ISSN: 1412-033X E-ISSN: 2085-4722

# BIODIVERSITAS Journal of Biological Diversity Volume 24 - Number 2 - February 2023

Front cover: Troides helena cerberus f. eumagos (C. & R. Felder 1865) (Photo: SierraSunrise)

**PRINTED IN INDONESIA** 





9177 1412

Published monthly



ISSN/E-ISSN: 1412-033X (printed edition), 2085-4722 (electronic)

### **EDITORIAL BOARD:**

Abdel Fattah N.A. Rabou (Palestine), Agnieszka B. Najda (Poland), Ajar Nath Yadav (India), Ajay Kumar Gautam (India), Alan J. Lymbery (Australia), Annisa (Indonesia), Bambang H. Saharjo (Indonesia), Daiane H. Nunes (Brazil), Darlina Md. Naim (Malaysia), Ghulam Hassan Dar (India), Hassan Pourbabaei (Iran), Joko R. Witono (Indonesia), Kartika Dewi (Indonesia), Katsuhiko Kondo (Japan), Kusumadewi Sri Yulita (Indonesia), Livia Wanntorp (Sweden), M. Jayakara Bhandary (India), Mahdi Reyahi-Khoram (Iran), Mahendra K. Rai (India), Mahesh K. Adhikari (Nepal), Maria Panitsa (Greece), Mochamad A. Soendjoto (Indonesia), Mohamed M.M. Najim (Srilanka), Mohib Shah (Pakistan), Nurhasanah (Indonesia), Praptiwi (Indonesia), Rasool B. Tareen (Pakistan), Seyed Aliakbar Hedayati (Iran), Seyed Mehdi Talebi (Iran), Shahabuddin (Indonesia), Shahir Shamsir (Malaysia), Shri Kant Tripathi (India), Shubhi Avasthi (India), Subhash C. Santra (India), Sugeng Budiharta (Indonesia), Sugiyarto (Indonesia), Taufiq Purna Nugraha (Indonesia), Wawan Sujarwo (Indonesia), Yosep S. Mau (Indonesia)

#### EDITOR-IN-CHIEF: Sutarno

#### **EDITORIAL MEMBERS:**

English Editors: **Suranto** (surantouns@gmail.com); Technical Editor: **Solichatun** (solichatun\_s@yahoo.com), **Artini Pangastuti** (pangastuti\_tutut@yahoo.co.id); Distribution & Marketing: **Rita Rakhmawati** (oktia@yahoo.com); Webmaster: **Ari Pitoyo** (aripitoyo@yahoo.com)

#### **MANAGING EDITORS:**

Ahmad Dwi Setyawan (unsjournals@gmail.com)

#### **PUBLISHER:**

The Society for Indonesian Biodiversity

#### **CO-PUBLISHER:**

Department of Biology, Faculty of Mathematics and Natural Sciences, Sebelas Maret University, Surakarta

**ADDRESS:** 

Jl. Ir. Sutami 36A Surakarta 57126. Tel. +62-271-7994097, Tel. & Fax.: +62-271-663375, email: editors@smujo.id

**ONLINE:** 

biodiversitas.mipa.uns.ac.id; smujo.id/biodiv

Society for Indonesia Biodiversity



Sebelas Maret University Surakarta

Published by Smujo International for The Society for Indonesian Biodiversity and Sebelas Maret University Surakarta

#### **GUIDANCE FOR AUTHORS**

Aims and Scope *Biodiversitas, Journal of Biological Diversity* or *Biodiversitas* encourages submission of manuscripts dealing with all aspects of biodiversity, including plants, animals, and microbes at the level of the gene, species, and ecosystem. Ethnobiology papers are also considered.

Article types The journal seeks original full-length: (i) Research papers, (ii) Reviews, and (iii) Short communications. Original research manuscripts are limited to 8,000 words (including tables and figures) or proportional to articles in this publication number. Review articles are also limited to 8,000 words, while Short communications should be less than 2,500 words, except for pre-study.

Submission: The journal only accepts online submissions through the open journal system (https://smujo.id/biodiv/about/submissions) or email the editors at **unsiournals@gmail.com**. Submitted manuscripts should be the original works of the author(s). Please ensure that the manuscript is submitted the **Biodiversitas** template. which can he found using at (https://biodiversitas.mipa.uns.ac.id/D/guidance.htm). The manuscript must be accompanied by a cover letter containing the article title, the first name and last name of all the authors, and a paragraph describing the claimed novelty of the findings versus current knowledge. Please also provide a list of five potential reviewers in your cover letter. Submission of a manuscript implies the submitted work has not been published (except as part of a thesis or report, or abstract) and is not being considered for publication elsewhere. When a group writes a manuscript, all authors should read and approve the final version of the submitted manuscript and its revision; and agree on the submission of manuscripts for this journal. All authors should have made substantial contributions to the concept and design of the research, acquisition of the data and its analysis, drafting the manuscript, and correcting the revision. All authors must be responsible for the work's quality, accuracy, and ethics.

