefore, efforts to raise awareness on the importance of the correct use of antibiotics regarding the dose and duration should be a concern of both government and communities.

CONCLUSION

The contamination of *L.monocytogenes* in chicken meat in supermarkets, mobile vendors and traditional markets in Surabaya indicates the potential listeriosis distribution. Moreover, the three isolates founded were included as distinctive lineages and already resistant to old generation antibiotics such as: sulfamethonazole-trimetophrim (SXT) and amoxyllin sulbactam (MAS). Therefore, surveillance policies for potential food contamination and antibiotic sensitivity of *L.monocytogenes* are required, while also ensuring effective antibiotic treatment.

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REFERENCES

Al-Nabulsi, A.A., Osaili, T.M., Shaker, R.R. 2015. Effects of osmotic pressure, acid, or cold stresses on antibiotic susceptibility of Listeria monocytogenes. Food Microbiology, 46: 154–160.

Ariyanti, T. 2010. Bacteria Listeria monocytogenes as food contaminants from animal origin (foodborne disease). Wartazoa. 20 (2): 94-102.

Antunes P, Reu C, Sousa JC, Pestana N, and Peixe L. 2002. Incidence and susceptibility to antimicrobial agents of Listeria spp and Listeria monocytogenes isolated from poultry carcasses in Porto, Portugal. J Food Prot 2002;65:1888–1893.

Lemaire, S., Bambeke, F.V., Tulkens, P., Mingeot-Leclercq, M.P. 2005. Activity of Three B-Lactams (Ertapenem, Meropenem, and Ampicillin) against intraphagocytic *Listeria monocytogenes* and *Staphylococcus aureus.* J. Antimicrobial Chemoterapy 2005: 1-5

Carryn, S., Van Bambeke, F., Mingeot-Leclercq, M. P. et al. 2003. Activity of b-lactams (ampicillin, meropenem), gentamicin, azithromycin and moxifloxacin against intracellular

Listeria monocytogenes in a 24 h THP-1 human macrophage model. Journal of Antimicrobial Chemotherapy 51, 1051–2.

Charpentier E, Gerbaud G, Jacquet C, Rocourt J, and Courvalin P. 1995.Incidence of antibiotic resistance in Listeria spp. J Infect Dis.172:277–281.

Chastre, J. 2008. Evolving problems with resistant pathogens. Clin. Microbiol. Infect. 14:3–14.

Chatterjee, SS, Hossain, H., Otten, S., Kuenne, C., Kuchmina, K., Machata, S., Domann, E., Chakraborty, T., and Hain, T. 2006. Intracellular gene expression profile of Listeria monocytogenes. Infect Immun. 74 (2): 1323–1338.

Chen, JQ., Regan, P., Laksanalamai, P., Healey, S., Hu, Z. 2017. Prevalence and methodologies for detection, characterization and subtyping of Listeria monocytogenes and L. ivanovii in foods and environmental sources. Food Science and Human Wellness. 6 (3). 97-120.

CDC -Centers for Disease Control and Prevention (CDC). 2010. Get smart: know when antibiotics work. CDC, Atlanta.

CDC - Centers for Disease Control and Prevention. 2011. Multistate outbreak of listeriosis associated with Jensen farms cantaloupe. United States of America

Etikaningrum and Iwantoro, S. 2017. Study of antibiotic residues in poultry products in Indonesia. Journal of Animal Husbandry Production and Technology. Vol. 09 (1). 29-33

Godreuil S, Galimand M, Gerbaud G, Jacquet C, and Courvalin P. 2003. Efflux pump lde is associated with fluroquinolone resistance in Listeria monocytogenes. Antimicrob Agents Chemother;47:704–708.

Gould, I. 2008. The epidemiology of antibiotic resistance. Int. J. Antimicrob. Agents 32:S2–S9.

Harsoyo and Andini, L. 2002. Effect of irradiation and storage of Listeria monocytogenes inoculated in goat meat. Pros. Nas. Animal Husbandry and Veterinary Technology. Bogor, 30 September-1 October 2002. Center for Animal Husbandry, Bogor. 334-337

Hershberger, E., S. F. Oprea, S. M. Donabedian, M. Perri, P. Bozigar, P. Bartlett, and M. J. Zervos. 2005. Epidemiology of antimicrobial resistance in enterococci of animal origin. J. Antimicrob. Chemother. 55:127–130

Jamali, H., Chai, L.C., Thong, K.L. 2013. Detection and isolation of Listeria spp. and Listeria monocytogenes in ready-to-eat foods with various selective culture media. Food Control. 32: 19-24.

Jamali, H., Paydar, M., Ismail, S., Looi, CY, Wong, WF, Radmehr, B., Abedini, A. 2015. Prevalence, antimicrobial susceptibility and virulotyping of Listeria species and Listeria monocytogenes isolated from open- water fish markets. BMC Microbiology. 15: 144.

Janzten, M.M., Navas, J., Mreno, J., Lpoez and Suarez, J.V. 2006. Detection of Listeria monocytogenes in food using commercial methods: Chromogen media-PCR. J. Agricultural Research. 4 (3): 235-247.

Liu, D. 2008. Epidemiology. In D. Liu (Ed.), Handbook of Listeria monocytogenes. New York: CRC Press: 27-30.

