



# UNIVERSITAS AIRLANGGA

## FAKULTAS KEDOKTERAN HEWAN

Kampus C Mulyorejo Surabaya 60115 Telp. (031) 5992785, 5993016 Fax (031) 5993015

Laman: <http://www.fkh.unair.ac.id>, e-mail: [info@fkh.unair.ac.id](mailto:info@fkh.unair.ac.id)

### SURAT KETERANGAN

Nomor : 856/UN3.1.6/KP/2023

Yang bertanda tangan di bawah ini :

Nama : Prof. Dr. Mustofa Helmi Effendi, drh., DTAPH  
NIP : 196201151988031002  
Pangkat / Golongan : Pembina TK I / (Gol. IV/b)  
Jabatan : Wakil Dekan III

Dengan ini menerangkan :

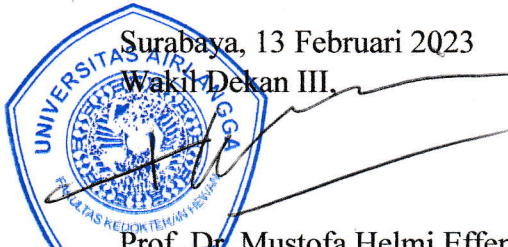
Nama : Dr. Eduardus Bimo Aksono H, drh., M.Kes  
NIP : 196609201992031003  
Pangkat / Golongan : Pembina (Gol. IV/a)  
Jabatan : Lektor Kepala

Telah melaksanakan penelitian dengan judul sebagai berikut :

No.	Judul Karya Ilmiah	Tahun Pelaksanaan Penelitian
1.	Phylogenetic Analysis and Antibiotics Resistance of <i>Listeria Monocytogenes</i> Contaminating Chicken Meat in Surabaya, Indonesia	2020
2.	Detection of bla TEM Gene of <i>Klebsiella pneumoniae</i> Isolated From Swab of Food-Producing Animals in East Java	2018
3.	Perbandingan Sekuens Nukleotid gZP3 Terhadap Sekuen Konsensus Nukleotid ZP3 Wanita dan Beberapa Spesies Untuk Pengembangan Bahan Imunokontrasepsi	2011
4.	Potentials of Nggorang Leaf Standard Extract ( <i>Salvia Ccidentalis</i> ) Study: Ethnopharmacology, Standardization, Antioxoxide, Sitotoxicity and Working Mechanisms	2019

Adapun penelitian tersebut tidak perlu dilakukan Uji Etical Clearence karena tidak menggunakan hewan coba.

Demikian Surat Keterangan ini kami buat untuk dapat dipergunakan sebagai persyaratan pengusulan Jabatan Fungsional **Guru Besar**

Surabaya, 13 Februari 2023  
Wakil Dekan III,  


Prof. Dr. Mustofa Helmi Effendi, drh., DTAPH  
NIP 196201151988031002



# Tropical Animal Science Journal

Authentication of Sumateran Wild Boar (*Sus scrofa vittatus*) Meat Contamination by Polymerase Chain Reaction Restriction Fragment Length Polymorphism (PCR-RFLP) Technique of *Cytochrome b* Gene

M. W. Adiningsih, R. D. Soejoedono, T. Purnawarman, H. Latif, O. N. Poetri, & D. D. Putri

Identification of Single Nucleotide Polymorphism and Pathway Analysis of Apolipoprotein A5 (APOA5) Related to Fatty Acid Traits in Indonesian Sheep

A. Gunawan, D. Anggrela, K. Listyarini, M. A. Abuzahra, Jakaria, M. Yamin, I. Inounu, & C. Sumantri

Detection of *bla<sub>TEM</sub>* Gene of *Klebsiella pneumoniae* Isolated from Swab of Food-Producing Animals in East Java

M. H. Effendi, I. G. Bintari, E. B. Aksono, & I. P. Hermawan

Growth and Production of *Brachiaria brizantha* cv. MG5 in Three Difference Regrowth Phase Treated by Gamma Radiation Dose

A. N. Respati, N. Umami, & C. Hanim

*In Vitro* and *in Vivo* Anthelmintic Activities of Aqueous Leaf Infusion of *Azadirachta indica* against *Haemonchus contortus*

A. A. Sakti, Kustantinah, & R. W. Nurcahyo

Anti-diabetic Potency and Characteristics of Probiotic Goat-Milk Yogurt Supplemented with Roselle Extract during Cold Storage

R. R. S. Wihansah, I. J. Arief, & I. Batubara

Isolation of Cellulolytic Lactic-Acid Bacteria from Mentok (*Anas moschata*) Gastro-Intestinal Tract

H. Herdian, L. Istiqomah, E. Damayanti, A. E. Suryani, A. S. Anggraeni, N. Rosyada, & A. Susilowati

Pre-Adipocyte Determination and Adipocyte Differentiation of Stromal Vascular Cells Isolated From Intramuscular Tissue of Hanwoo Beef Cattle Treated by Acetate and Propionate

T. G. Wandita, N. Joshi, H. H. Kim, S. J. An, & S. G. Hwang

Carcass and Meat Characteristics of Male Bali Cattle in Indonesian Smallholder Farms Fed Ration with Different Protein Levels

P. K. Tahuk, S. P. S. Budhi, Panjono, & E. Baliarti

Analysis of Domestic Resources Cost Based on Farm Typology of Bali Cattle in Plampang Sumbawa Regency, West Nusa Tenggara