**Ethics** Author(s) must be obedient to the law and/or ethics in treating the object of research and pay attention to the legality of material sources and intellectual property rights.

**Copyright** If the manuscript is accepted for publication, the author(s) still hold the copyright and retain publishing rights without restrictions. Authors can reproduce articles as long as they are not used for commercial purposes. For the new invention, authors must manage its patent before publication.

**Open Access** The journal is committed to free-open access that does not charge readers or their institutions for access. Readers are entitled to read, download, copy, distribute, print, search, or link to the full texts of articles, as long as not for commercial purposes. The license type is CC-BY-NC-SA.

Acceptance Only articles written in US English are accepted for publication. Manuscripts will be reviewed by editors and invited reviewers (double-blind review) according to their disciplines. Authors will generally be notified of acceptance, rejection, or need for revision within 1 to 2 months of receipt. Manuscripts will be rejected if the content does not align with the journal scope, does not meet the standard quality, is in an inappropriate format, or contains complicated grammar, dishonesty (i.e., plagiarism, duplicate publications, fabrication of data, citations manipulation, etc.), or ignoring correspondence in three months. The primary criteria for publication are scientific quality and biological significance. **Uncorrected proofs** will be sent to the corresponding author by email as .doc or .docx files for checking and correcting typographical errors. The accepted proofs should be returned in 7 days to avoid publication delays. The accepted papers will be published online in chronological order at any time but printed at the end of each month.

A charge Authors are charged USD 320 (IDR 4,500,000). Additional charges may be billed for language improvement, USD 75-150 (IDR 1,000,000-2,000,000).

Reprints The sample journal reprint is only available by special request. Additional copies may be purchased when ordering by email and sending back the uncorrected proofs. Manuscript preparation Manuscript is typed on A4 (210x297 mm<sup>2</sup>) paper size, in a single column, single space, 10-point (10 pt) Times New Roman font. The margin text is 3 cm from the top, 2 cm from the bottom, and 1.8 cm from the left and right. Smaller lettering sizes can be applied in presenting tables and figures (9 pt). Word processing program or additional software can be used: however, it must be PC compatible, use the Biodiversitas template, and be Microsoft Word based (.doc or .rtf; not .docx). Scientific names of species (incl. subspecies, variety, etc.) should be written in italics, except in italicized sentences. Scientific names (genus, species, author) and cultivar or strain should be mentioned completely for the first time mentioning it in the body text, especially for taxonomic manuscripts. The Genus name can be shortened after the first mention, except where this may generate confusion. name of the author can be eliminated after the first mention. For example, Rhizopus oryzae L. UICC 524 can be written hereinafter as R. oryzae UICC 524. Using trivial names should be avoided. Biochemical and chemical nomenclature should follow the order of the IUPAC - IUB. For DNA sequences, it is better to use Courier New font. Standard chemical abbreviations can be applied for common and clear used, for example, completely written butilic hydroxyl toluene (BHT) to be BHT hereinafter. Metric measurements should use IS denominations, and other systems should use equivalent values with the denomination of IS mentioned first. A dot should not follow abbreviations like g, mg, mL, etc. Minus index (m<sup>-2</sup>, L<sup>-1</sup>, h<sup>-1</sup>) suggested being used, except in things like "per-plant" or "perplot." Mathematical equations can be written down in one column with text; in that case, they can be written separately. **Numbers** one to ten are written in words, except if it relates to measurement, while values above them are written in number, except in early sentences. The fraction should be expressed in decimal. In the text, it should be used "%" rather than "percent." Avoid expressing ideas with complicated sentences and verbiage/phrasing, and use efficient and effective sentences.

The **article's title should be written in a** compact, clear, and informative sentence, preferably not more than 20 words. Author name(s) should be completely written. Name and institution address should also be completely written with street name and number (location), postal code, telephone number, facsimile number, and email address. Manuscripts written by a group, author for correspondence, and address are required. The first page of the manuscript is used for writing the above information.

The Abstract should not be more than 200 words. Include between five and eight Keywords, using scientific and local names (if any), research themes, and special methods used, and sorted from A to Z. All important abbreviations must be defined at their first mention. The running title is about five words. The Introduction is about 400-600 words, covering the background and aims of the research. Materials and Methods should emphasize the procedures and data analysis. Results and Discussion should be written as a series of connecting sentences; however, manuscripts with long discussions should be divided into subtitles. Thorough discussion represents the causal effect that mainly explains why and how the research results occurred and did not only re-express the mentioned results in sentences. A Conclusion should be given at the end of the Discussion. Finally, acknowledgments are expressed in brief; all sources of institutional, private, and corporate financial support for the work must be fully acknowledged, and any potential conflicts of interest must be noted.