Meloni, D., Galluzzo, P., Mureddu, A., Piras, F., Griffiths, M., & Mazzette, R. 2009. Listeria monocytogenes in RTE foods marketed in Italy: prevalence and automated EcoRI ribotyping of the isolates. International Journal of Food Microbiology. 129(2): 166-172

Miller, W.R., Munita, J.M., Arias, C.A. 2014. Mechanisms of antibiotic resistance in enterococci. Expert Review of AntiInfective Herapy, 12(10): 1221–1236

Mohamed, Y., Reda, W.W., Abdel-Moein, K., El-Razik, K.A.A., Barakat, A.M.A., El Fadaly, H.A., Hassanain, N.A., Hegazi, A.G. 2016. Prevalence and phylogenetic characterization of Listeria monocytogenes isolated from processed meat marketed in Egypt. Journal of Genetic Engineering and Biotechnology. 14: 119-123

Muaz, K., Riaz, M., Akhtar, S., Park, S., Ismail, A. Antibiotic Residues in Chicken Meat: Global Prevalence, Threats, and Decontamination Strategies: a Review. J. Food Protection, 8(14): 619 - 627

National Standardization Board. 2008. Indonesian National Standard (SNI). SNI No. 2897: 2008 concerning methods of testing microbial contamination in meat, eggs and milk, and their processed products. Jakarta. BSN.

Pradipta IS., Ronasih, E., Kartikawati, AD., Hartanto, H., Amelia, R., Febrina, E., and Abdulah, E. 2015. Three years of antibacterial consumption in Indonesian Community Health Centers: The application of anatomical therapeutic chemical / defined daily doses and drug utilization 90% method to monitor antibacterial use. J Family Community Med. Vol. 22 (2): 101-105.

Nwachukwu, N.C., Orji, F.A., Iheukwumere, I., Ekeleme, U.G. 2010. Antibiotic resistant environmental isolates of Listeria monocytogenes from anthropogenic lakes in Lokpa-Ukwu, Abia State of Nigeria.Aust. J. Basic Appl. Sci 4: 1571-1576.

Pacifici, G.M., Marchini, G. Clinical Pharmacology of Cefotaxime in Neonates and Infants: Effects and Pharmacokinetics. Int J Pediatr 2017; 5(11): 6111-6138

Paziak-Domanska, B., Bogulawska, E., Wiekowska-Szakiel, M., Kotlowski, R., Rozalska, B., Chmiela, M., Kur, J., Dabrowksi, W., and Rudnicka, W. 1999 Evaluation of the API test, phathylinlinositol-specific phospholipase C activity and PCR method in identification of Listeria monocytogenes in meat foods. FEMS. Mircobiol. Lett. 171: 209-214.

Ponniah, J., Robin, T., Paie, M. S., Radu, S., Ghazali, F. M., Kqueen, C. Y., et al. 2010. Listeria monocytogenes in raw salads vegetables sold at retail level in Malaysia. Journal of Food Control. 21: 774-778.

Rafikah, N., Irmawaty., Kiramang, K. 2018. The level of contamination of Escherichia coli bacteria in chicken meat sold in Makassar traditional markets. Proceedings of the Indonesian National Megabiodiversity Seminar. April 9, 2018.

Rivoal, K., Queguiner, S., Boscher, E., Bougeard, S., Ermel, G., Salvat, G., Federighi, M., Jugiau, F and Protais, J. 2010. Detection of Listeria monocytogenes in raw and pasteurized liquid whole eggs and characterization by PFGE. Int. J. Food Microbiol. 138: 56 - 62.

Riwu, K.H.P. 2018. Identification of Listeria monocytogenes bacteria in chicken meat in several supermarkets and traditional markets in the city of Surabaya by using the polymerase chain reaction (PCR) method. Thesis. Master of Science Disease and Veterinary Health Study Program, Faculty of Veterinary Medicine, Airlangga University.

Rodrigues, C.S., de Sa, C.V.G.C., de Melo, C.B. 2017. An overview of Listeria monocytogenes contamination in ready to eat meat, dairy and fishery foods. Ciencia Rural, Santa Maria. 47 (2): 1-8

Sanlibaba, P., Cakmak, G.A., Tezel, B.U. 2018 Prevalence and Antibiotic Resistantce of *Listeria monocytogenes* isolated from Ready-to-Eat Food in Turkey. Journal od Food Quality 2019: 1-11

Soni, D.K., Dubey, S.K. 2014. Phylogenetic analysis of the Listeria monocytogenes based on sequencing of 16SrRNA and hlyA genes. Molecular Biology Reports. 41 (12). 8219-8229.

Srinivasan V, Nam HM, Nguyen LT, Tamilselvam B, Murinda SE, and Oliver SP. 2005. Prevalence of antimicrobial resistance genes in Listeria monocytogenes isolated from dairy farms. Foodborne Pathog Dis 2005;2:201–210.

Sugiri, Y.D., Gölz, G., Meeyam, T., Baumann, M.P., Kleer J., Chaisowwong W., Alter T. 2014. Prevalence and antimicrobial susceptibility of Listeria monocytogenes on chicken carcasses in Bandung, Indonesia. J. Food Prot. 77 (8). 1407-1410.

Swaminathan B and Gerner-Smidt P. 2007. The epidemiology of human listeriosis. Microbes Infect ;9:1236–1243

Van Den Bogaard, A. E., and E. E. Stobberingh. 2000. Epidemiology of resistance to antibiotics: links between animals and humans. Int. J. Antimicrob. Agents 14:327–335

Vazquez-Boland, JA, Kuhn, M., Berche, P., Chakraborty, T., Domínguez-Bernal, G., Goebel, W., González-Zorn, B., Wehland, J., and Kreft, J. 2001. Listeria pathogenesis and molecular virulence determinants. Clin Microbiol Rev. 14 (3): 584–640.

Yucel N, Citak S, and Onder M. 2005. Prevalence of antibiotic resistance of Listeria species in meat products in Ankara, Turkey. Food Microbiol;22:241–245.