Sudirman, B. Hartono, I. Subagiyo, & B. A. Nugroho



8

# Tropical Animal Science Journal

Authentication of Sumateran Wild Boar (*Sus scrofa vittatus*) Meat Contamination by Polymerase Chain Reaction Restriction Fragment Length Polymorphism (PCR-RFLP) Technique of *Cytochrome b* Gene

M. W. Adiningsih, R. D. Soejoedono, T. Purnawarman, H. Latif, O. N. Poetri, & D. D. Putri

Identification of Single Nucleotide Polymorphism and Pathway Analysis of Apolipoprotein A5 (APOA5) Related to Fatty Acid Traits in Indonesian Sheep

A. Gunawan, D. Anggrela, K. Listyarini, M. A. Abuzahra, Jakaria, M. Yamin, I. Inounu, & C. Sumantri

Detection of *bla<sub>TEM</sub>* Gene of *Klebsiella pneumoniae* Isolated from Swab of Food-Producing Animals in East Java

M. H. Effendi, I. G. Bintari, E. B. Aksono, & I. P. Hermawan

Growth and Production of *Brachiaria brizantha* cv. MG5 in Three Difference Regrowth Phase Treated by Gamma Radiation Dose

A. N. Respati, N. Umami, & C. Hanim

*In Vitro* and *in Vivo* Anthelmintic Activities of Aqueous Leaf Infusion of *Azadirachta indica* against *Haemonchus contortus*

A. A. Sakti, Kustantinah, & R. W. Nurcahyo

Anti-diabetic Potency and Characteristics of Probiotic Goat-Milk Yogurt Supplemented with Roselle Extract during Cold Storage

R. R. S. Wihansah, I. I. Arief, & I. Batubara

Isolation of Cellulolytic Lactic-Acid Bacteria from Mentok (*Anas moschata*) Gastro-Intestinal Tract

H. Herdian, L. Istiqomah, E. Damayanti, A. E. Suryani, A. S. Anggraeni, N. Rosyada, & A. Susilowati

Pre-Adipocyte Determination and Adipocyte Differentiation of Stromal Vascular Cells Isolated From Intramuscular Tissue of Hanwoo Beef Cattle Treated by Acetate and Propionate

T. G. Wandita, N. Joshi, H. H. Kim, S. J. An, & S. G. Hwang

Carcass and Meat Characteristics of Male Bali Cattle in Indonesian Smallholder Farms Fed Ration with Different Protein Levels

P. K. Tahuk, S. P. S. Budhi, Panjono, & E. Baliarti

Analysis of Domestic Resources Cost Based on Farm Typology of Bali Cattle in Plampang Sumbawa Regency, West Nusa Tenggara

Sudirman, B. Hartono, I. Subagiyo, & B. A. Nugroho

Scopus®

Clarivate  
Analytics  
EMERGING SOURCES  
CITATION INDEX

SJR

DOAJ

DIRECTORY OF  
OPEN ACCESS  
JOURNALS  
ASEAN  
CITATION  
INDEX

Crossref

CAB Abstracts

EBSCO  
PUBLISHING

Google  
scholar

sinta

Published by Faculty of Animal Science, Bogor Agricultural University, associated with  
Animal Scientist's Society of Indonesia

# Tropical Animal Science Journal

Accredited by Directorate General of Research and Development Strengthening,  
Ministry of Research, Technology and Higher Education of the Republic of Indonesia  
No: 36b/E/KPT/2016

Vol. 41 No. 3, December 2018

## PUBLISHER

Faculty of Animal Science, Bogor Agricultural University associated with Animal Scientist's Society of Indonesia

## CHIEF EDITOR

Komang G. Wiryawan

## ASSOCIATE EDITORS

Tuti Suryati  
Sri Suharti  
Jakaria

## EDITORIAL BOARDS

Abdul Razak Alimon (Malaysia) ~2019  
Anjas Asmara B. Samsudin (Malaysia) ~2021  
Anuraga Jayanegara (Indonesia) ~2019  
Arief Boediono (Indonesia) ~2019  
Armagan Hayirli (Turkey) ~2019  
Asep Gunawan (Indonesia) ~2018  
Beben Benyamin (Australia) ~2020  
Budi Santoso (Indonesia) ~2021  
Burhanudin Sundu (Indonesia) ~2018  
Cahyo Budiman (Indonesia) ~2018

Cece Sumantri (Indonesia) ~2019  
Elizabeth Wina (Indonesia) ~ 2020  
Epi Taufik (Indonesia) ~2021  
Irma Isnafia Arief (Indonesia) ~2019  
Jong K. Ha (Korea) ~2019  
Junichi Takahashi (Japan) ~2019  
Karl Schellander (Germany) ~ 2019  
Kenji Fukuda (Japan) ~2019  
Loh Teck Chwen (Malaysia) ~ 2019  
Lucia Cyrilla (Indonesia) ~2019

Luki Abdullah (Indonesia) ~ 2021  
Mulyoto Pangestu (Australia) ~ 2020  
Myunggi Baik (Korea) ~2021  
Nahrowi (Indonesia) ~2019  
Rita Mutia (Indonesia) ~2019  
Sri Mulatsih (Indonesia) ~2021  
Tadasu Urashima (Japan) ~2018  
Toto Toharmat (Indonesia) ~2018  
Wasmen Manalu (Indonesia) ~2018

## TECHNICAL EDITORS

Irma Nuranthy Purnama  
Widya Hermana

## SCOPE OF JOURNAL

Tropical Animal Science Journal receives original manuscripts encompass a broad range of research topics in tropical animal sciences: breeding and genetics, reproduction and physiology, nutrition, feed sciences, agrostology, animal products, biotechnology, behaviour, welfare, health, livestock farming system, socio-economic, animal waste management, and policy.