**Figures and Tables** of three pages maximum should be clearly presented. Include a label below each figure and above each table (see example). Colored figures can only be accepted if the manuscript's information can be lost without those images; the chart is preferred to use black and white images. The author could consign any picture or photo for the front cover, although it does not print in the manuscript. All images property of others should be mentioned as sources. Although there is no **Appendix**, all data or data analysis is incorporated into Results and Discussions. For comprehensive/broad data, supplementary information can be provided on the website.

**References** In the text, give the author names followed by the year of publication and arrange from oldest to newest and from A to Z; in citing an article written by two authors, both of them should be mentioned; however, for three and more authors only the first author is mentioned followed by et al. For example, Saharjo and Nurhayati (2006) or (Boonkerd 2003a, b, c; Sugiyarto 2004; El-Bana and Nijs 2005; Balagadde et al. 2008; Webb et al. 2008). Extent citation should be avoided, as shown with the word "cit." Reference to unpublished data and personal communication should not appear in the list but should be cited in the text only (e.g., Rifai MA 2007, pers. com. (personal communication); Setyawan AD 2007, unpublished data). In the reference list, the references should be listed in alphabetical order. Names of journals should be abbreviated. Always use the standard abbreviations (www.issn.org/2-22661-LTWA-online.php). Please include DOI links for journal papers. The following examples are for guidance.

#### Journal:

Saharjo BH, Nurhayati AD. 2006. Domination and composition structure change at hemic peat natural regeneration following burning; a case study in Pelalawan, Riau Province. Biodiversitas 7: 154-158. DOI: 10.13057/biodiv/d070213

Book:

Rai MK, Carpinella C. 2006. Naturally Occurring Bioactive Compounds. Elsevier, Amsterdam.

#### Chapter in the book:

Webb CO, Cannon CH, Davies SJ. 2008. Ecological organization, biogeography, and the phylogenetic structure of rainforest tree communities. In: Carson W, Schnitzer S (eds.). Tropical Forest Community Ecology. Wiley-Blackwell, New York.

#### Abstract:

Assaeed AM. 2007. Seed production and dispersal of *Rhazya stricta*. 50th annual symposium of the International Association for Vegetation Science, Swansea, UK, 23-27 July 2007.

#### Proceeding:

Alikodra HS. 2000. Biodiversity for development of local autonomous government. In: Setyawan AD, Sutarno (eds.). Toward Mount Lawu National Park; Proceeding of National Seminary and Workshop on Biodiversity Conservation to Protect and Save Germplasm in Java Island. Universitas Sebelas Maret, Surakarta, 17-20 July 2000. [Indonesian]

#### Thesis, Dissertation:

Sugiyarto. 2004. Soil Macro-invertebrates Diversity and Inter-Cropping Plants Productivity in Agroforestry System based on Sengon. [Dissertation]. Universitas Brawijaya, Malang. [Indonesian]

Information from the internet: Balagadde FK, Song H, Ozaki J, Collins CH, Barnet M, Arnold FH, Quake SR, You L. 2008. A synthetic *Escherichia coli* predator-prey ecosystem. Mol Syst Biol 4:187. www.molecularsystembiology.com

ISSN: 1412-033X E-ISSN: 2085-4722



Short Communication: Tabanid and muscoid hematophagous flies as potential vectors of Surra Disease in Yogyakarta, Indonesia NILA QUDSIYATI, RADEN WISNU NURCAHYO, DWI PRIYOWIDODO, SOEDARMANTO INDARJULIANTO	675-680
Moss from Sumbawa District, Indonesia FLORENTINA INDAH WINDADRI	681-689
Characteristics of maleo bird spawning nests ( <i>Macrocephalon maleo</i> ) in Lake Towuti, South Sulawesi	690-696
HADIJAH AZIS KARIM, NARDY NOERMAN NAJIB, SRIDA MITRA AYU, FIDEL	
Changes in reef benthic communities in Sumba Timur, East Nusa Tenggara, Indonesia RIZKIE SATRIYA UTAMA, TRI ARYONO HADI, BAMBANG HERMANTO, GIYANTO, AGUS BUDIYANTO	697-707
Abundance and diversity of butterfly in the Lombok Forest Park, Indonesia MOHAMMAD LIWA ILHAMDI, AGIL AL IDRUS, DIDIK SANTOSO, GITO HADIPRAYITNO, MUHAMMAD SYAZALI, ISMAWAN HARIYADI	708-715
Phylogenetic of red snapper (Lutjanidae) in Yapen Island Waters, Papua, Indonesia RIDWAN SALA, ARADEA BUJANA KUSUMA, BAYU PRANATA,	716-723
Sex-linked Single Nucleotide Polymorphism (SNP) identification and molecular marker development of Salacca (Salacca zalacca (Gaertn.) Voss) RIRY PRIHATINI, DINY DINARTI, AGUS SUTANTO, SUDARSONO,	724-732
Analysis of phytochemical constituents and antioxidant activity from the fractions of <i>Luvunga sarmentosa</i> root extract using LCMS/MS SYARPIN, SILVANI PERMATASARI, DWI ARI PUJIANTO	733-740
Determination of Begomovirus on chili plants ( <i>Capsicum</i> sp.) in Buton and Muna Islands, Southeast Sulawesi, Indonesia CATUR JOKO WIDODO, MUHAMMAD TAUFIK, ANDI KHAERUNI, RAHAYU MALLARANGENG	741-751
Molecular identification of thermophilic bacteria with antimicrobial activity isolated from hot springs in North Sumatra, Indonesia CHRISMIS NOVALINDA GINTING, FINNA PISKA, HARMILENI, EDY FACHRIAL	752-758
Short Communication: Characteristics of the endangered Javan banteng	759-766
(Bos javanicus) spermatozoa VINCENTIA TRISNA YOELINDA, RADEN IIS ARIFIANTINI, DEDY DURYADI SOLIHIN, MUHAMMAD AGIL, DEDI RAHMAT SETIADI, YOHANA TRI HASTUTI, JANSEN MANANSANG, DONDIN SAJUTHI	
Ethnobotany of medicinal plants in the Dayak Linoh Tribe in Sintang District, Indonesia HENDRIKUS JULUNG, MARKUS IYUS SUPIANDI, BENEDIKTUS EGE, SITI ZUBAIDAH, SUSRIYATI MAHANAL	767-775
Fishing methods and fishing season of the tropical lobster fisheries of southern Java, Indonesia ARIEF SETYANTO, SUMARNO, DGR. WIADNYA, C. PRAYOGO, Z. KUSUMA, R.J. WEST, M. TSAMENYI	776-783
Flowering and fruiting phenology of <i>Anaxagorea Iuzonensis</i> A. Gray (Annonaceae) DEWI AYU LESTARI, LINDA WIGE NINGRUM, F. MAFTUKHAKH HILMYA NADA, NARESVARA NIRCELA PRADIPTA, DIAN RAKHMAWATI HARSONO4	784-792

The influence of inoculum types on the chemical characteristics and of tempe Gembus SAMSUL RIZAL, MARIA ERNA KUSTYAWATI, MURHADI, MUHAMAMAD	β-glucan content 7 AMIN	'93-798
Forest structures and carbon stocks of community forests with different management in Maha Sarakham Province, Thailand WANNACHAI WANNASINGHA, BHUVADOL GOMONTEAN, YANNAWUT	nt forest 7 UTTARUK	'99-809
Diversity of aquatic plants in the Rote Dead Sea area, East Nusa Tenge based on <i>rbcL</i> marker DESY WULANSARI LONTHOR, MIFTAHUDIN, KAYAT, ATRIYON JULZAR SUBEHI, ELISA ISWANDONO, ALFRED O. M DIMA7, AAN DIANTO, FAJA MEDIA FITRI ISMA NUGRAHA8,	gara, Indonesia, 8 RIKA, LUKI AR SETIAWAN,	810-818
Agronomic performance and selection of green super rice doubled has anther culture SITI NURHIDAYAH, BAMBANG SAPTA PURWOKO, ISWARI SARASWAT BAYUARDI SUWARNO, ISKANDAR LUBIS	ploid lines from 8	819-826
Mapping coral cover using Sentinel-2A in Karimunjawa, Indonesia ERIDHANI DHARMA SATYA, AGUS SABDONO, DIAH PERMATA WIJAYA HELMI, RIKHA WIDIARATIH	8 Anti, muhammad	827-836
State of human tiger conflict around Gunung Leuser National Park in L Landscape, North Sumatra, Indonesia PINDI PATANA, HADI S. ALIKODRA, HERMAN MAWENGKANG, R. HAMI	<b>-angkat 8</b> DANI HARAHAP4	37-846
Short Communication: Biological control of <i>Meloidogyne javanica</i> by <i>penetrans</i> and <i>Trichoderma harzianum</i> on tomato plants HAMOOD M. SALEH, AHMED FAWZI SHAFEEQ, MAHER A. KHAIRI	Pasteuria 8	847-851
Seasonal litter production patterns in three tropical forests in Sulawes Implications for managing secondary forests PUTU SUPADMA PUTRA, AMRAN ACHMAD, TOSHIHIRO YAMADA, PUT	i <b>, Indonesia:</b> 8	852-860
Spatial distribution of <i>Vespa affinis</i> based on land use and population Bojonegoro District, East Java Province, Indonesia LAILY AGUSTINA RAHMAWATI, DEWI HIDAYATI, DIAN SAPTARINI, MR. RENDRA, BELLA LUTFIANI AL ZAKINA	density in 8 ABAWANI INSAN	861-868
Determinants of symptom variation of <i>Pepper yellow leaf curl Indones</i> pepper and its spread by <i>Bemisia tabaci</i> DEWA GEDE WIRYANGGA SELANGGA, LISTIHANI LISTIHANI, I GEDE F TEMAJA, GUSTI NGURAH ALIT SUSANTA WIRYA, I PUTU SUDIARTA, K YULIADHI	<i>ia virus</i> in bell 8 RAI MAYA ŒTUT AYU	869-877
Low-density polyethylene sheet biodegradation by <i>Tenebrio molitor</i> an <i>morio</i> larvae and metagenome studies on their gut bacteria BERNADETTA OCTAVIA, ANNA RAKHMAWATI, SUHARTINI, LUTHFIA D TITAN DWIKAMA PUTRA	nd <i>Zophobas</i> 8 WI RACHMANI,	878-886
The effects of flowering trap crops on diversity and longevity of pollina visitation on chili plants TIEN AMINATUN, BUDIWATI, YUNITA FERA RAHMAWATI, DEVITA NUR WIDIYANTI, LUFI ATHIFAH FAHDAH	ator insect 8	87-893
Morphological characteristic of dengue vectors Aedes aegypti and Ae. (Family: Culicidae) using advanced light and scanning electron micros SUPRIYONO, SUSI SOVIANA, MUHAMMAD FALIKHUL MUSYAFFA, DIM UPIK KESUMAWATI HADI	. <i>albopictus</i> 8 scope AS NOVIATO,	94-900
Serratia marcescens strain NPKC3_2_21 as endophytic phosphate sol and entomopathogen: promising combination approach as rice biofert biopesticide G. SUTIO, A. NUR AFIFAH, R. MAHARANI, M. BASRI	ubilizing bacteria 9 ilizer and	01-909