Authors have to prepare the manuscripts according to Tropical Animal Science Journal's guidelines. Manuscript and statement of originality and copyright release form (Form A) should be submitted via online submission through journal website: <http://journal.ipb.ac.id/index.php/tasj>.

## ABOUT JOURNAL

Tropical Animal Science Journal (Trop. Anim. Sci. J.) previously Media Peternakan is a scientific journal covering broad aspects of tropical animal sciences. Started from 2018, the title is changed from Media Peternakan in order to develop and expand the distribution as well as increase the visibility of the journal. The journal is published three times a year in April, August, and December by Faculty of Animal Science, Bogor Agricultural University, associated with Animal Scientist's Society of Indonesia. Tropical Animal Science Journal is a peer reviewed journal that has been indexed and abstracted in Elsevier products (Scopus, Reaxys), Clarivate Analytics product (Emerging Sources Citation Index), Scimago Journal Rank, ASEAN Citation Index, DOAJ, CAB, EBSCO, Science and Technology Index (SINTA), Google Scholar, and other scientific databases. The journal already used Similarity Check to prevent any suspected plagiarism in the manuscripts.

## SECRETARIAT OF TROPICAL ANIMAL SCIENCE JOURNAL

Faculty of Animal Science Building, Bogor Agricultural University  
Jalan Agatis, Kampus IPB Darmaga, Bogor 16680, Indonesia  
Phone/Fax: +62-251-8421692

e-mail: [mediapeternakan@apps.ipb.ac.id](mailto:mediapeternakan@apps.ipb.ac.id); [mediapeternakan@yahoo.co.id](mailto:mediapeternakan@yahoo.co.id)  
website: <http://journal.ipb.ac.id/index.php/tasj>

## Subscription Rate (Not including shipping and handling):

IDR 100.000/ edition and IDR 250.000/ year (Indonesian subscribers); 50 US\$ (Foreign subscribers)  
Bank account: BNI BOGOR No. 0668269871, o.b. Irma Nuranthy Purnama, SWIFT CODE: BNINIDJABGR

# Tropical Animal Science *Journal*

Accredited by Directorate General of Research and Development Strengthening,  
Ministry of Research, Technology and Higher Education of the Republic of Indonesia  
No: 36b/E/KPT/2016

---

## HISTORY

Tropical Animal Science Journal (Trop. Anim. Sci. J.) (p-ISSN 2615-787X and e-ISSN 2615-790X) previously Media Peternakan (published from 1967-2017 with p-ISSN 0126-0476 and e-ISSN 2087-4634) is a scientific journal covering broad aspects of tropical animal sciences. Started from 2018, the title is changed from Media Peternakan in order to develop and expand the distribution as well as increase the visibility of the journal. The journal is published three times a year in April, August, and December by Faculty of Animal Science, Bogor Agricultural University, associated with Animal Scientist's Society of Indonesia.

The first edition with the new title was published in April 2018 edition (Vol 41 No 1 2018), while the previous edition (up to 2017 edition) still use Media Peternakan as the title. The online version of Tropical Animal Science Journal could be accessed in the new website (<http://journal.ipb.ac.id/index.php/tasj>) and the previous editions are available in the old website (<http://medpet.journal.ipb.ac.id/>).

Tropical Animal Science Journal has been indexed and abstracted in Elsevier products (Scopus, Reaxys), Clarivate Analytics products (Emerging Sources Citation Index), Scimago Journal Rank, ASEAN Citation Index, DOAJ, CABI, EBSCO, Science and Technology Index (SINTA), Google Scholar, and other scientific databases. The journal also used Similarity Check to prevent any suspected plagiarism in the manuscripts.

# Tropical Animal Science Journal

Accredited by Directorate General of Research and Development Strengthening,  
Ministry of Research, Technology and Higher Education of the Republic of Indonesia  
No: 36b/E/KPT/2016

## LIST OF CONTENT

<b>Authentication of Sumateran Wild Boar (<i>Sus scrofa vittatus</i>) Meat Contamination by Polymerase Chain Reaction Restriction Fragment Length Polymorphism (PCR-RFLP) Technique of <i>Cytochrome b</i> Gene</b>	157
M. W. Adiningsih, R. D. Soejoedono, T. Purnawarman, H. Latif, O. N. Poetri, & D. D. Putri	
<b>Identification of Single Nucleotide Polymorphism and Pathway Analysis of Apolipoprotein A5 (APOA5) Related to Fatty Acid Traits in Indonesian Sheep</b>	165
A. Gunawan, D. Anggrela, K. Listyarini, M. A. Abuzahra, Jakaria, M. Yamin, I. Inounu, & C. Sumantri	
<b>Detection of <i>bla</i><sub>TEM</sub> Gene of <i>Klebsiella pneumoniae</i> Isolated from Swab of Food-Producing Animals in East Java</b>	174
M. H. Effendi, I. G. Bintari, E. B. Aksono, & I. P. Hermawan	
<b>Growth and Production of <i>Brachiaria brizantha</i> cv. MG5 in Three Difference Regrowth Phase Treated by Gamma Radiation Dose</b>	179
A. N. Respati, N. Umami, & C. Harim	
<b><i>In Vitro</i> and <i>in Vivo</i> Anthelmintic Activities of Aqueous Leaf Infusion of <i>Azadirachta indica</i> against <i>Haemonchus contortus</i></b>	185
A. A. Sakti, Kustantinah, & R. W. Nurcahyo	
<b>Anti-diabetic Potency and Characteristics of Probiotic Goat-Milk Yogurt Supplemented with Roselle Extract during Cold Storage</b>	191
R. R. S. Wihansah, I. I. Arief, & I. Batubara	
<b>Isolation of Cellulolytic Lactic-Acid Bacteria from Mentok (<i>Anas moschata</i>) Gastro-Intestinal Tract</b>	200
H. Herdian, L. Istiqomah, E. Damayanti, A. E. Suryani, A. S. Anggraeni, N. Rosyada, & A. Susilowati	
<b>Pre-Adipocyte Determination and Adipocyte Differentiation of Stromal Vascular Cells Isolated From Intramuscular Tissue of Hanwoo Beef Cattle Treated by Acetate and Propionate</b>	207
T. G. Wandita, N. Joshi, H. H. Kim, S. J. An, & S. G. Hwang	
<b>Carcass and Meat Characteristics of Male Bali Cattle in Indonesian Smallholder Farms Fed Ration with Different Protein Levels</b>	215
P. K. Tahuk, S. P. S. Budhi, Panjono, & E. Baliarti	
<b>Analysis of Domestic Resources Cost Based on Farm Typology of Bali Cattle in Plampang Sumbawa Regency, West Nusa Tenggara</b>	224
Sudirman, B. Hartono, I. Subagiyo, & B. A. Nugroho	