Characterization, and screening of urease activity of ureolytic bacteria from landfills soil in Banda Aceh, Indonesia LENNI FITRI, TEUKU BUDI AULIA, AMIR FAUZI, GALIH AHMAD KAMIL	910-915
Amplicon metabarcoding analysis of bio-extract and its potential on plant growth promotion THANAKORN SAENGSANGA, WASSANA PHANURAK	916-922
Predicting the current potential geographical distribution of <i>Baccaurea</i> ( <i>B. lanceolata</i> and <i>B. motleyana</i> ) in South Kalimantan, Indonesia GUNAWAN, KHOERUL ANWAR, ABDUL GAFUR, RAUDATUL HILALIYAH, AZMIL AQILATUL WARO, NUR HIKMAH, SAKINAH, MUHAMMAD ERWANSYAH, DIAN SUSILAWATI, RATNA DWI LESTARI, DINDA TRIANA	930-939
Phenotypic detection strategies of multidrug-resistant <i>Staphylococcus aureus</i> isolated from cat nasal swab in Madiun city, Indonesia SAUMI KIREY MILLANNIA, ASWIN RAFIF KHAIRULLAH, MUSTOFA HELMI EFFENDI, SUZANITA UTAMA, SHENDY CANADYA KURNIAWAN, DANIAH ASHRI AFNANI, OTTO SAHAT MARTUA SILAEN, SAFIRA RAMADHANI, SANCAKA CHASYER RAMANDINIANTO, YUSAC KRISTANTO KHODA WARUWU, AGUS WIDODO9, GIOVANNI DWI SYAHNI PUTRA, MUHAMMAD THORIQ IHZA FARIZQI, KATTY HENDRIANA PRISCILIA RIWU	940-946
Ethnobotany of wild and semi-wild edible plants of the Madurese Tribe in Sampang and Pamekasan Districts, Indonesia THOBIB HASAN AL YAMINI, NINA RATNA DJUITA, TATIK CHIKMAWATI, YOHANES PURWANTO	947-957
Inhibitor α-glucosidase activity of <i>Pediococcus acidilactici</i> DNH16 isolated from Dali ni Horbo, a traditional food from North Sumatra, Indonesia EDY FACHRIAL, SARI ANGGRAINI, HARMILENI, SARYONO, TITANIA T. NUGROHO4	958-965
Morphometric characterization and zoometric indices of female Bali cattle reared in Lombok Tengah District, West Nusa Tenggara, Indonesia ADI TIYA WARMAN, GALIH TRIE FADHILAH, ALEK IBRAHIM, BAYU ANDRI ATMOKO, ENDANG BALIARTI, PANJONO,	966-974
Short Communication: Notes on a new distribution record of the Critically Endangered Sunda Pangolin ( <i>Manis javanica</i> ) in Sabah, Malaysian Borneo JEPHTE SOMPUD, NURHIDAYAH SAHAR, CARMEN ADROS, EVELYN RICHARD, CYNTHIA BOON SOMPUD	975-981
Population survey and red list assessment of <i>Kandelia candel</i> (L.) Druce, a critically endangered mangrove species in the Philippines Pastor L. Malabrigo Jr., Gerald T. Eduarte, Damasa M. Macandog, LERMA SJ. MALDIA, NELSON M. PAMPOLINA, RACHEL C. SOTTO, INOCENCIO E. BUOT JR.	982-989
Visualization of paddy in Panel 65 of the Karmawibhangga section of Borobudur Temple ARYO ANGGOROJATI, RAHMANU WIDAYAT, SETYO BUDI	990-1003
Short Communication: Avian sanctuary within the city: the case of Timaco Hill, Cotabato City, Bangsamoro Autonomous Region in Muslim Mindanao (BARMM), Mindanao Island, Philippines PETER JAN D. DE VERA, JOHN PAUL C. CATIPAY, MARIAN DARA T. TAGOON, ELSA MAY DELIMA-BARON, BENITO ANTHONY PINGGOY5	1004-1009
Abundance and diversity of plant parasitic nematodes associated with vegetable cultivation on various types of organic fertilizers SIWI INDARTI, TARYONO, CHANDRA WAHYU PURNOMO, AYU SUCI WULANDARI, RINA MAHARANI	1010-1016

Stakeholder and social networks analysis of conservation partnership in Bantimurung Bulusaraung National Park, South Sulawesi, Indonesia ADRAYANTI SABAR, RISMA ILLA MAULANY, YUSRAN, ANDY KURNIAWAN, RIO AKBAR RAHMATULLAH, MUH. ARIEF SYAM, MUHAMMAD AKBAR SYAWAL, ARDIAN HALIS, MUHAMMAD TEGAR RAFIADI	1017-1024
The effect of different honey concentrations on the ultrastructure profile of spermatozoa in Dewa Mahseer ( <i>Neolissochilus soro</i> ) A. ABINAWANTO, SUCI LESTARI, ANOM BOWOLAKSONO, ASTARI DWIRANTI, RETNO LESTARI, RUDHY GUSTIANO, ANANG HARI KRISTANTO4	1025-1031
The heterosis, heterobeltiosis, and the yielding ability of hybrids of three parental crossings of soybean ( <i>Glycine max</i> )] DIANA SOFIA HANAFIAH, LUTHFI AZIZ MAHMUD SIREGAR, KHAIRUNNISA LUBIS, HARYATI, FADILLAH RAHMADANA	1032-1038
Nutritional values of <i>Avicennia marina</i> leaves and its application as fodder for Kacang goat ( <i>Capra aegagrus</i> ) MOHAMMAD BASYUNI, MUHAMMAD RISKY RIZALDI, RIZKA AMELIA, YUNTHA BIMANTARA, NURDIN SULISTIYONO, BEJO SLAMET, SHOFIYAH SABILAH AL MUSTANIROH	1039-1048
Application of remote sensing and GIS for mapping changes in land area and mangrove density in the Kuri Caddi Mangrove tourism, South Sulawesi Province, Indonesia DWI ROSALINA, HAWATI, KATARINA HESTY ROMBE, AGUS SURACHMAT, AWALUDDIN, MUCHTAR AMILUDDIN, ANI LEILANI, ASRIYANTI	1049-1056
Recorded and predicted butterflies in the Padang Bindu Karst, South Sumatra, Indonesia AGMAL QODRI, ENCILIA, YULIZAH, DEDEN GIRMANSYAH, SUNARDI, WAHYUDI SANTOSO, MEGAWATI, RINA RACHMATIYAH, FATIMAH, DARMAWAN, SARINO, DJUNIJANTI PEGGIE	1057-1082
Diversity of dinoflagellate cysts isolated from estuarine sediments of the Bengawan Solo and Brantas rivers, Indonesia SAPTO ANDRIYONO, NITA RUKMINASARI, ANDI ALIAH HIDAYANI, INDRA JUNAIDI ZAKARIA, MD. JOBAIDUL ALAM, HYUN-WOO KIM	1083-1091
Phylogeny and taxonomic status evaluation of Dark-sided Narrow-mouthed Frog, Microhyla heymonsi, (Anura: Microhylidae) from Sumatra, Indonesia RURY EPRILURAHMAN, VESTIDHIA Y. ATMAJA, MISBAHUL MUNIR, ROSICHON UBAIDILLAH, TUTY ARISURYANTI, ERIC N. SMITH, AMIR HAMIDY	1092-1103
Inventory of terrestrial vertebrate wildlife species in a private-owned forest patch in Tagum City, Mindanao, Philippines LIEF ERIKSON D. GAMALO, SHIELA MAE E. CABRERA, NOEL CARL L. DE LOS REYES, ALEYLA E. DE CADIZ, JOSELITO B. CHAVEZ JR., AARON FROILAN M. RAGANAS, MA. NIÑA REGINA M. QUIBOD	1104-1116
Next Generation Sequencing (NGS) for Cyanobacterial study in Agung and Sunter Barat Lakes, North Jakarta, Indonesia DIAN HENDRAYANTI, NINING BETAWATI PRIHANTINI, FITRIA NINGSIH, FADHLURAHMAN MAULANA	1117-1124
Diversity and distribution pattern of bioactive compound potential seaweed in Menganti Beach, Central Java, Indonesia DWI SUNU WIDYARTINI, HEXA APRILIANA HIDAYAH, ACHMAD ILALQISNY INSAN	1125-1135
Antagonist and plant growth promoting potential of indigenous bacteria isolated from oil palm empty fruit bunches DERMIYATI, RADIX SUHARJO, MARELI TELAUMBANUA, RULLY YOSITA, ANGGI WINANDA SARI, ANIS PUJI ANDAYANI	1136-1142