## Detection of *bla*<sub>TEM</sub> Gene of *Klebsiella pneumoniae* Isolated from Swab of Food-Producing Animals in East Java

M. H. Effendi<sup>a,\*</sup>, I. G. Bintari<sup>b</sup>, E. B. Aksono<sup>c</sup>, & I. P. Hermawan<sup>b</sup>

<sup>a</sup>Department of Veterinary Public Health, Faculty of Veterinary Medicine, Airlangga University

<sup>b</sup>Student, Faculty of Veterinary Medicine, Airlangga University

<sup>c</sup>Department of Basic Veterinary Medicine, Faculty of Veterinary Medicine, Airlangga University  
Jalan Mulyorejo, Kampus C Mulyorejo Surabaya 60115, Indonesia

\*Corresponding author: [mheffendi@yahoo.com](mailto:mheffendi@yahoo.com)

(Received 22-01-2018; Reviewed 06-04-2018; Accepted 03-08-2018)

### ABSTRACT

*Klebsiella pneumoniae* is one of 9 bacteria resistance to antibiotics in concern. This research aimed to detect any gene of *bla*<sub>TEM</sub> in bacteria of the *K. pneumoniae* isolated from swab of food-producing animals. In this study, 195 swab samples were taken from 17 sampling locations. Samples obtained were cultivated on selective medium and had several tests including identification, antibiotic sensitivity test using Kirby-Bauer method against antibiotics of ampicillin, cefotaxime, amoxicillin, meropenem, and trimethoprim-sulfamethoxazole, and followed by PCR test for detecting the gene that was responsible for the antibiotic resistances. The results showed that 10 out of 195 samples were found to be *K. pneumoniae*, those were 4 samples originated from dairy cows (SP-S1, SP-S3, SP-B2, SP-G4), 2 samples originated from beef (SPT-K1, SPT-K2), 1 sample originated from chickens (A-W5), and 3 samples originated from fish (IN-P2, IN-P3, IN-S3). Most of isolates (9/10) were found to be resistant toward amoxicillin. These isolates were SP-S3, SP-B2, SP-G4, SPT-K1, SPT-K2, A-W5, IN-P2, IN-P3, and the IN-S3 and all of them also showed to be positive of *bla*<sub>TEM</sub> gene. It could be concluded that most of *K. pneumoniae* isolates from food animals harbour had Extended Spectrum Beta-Lactamase (ESBL) encoding gene.

**Keywords:** antibiotic resistance, ESBL, food-producing animals, *Klebsiella pneumoniae*, amoxicillin

### INTRODUCTION

*Klebsiella pneumoniae* is a bacterium belonging to the genus of *Klebsiella* a member of the family of Enterobacteriaceae. The bacterium is a normal organism living in traktus digestivus so that it can be isolated from animal or human feces (Susilo *et al.*, 2004). *K. pneumoniae* is a Gram-negative, basil, nonmotile, and one of the important pathogenic bacteria. This species is the agents of various diseases, such as pneumonia, urinary tract infection, bakteremia, infection in wounds, and abscesses of the liver (Rahamathulla *et al.*, 2016). *K. pneumoniae* pose a great impact on the health sector. In a report on global surveillance on antimicrobial resistance carried out by the World Health Organization, *K. pneumoniae* is one of nine bacteria concerned in resistance to antibiotics (WHO, 2014). *K. pneumoniae* was found to be capable of being resistant towards many of the third-generation of cephalosporin antibiotics especially cefotaxime, ceftazidime, and ceftriaxone (Yeh *et al.*, 2007).

Each bacteria employs different mechanisms in causing resistance to antibiotics. The transfer of antibiotic-resistant genes, especially through plasmids is considered to be one of the important mechanisms in the spreading of antibiotic resistance in bacteria (Apriliani & Pinatih, 2017). This event is known to be mediated by an enzyme of beta-

lactamase. The beta-lactamase enzyme is firstly identified in *Escherichia coli*, which is encoded by the *bla*<sub>TEM</sub> gene. In addition, *bla*<sub>TEM</sub> gene was also currently found in *K. pneumoniae*. (Lalzampuia *et al.*, 2014). Treatments of bacterial infections of beta-lactamase producers have so far involved the use of cephalosporins and aztreonam which also belong to the group of beta-lactam antibiotics. In fact, this drug can not kill lactamase producing bacteria as a results of it spread of its resistance spectrum, to penicillin, cephalosporins, and aztreonam so that it called as the *Extended Spectrum Beta-Lactamase* (ESBL) bacteria. The ability of ESBL strains to hydrolyze betalactam antibiotics is generally due to a number of mutations in the gene, and one of which is *bla*<sub>TEM</sub> gene. These mutations are generally found to be at the active site of the enzyme that leads to a higher enzymatic activity (Yuwono, 2011). The existence of ESBL strain in food-producing animals is reported by Overdevest *et al.* (2011).