Incidence of Escherichia coli producing Extended-spectrum beta-lactamase in wastewater of dairy farms in East Java, Indonesia FIDI NUR AINI EKA PUJI DAMEANTI, SHEILA MARTY YANESTRIA, AGUS WIDODO, MUSTOFA HELMI EFFENDI, HANI PLUMERIASTUTI, WIWIEK TYASNINGSIH, RAHAYU SUTRISNO, M. ALI AKRAMSYAH	1143-1150
Antibacterial activity of sponge-associated bacteria from Torosiaje marine area, Gorontalo, Indonesia YULIANA RETNOWATI, ABUBAKAR SIDIK KATILI	1151-1156
Immunomodulatory effects of probiotics isolated from traditional fermented foods and beverages of Sumatra (Indonesia) and synbiotics in mice NUR INDAH PERMATASARI HARAHAP, ERMAN MUNIR, SALOMO HUTAHAEAN	1157-1162
Ethnomedicine exploration of medicinal plants in Dayak Bakumpai and Ngaju Tribes, Central Kalimantan, Indonesia NANIK LESTARININGSIH, MUHAMAD JALIL, AYATUSA'ADAH, RIDHA NIRMALASARI	1163-1174
Genotypes assessment for developing varieties on multi-canopy rice cultivation system MIFTAKHUL BAKHRIR ROZAQ KHAMID, AHMAD JUNAEDI, HENI PURNAMAWATI, HAJRIAL ASWIDINNOOR, LILIK BUDI PRASETYO4	1175-1185
Short Communication: The flowering process of <i>Prunus cerasoides</i> in Bali Botanic Gardens, Indonesia	1186-1191
Characterization and virulence of two indigenous entomopathogenic fungal isolates from decayed oil palm empty fruit bunches against <i>Spodoptera litura</i> (Lepidoptera: Noctuidae)	1192-1199
Amylase enzyme production with variation of carbon sources and molecular identification of thermophilic fungus <i>Aspergillus</i> sp. LBKURCC304 from Bukik Gadang, West Sumatra, Indonesia SARYONO, SILVERA DEVI, TITANIA T NUGROHO, WIDYLIA FITRI FADHILA, LORENA LORENITA, FIDA SELFIANA NASUTION, NABELLA SURAYA	1200-1205
Analysis of land cover change due to mining and its potential economic loss: A case study in the Bukit Soeharto Forest Park, East Kalimantan, Indonesia SUNARTO, MARLON I. AIPASSA, RUJEHAN, ALI SUHARDIMAN, ROCHADI KRISTININGRUM, YOSEP RUSLIM, WULAN IR SARI	1206-1214
Exploration of indigenous copper and dye-resistant bacteria isolated from Citarum River, West Java, Indonesia WAHYU IRAWATI, DWI NINGSIH SUSILOWATI, INDAH SOFIANA, VALENTINE LINDARTO, REINHARD PINONTOAN, TRIWIBOWO YUWONO	1215-1223
Short communication: Macrofungi assemblage in Rawa Bento Forest, Kerinci Seblat National Park, Indonesia IRDA SAYUTI, ZULFARINA, ZATRI RAHAYU	1224-1230
Ecological index and economic potential of mollusks (Gastropods and Bivalves) in Ayah Mangrove Forest, Kebumen District, Indonesia MINI AMBARWATI KUSUMA DEWI, DEVI MAYANG AURINA, AQRA DANIAL FATURRAHMAN, LAYYINATUSSYIFA A'YUNI FATIKHA, FAYZA RACHMALIA, FADIA AULIANISSA AINAYA, ASIH KINANTHI, CAHYA MAULIDTA ROHMAN, FARIZ PRADHANA ADIL FADZILAH, DESMA ASTY PRAMUDITA, MUHAMMAD FADHIL RAMADHAN, EDWI MAHAJOENO, GILANG DWI NUGROHO, PUGUH SUJARTA, MUH. SULAIMAN DADIONO, CHEE KONG YAP, KHAIRUL ADHA BIN A. RAHIM, AHMAD DWI SETYAWAN	1231-1241
Butterfly species in Bogani Nani Wartabone National Park, North Sulawesi, Indonesia RONI KONERI, MEIS JACINTA NANGOY, PIENCE VERALYN MAABUAT, WAKHID	1242-1251