Animals can carry harmful bacteria in their intestines. When antibiotics are given to animals, antibiotics kill most of the bacteria. However, resistant bacteria are survive and multiply. Food-producing animals have been known as reservoirs for ESBL-producing bacteria. Food-producing animals are capable of spreading bacteria that are resistant to antibiotics through feces. Through feces, resistant bacteria contained in

animal waste and they can migrate around the farms, slaughterhouses or poultry slaughterhouses, and during meat processing. The surroundings of farms and slaughterhouses or chicken slaughterhouses will also be contaminated even though it is far from the source of contamination (Doosti *et al.*, 2014).

Attempts to detect the existence of resistant genes in bacteria from food-producing animals in Indonesia, to our knowledge, remains limited. In fact, the gene detection should allow us to understand the pattern of genes spreading and possibility of the bacteria to gain further antibiotic resistance from certain groups. Accordingly, detection of the *bla*<sub>TEM</sub> gene group in *K. pneumoniae* strain is important to do.

## MATERIALS AND METHODS

### Sample Collection and Preparation

In this study 195 swab samples were obtained from 17 locations in East Java. Sampling was done by using swab aseptically from dairy cows, beef cattle, broiler, and tilapia in 17 sampling locations. The samples were directly analysed within 30-40 min or then transferred to the lab for further analysis in a cool box.

As many as 195 samples of dairy cows, beef cattle, broilers, and tilapia were cultivated by taking 1 ose and then streaked by streak plate technique method on selective media of Mac Conkey and EMBA then incubated at 37°C for 24 h. In Mac Conkey media, *K. pneumoniae* looks pink with colonies culture looks very mucoid and in EMBA media *K. pneumoniae* looks red to brick (Masruroh *et al.*, 2016).

### Characterization of Isolates

Pure bacterial isolates were identified based on morphological characters that included colony morphology, cell morphology, and gram staining tests. Observation of colony morphology was based on the shape, color, and the edge of bacterial colonies. The morphological observations of bacterial cells include the shape and structure of bacterial cells. Furthermore, each isolate was biochemically characterized including carbohydrate fermentation test (glucose, lactose, mannitol, maltose, and sucrose), indole, motility, and citrate (Lestari *et al.*, 2016).

### Antibiotic Test

The antibiotic sensitivity test was performed using Kirby-Bauer agar diffusion method (Ningrum *et al.*, 2016). The resulting clear zone was then grouped into sensitive groups (S), intermediates (I) or resistant (R) (Sagita *et al.*, 2015; Kusumaningrum

*et al.*, 2016). Selection of antibiotic discs (disks) used previous research and based on some journals for reference. The types of antibiotics used were ampicillin, amoxicillin, cefotaxim, meropenem, and sulfamethonazole-trimetrophrim.

Pure cultures were prepared in suspensions with an equivalent of 0.5 McFarland ( $1-2 \times 10^8$  CFU/mL) turbidity. The cultures were taken using sterile swab cotton and distributed by means of a diole on the surface of the Mueller Hinton agar (MHA), and allowed to stand for  $\pm 5$  min. The antibiotic-containing discs were placed on the top of the MHA, which had been dispersed with pure cultures, at a distance of 25-30 mm. Furthermore, the culture was incubated at 35°C for 24 h (Masruroh *et al.*, 2016).

### Genomic DNA Extraction

DNA extraction by adding *K. pneumoniae* bacteria was performed using QIAamp DNA mini kit 50 (Qiagen, USA) according to manufacturer protocol. Briefly, samples were added with 5  $\mu$ L lysozyme (5 mg/mL) enzyme and incubated for 30 min at 56°C. Furthermore, the extracted DNA was diluted to 100  $\mu$ L with a buffer kit. DNA solution used for PCR amplification was as much as 1  $\mu$ L.

### Amplification of *bla*<sub>TEM</sub> gene: Polymerase Chain Reaction

For the amplification was performed using Qiagen HotStarTag Master Mix (Qiagen, USA) according to manufacturer protocol with pure genomic DNA of *K. pneumoniae* was used as a template. The primers used in this study were shown in Table 1. The amplification steps involved a denaturation process at 95°C for 15 min, 30 cycles denaturation at 94°C for 30 s, annealing at 50°C for 30 s, extension at 72°C for 2 min followed by the final extension on temperature of 72°C for 10 min (Moenstein *et al.*, 2007).

Amplification product was then separated on 2% gel agarose, stained with gel-red, and visualized using UV light. *bla*<sub>TEM</sub> gene detection was considered to be positive when bands at 445 bp of apparent size was observed in the gel.

## RESULTS

The result showed that 10 out of 195 samples isolated from swab dairy cows, beef cattle, broiler chickens, and fish tilapia were positive *K. pneumoniae*. The presence of these bacteria on swab samples consisted of dairy cows by 4.8% (4/83), beef cattle by 40% (2/5), chicken broiler by 10% (1/10), and fish by 3% (3/97). All isolates also showed 90% resistance to the amoxicillin

Table 1. The primers used in this study

Gene target	Primary sequences	Amplicon (bp)	Reference
<i>bla</i> <sub>TEM</sub>	F- 5'-TCGCCGCATACACTATTCTCAGAATGA-3' R-5'-ACGCTCACCGGCTCCAGATTTAT-3'	445	Monstein <i>et al.</i> , 2007



(9/10) and sensitive to other types of antibiotics (Table 2, Figure 1).

Examination of DNA from samples using agarose electrophoresis gel (Figure 2) showed that the *bla*<sub>TEM</sub> gene was successfully amplified with *bla*<sub>TEM</sub>-F and *bla*<sub>TEM</sub>-R primaries. The 10 samples tested by PCR in the study showed a positive result of 9 samples (90%) of the *bla*<sub>TEM</sub> gene (Figure 2 and Figure 3).

**DISCUSSION**

The results of biochemical identification showed that *K. pneumoniae* bacteria did not contain indole (-) and Methyl Red (-), contained urea (+), Simmon's Citrate (+), Voges Proskauer (+), and positive fermentation test of carbohydrate (+). The isolation and identification results were confirmed by *K. pneumoniae* character according to Holt *et al.* (2000).

*K. pneumoniae* bacteria is a bacteria with a size of 2.0-3.0 x 0.6 µm and this bacterium is a normal flora in the intestinal and respiratory tracts. *K. pneumoniae* has a large capsule so that in its colonies culture looks very mucoid (Brooks *et al.*, 2005).

Based on the morphology of colonies grown on Mac Conkey agar and biochemical test, *K. pneumoniae* isolates were found from a dairy cow feces, chicken broiler, and tilapia fish as much as 10 out of 45 stool samples. The presence of bacteria of the *K. pneumoniae* on swab samples were 4.8% (4/83) in dairy cows, 40% (2/5) in beef cattle, 10% (1/10) in the broiler chicken, and 3% (3/97) in the tilapia fish.

*K. pneumoniae* bacteria resistance test against antibiotics indicated as much as 90% (9/10) resistant to amoxicillin. The results are similar to the results found by Sagita *et al.* (2015) that the bacteria *K. pneumoniae* was resistant to the antibiotic amoxicillin. Resistance occurs due to the ability of the bacteria to produce penicillinase enzymes that are capable of breaking down the beta lactam ring. With this effect, penicillin is converted into penicilloid acid that is not so active. Resistance is produced by taking action against degraded penicillin by beta-lactamase. Beta lactamase enzymes protect Gram-positive and Gram-negative bacteria. In a Gram-positive bacteria, the enzyme is liberated in the medium and destroys antibiotics before it reached the cell and in gram negative it is located on the route where antibiotics must

Table 2. Antibiotic inhibition zone interpretation

Isolate code	Antibiotic discs									
	SAM		AML		SXT		MEM		CTX	
	D (mm)	R,I,S	D (mm)	R,I,S	D (mm)	R,I,S	D (mm)	R,I,S	D (mm)	R,I,S
SP-S1	25	S	17	S	28	S	29	S	34	S
SP-S3	24	S	11	R	25	S	30	S	36	S
SP-B2	24	S	13	R	29	S	30	S	35	S
SP-G4	22	S	13	R	23	S	32	S	34	S
SPT-K1	25	S	13.7	R	19	S	34.5	S	40	S
SPT-K2	24.5	S	10	R	20.1	S	30.2	S	30	S
AW-5	22	S	11.2	R	29.7	S	27.7	S	33	S
IN-P2	23	S	12	R	19.9	S	29	S	28	S
IN-P3	21.1	S	10.5	R	22.9	S	27.8	S	29.4	S
IN-S3	24	S	11	R	18	S	34	S	37	S

Note: R (resistant), I (intermediates), S (sensitive), SP-S (dairy cow in Senduro), SP-B (dairy cow in Batu), SP-G (dairy cow in Grati), SPT-K (beef cattle), AW (broiler in Wonokromo), IN-P (customs tilapia), IN-S (tilapia in Sedila), SAM (ampicillin 10µg), SXT (sulfamethonazole-trimetrophrim 27,75 µg), CTX (cefotaxime 30 µg), MEM (meropenem 10 µg), AML (amoxycilin 15 µg).

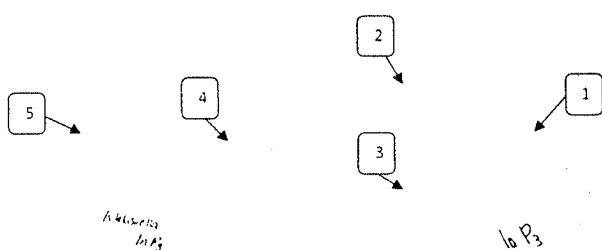


Figure 1. Inhibition zone diameter interpretation. (1) SAM (ampicillin 10µg), (2) SXT (sulfamethonazole-trimetrophrim 27,75 µg), (3) CTX (cefotaxime 30 µg), (4) MEM (meropenem 10 µg), (5) AML (amoxycilin 15 µg).

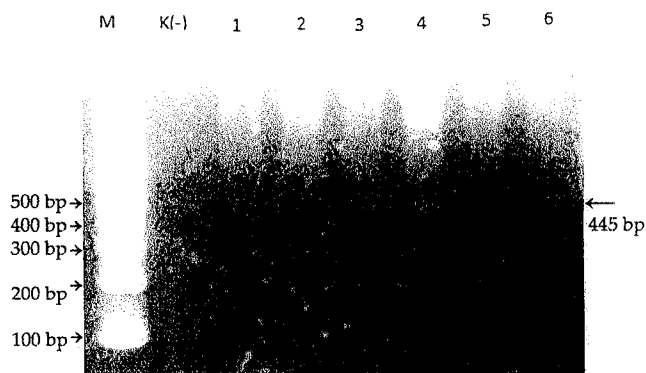


Figure 2. Agarose gel electrophoresis 2% product used primer for *bla*<sub>TEM</sub> gene detection in *Klebsiella pneumoniae* bacteria. Lane M: marker; lane 1-6 sample; lane K (-): negative control. Note: lane 1= SP-S1; lane 2= SP-S3; lane 3= SP-B2; lane 4= SP-G4; lane 5= IN-P2; lane 6= SPT-K1.

Handwritten signature or initials.

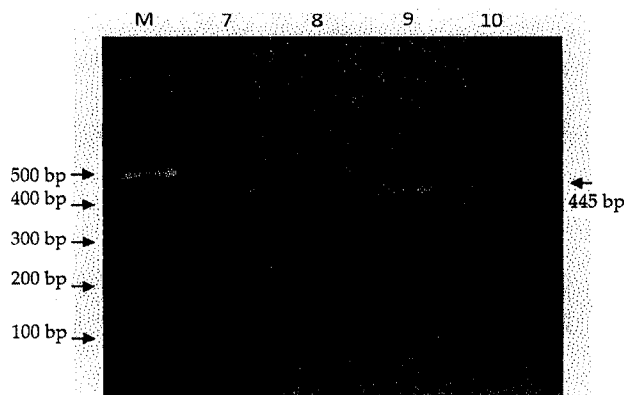


Figure 3. Agarose gel electrophoresis 2% product used primer for *bla*<sub>TEM</sub> gene detection in *Klebsiella pneumoniae* bacteria. Lane M: marker; lane 7-10 sample; lane K (-): negative control. Note: lane 7= SPT-K2; lane 8= IN-P3; lane 9=IN-S3; lane 10=AW-5.

proceed to reach the target (Sagita *et al.*, 2015). The bacterial isolate that were resistance in this study only occurred against amoxicillin and showed sensitive against ampicillin sulbactam, cefotaxim, meropenem, and sulfamethonazole trimethoprim. These results are contrary to the study conducted by Sagita *et al.* (2015) and Ghasemi *et al.* (2013) stating that the bacteria *K. pneumoniae* are resistant to cefotaxime antibiotics. Ghasemi *et al.* (2013) also states that the bacterium *K. pneumoniae* are 100% resistant to ampicillin antibiotics.

Research conducted by Ahmed & Shimamoto (2011) shows that *bla*<sub>TEM</sub> genes as antimicrobial resistance found as many as 23 isolates (67. 6%) of 34 isolates Gram-negative in case of mastitis in Egypt, and research conducted by Aljanaby & Alhasani (2016) also showed 30 isolates (93.75%) found *bla*<sub>TEM</sub> genes from 32 bacterial isolates *K. pneumoniae* isolated from patients with different clinias infections in Iraq. The majority of ESBL enzymes derived from the TEM type decoded by gene *bla*<sub>TEM</sub>. *bla*<sub>TEM</sub> gene is a gene causes antibiotic resistance in the plasmids, and it is most often detected in clinical populations of Gram-negative microorganisms (Wilopo *et al.*, 2015).

### CONCLUSION

*K. pneumoniae* can be isolated from swab samples of food-producing animals that is equal to 5.12% (10/195). All of the isolates showed a tendency to be resistant to amoxicillin 90% (9/10). Their resistances also be confirmed by detecting the ESBL-encoding gene ie *bla*<sub>TEM</sub> genes. Further research needs to be conducted to study that these bacteria have other ESBL-encoding genes such as CTX and SHV genes. In addition, it is necessary to detect the ESBL-producing *K. pneumoniae* bacteria from the meat of food-producing animals, farm waste, and slaughterhouses, as well as human feces.

### CONFLICT OF INTEREST

The Authors declare that there is no conflict of interest with any financial, personal, or other relationships

with other people or organization related to the material discussed in the manuscript.

### ACKNOWLEDGEMENT

Thanks to the Integrated Applied Research Flagship Universities of the Ministry of Research, Technology and Higher Education of the Republic of Indonesia the 2017 Budget that supported this research.

### REFERENCES

- Ahmed, A.M. & T. Shimamoto. 2011. Molecular characterization of antimicrobial resistance in gram-negative bacteria isolated from bovine mastitis in Egypt. *Microbiol. Immunol.* 55: 318-327. <https://doi.org/10.1111/j.1348-0421.2011.00323.x>
- Aljanaby, A.A.J & A.H.A. Alhasani. 2016. Virulence factors and antibiotic susceptibility patterns of multidrug resistance *Klebsiella pneumoniae* isolated from different clinical infections. *African Journal of Microbiology Research* 10: 829-843. <https://doi.org/10.5897/AJMR2016.8051>
- Apriliani, N. P. E. U. & K. J. P. Pinatih. 2017. Prevalensi kelompok gen *bla*<sub>CTX-M-1</sub> pada *Klebsiella pneumoniae* di Rumah Sakit Umum Pusat Sanglah Denpasar. *E-Jurnal Medika* 6: 1-7.
- Brooks, G. F., J.S. Butel, & S.A. Morse. 2005. *Medical Microbiology*. Edisi I. Alih Bahasa: Bagian Mikrobiologi, FKU Unair. Salemba Medika. Jakarta.
- Doosti, A., M. Pourabbas, A. Arshi, M. Chehelgerdi, & H. Kabiri. 2014. TEM and SHV genes in *Klebsiella pneumoniae* isolated from cockroaches and their antimicrobial resistance pattern. *Osong Public Health and Research Perspectives* 6: 3-8. <https://doi.org/10.1016/j.phrp.2014.10.011>
- Ghasemi, Y., T.Archin, M.Kargar, & M.Mohkam. 2013. A Simple multiplex PCR for assessing prevalence of extended spectrum beta lactamases producing *Klebsiella pneumoniae* in Intensive Care Units of a referral hospital in Shiraz Iran. *Asian Pasific Journal of Tropical Medicine*. 703-708.
- Holt, S.R., E.G.D. Murray, & R.N. Smith. 2000. *Bergey's Manual Determinative of Bacteriology*. 9th ed. Waverly Press, Baltimore.
- Kusumaningrum, H. D., L. Handayani, & R. Novrianti. 2016. Partial sequencing of 16S rRNA gene of selected *Staphylococcus aureus* isolates and its antibiotic resistance. *Med. Pet.* 39: 67-74. <http://dx.doi.org/10.5398/medpet.2016.39.2.67>
- Lalzampuaia, H., T. K. Dutta, I. Warjri & R. Chandra. 2014. Detection of extended-spectrum  $\beta$ -lactamases (*bla*<sub>CTX-M-1</sub> and *bla*<sub>TEM</sub>) in *Escherichia coli*, *Salmonella* spp., and *Klebsiella pneumoniae* isolated from poultry in North Eastern India. *Veterinary World* 7: 1026-1031. <https://doi.org/10.14202/vetworld.2014.1026-1031>
- Lestari, N. W., A. Budiharjo, & A. Pangastuti. 2016. Bakteri Heterotrof aerobik asal saluran pencernaan ikan sidat (*Anguilla bicolor bicolor*) dan potensinya sebagai probiotik. *Biotechnologi* 13: 9-17.
- Masruroh, C. A., M. B. Sudarwanto, & H. Latif. 2016. The Occurrence of extended spectrum *B-Lactamase*-producing *Escherichia coli* from broiler feces in Bogor. *JSV* 34: 42-49
- Monstein, H.J., A. Osthlm-Bulkhed, M.V. Nilsson, M. Dombusch, & L.E. Nilsson. 2007. Multiplex PCR amplification assay for the detection of *bla*<sub>SHV</sub>, *bla*<sub>TEM</sub>, and *bla*<sub>CTX-M</sub> genes in *Enterobacteriaceae*. *APMIS*. 115:1400-1408. <https://doi.org/10.1111/j.1600-0463.2007.00722.x>
- Ningrum, S.G., R. D. Soejoedono, H. Latif, W. Arnafia, & I. W. T. Wibawan. 2016. Prevalence and characterization of shiga toxin-producing *Escherichia coli* isolated from

- slaughtered qurban animal in Jakarta Province. *Med. Pet.* 39: 90-94. <http://dx.doi.org/10.5398/medpet.2016.39.2.90>
- Overdevest, L., I. Willemsen, M. Rijnsburger, A. Eustace, X. Li, P. Hawkey, M. Heck, P. Savelkoul, C. Vandenbroucke-Grauls, K. van der Zwaluw, X. Huijsdens, & J. Klutmans. 2011. Extended-Spectrum  $\beta$ -laktamase genes of *Escherichia coli* in chicken meat and humans, the Netherlands. *Emerg. Infect. Dis.* 17: 1216-1222. <https://doi.org/10.3201/eid1707.110209>
- Rahamathulla, M.P., B.N. Harish, L. Mataseje, & M.R. Mulvey. 2016. Carbapenem resistance mechanisms among blood isolates of *Klebsiella pneumoniae* and *Escherichia coli*. *Journal. Microbiol.* 10:45-53.
- Sagita, D., L. Azizah, & Y. Sari. 2015. Identification of bacteria and antibiotic sensivity test of pus of post surgical wound infection in Jambi Public Hospital in the period August-October 2014. *Sains Farmasi* 1: 6-7.
- Susilo, J., T. R. Sartono, & Sumarno. 2004. Deteksi bakteri *Klebsiella pneumoniae* pada sputum dengan metode imunohistokimia menggunakan anti outer membrane protein berat molekul 40 kDA *Klebsiella pneumoniae* sebagai antibodi. *Jurnal Kedokteran Brawijaya* 20: 12-18. <https://doi.org/10.21776/ub.jkb.2004.020.01.3>
- Wilopo, B.A.P., S. Sudigdoadi, E. Sahiratmadja, & I.M.W. Dewi. 2015. Loop-mediated isothermal amplification untuk mendeteksi gen *blaTEM* sebagai penyandi *extended-spectrum beta-lactamase* pada isolat enterobacteriaceae. *Majalah Kedokteran Bandung* 47: 243-244. <https://doi.org/10.15395/mkb.v47n4.618>
- World Health Organization. 2014. *Antimicrobial resistance: global report on surveillance*.
- Yeh, K, M., A. Kurup, L.K. Siu, Y.L.Koh, C.P. Fung, J.C. Lin, T.L. Chen, F.Y. Chang, & T.H. Koh. 2007. Capsular serotype K1 or K2, rather than *magA* and *rmpA*, is a major virulence determinant for *Klebsiella pneumoniae* liver abscess in singapore and Taiwan. *J. Clin. Microbiol.* 45:466-471. <https://doi.org/10.1128/JCM.01150-06>
- Yuwono, J. 2011. Prevalensi gen TEM pada extended spectrum beta lactamase producing enterobactericeae. *Jurnal Kedokteran dan Kesehatan* 43: 3098-3102.