Effect of environmental temperature and relative humidity towards postural behavior and activity patterns of female <i>Pongo pygmaeus</i> at Bukit Merah Orang Utan Island, Perak, Malaysia NORAMIRA NOZMI, SABAPATHY DHARMALINGAM, NUR NADIAH MD YUSOF, FARIDA ZURAINA MOHD YUSOF	1252-1263
Characteristics of vegetation in the <i>gumuk</i> ecosystem in Jember District, East Java, Indonesia WIWIN MAISYAROH, LUCHMAN HAKIM, SUDARTO, JATI BATORO	1264-1271
Exploration of phosphate solubilizing bacteria from mangrove soil of Lamongan, East Java, Indonesia FATIMAH, NUR AULA, SYARIFAH SALSABILA, ZAKIA ASRIFAH RAMLY, SHERINA YULIA ROSE, TINI SURTININGSIH, TRI NURHARIYATI	1272-1278
Isolation and characterization of endophytic bacteria from some halophytes in saline desert regions of Uzbekistan BEGALI ALIKULOV, DOSTON GULBOEV, DILNOZA MAXAMMADIEVA, ZARRINA TILLAEVA, SADOKAT OLIMJONOVA, ZAFAR ISMAILOV	1279-1288
Comparative study on Pb absorption ability of five shade plants species in Industrial Estate and Urban Forests of Bekasi, Indonesia DAYANA ZULFADILLAH INTAN, RATNA YUNIATI, RETNO LESTARI	1289-1295
Habitat suitability model for <i>Banteng</i> ( <i>Bos javanicus</i> d'Alton, 1823) in Meru Betiri National Park, Indonesia ARIF MOHAMMAD SIDDIQ, WACHJU SUBCHAN, HAIKAL IDRIS MAULAHILA, NUR KHOLIQ	1296-1302
The false alarm of experimentally companion plants to the cabbage pests with their ecological effects AGUNG SIH KURNIANTO, LUHUR SEPTIADI, NANANG TRI HARYADI, WILDAN MUHLISON, IRWANTO SUCIPTO, NILASARI DEWI, AHMAD ILHAM TANZIL, AYU LESTARI, ROSE NOVITA SARI HANDOKO	1303-1312
α-amylase and α-glucosidase inhibitory effects of four piper species and GC-MS analysis of <i>Piper crocatum</i> YUSTINA SRI HARTINI, DEWI SETYANINGSIH	1313-1319
Antimicrobial activity of <i>Kaempferia galanga</i> against plant pathogen on rice WORO SRI SUHARTI, ETIK WUKIR TINI, DINA ISTIQOMAH	1320-1326

**BIODIVERSITAS** Volume 24, Number 2, February 2023 Pages: 1143-1150

## Incidence of *Escherichia coli* producing Extended-spectrum betalactamase in wastewater of dairy farms in East Java, Indonesia

### FIDI NUR AINI EKA PUJI DAMEANTI<sup>1,2</sup>, SHEILA MARTY YANESTRIA<sup>1</sup>, AGUS WIDODO<sup>1</sup>, MUSTOFA HELMI EFFENDI<sup>3,•</sup>, HANI PLUMERIASTUTI<sup>3</sup>, WIWIEK TYASNINGSIH<sup>3</sup>, RAHAYU SUTRISNO<sup>2</sup>, M. ALI AKRAMSYAH<sup>4</sup>

<sup>1</sup>Doctoral Student of Veterinary Science, Faculty of Veterinary Medicine, Universitas Airlangga. Jl. Mulyorejo, Kampus C, Surabaya 60115, East Java, Indonesia

Indonesi

<sup>2</sup>Laboratory of Veterinary Microbiology and Immunology, Faculty of Veterinary Medicine, Universitas Brawijaya. Jl. Veteran, Malang 65145, East Java, Indonesia

<sup>3</sup>Faculty of Veterinary Medicine, Universitas Airlangga. Jl. Mulyorejo, Kampus C, Surabaya 60115, East Java, Indonesia. Tel./fax.: +62-31-5993016, \*email: mhelmieffendi@gmail.com

<sup>4</sup>Veterinary Professional Program, Faculty of Veterinary Medicine, Universitas Brawijaya. Jl. Veteran, Malang 65145, East Java, Indonesia

Manuscript received: 16 December 2022. Revision accepted: 13 February 2023.

Abstract. Dameanti FNAP, Yanestria SM, Widodo A, Effendi MH, Plumeriastuti H, Tyasningsih W, Sutrisno R, Akramsyah MA. 2023. Incidence of Escherichia coli producing Extended-spectrum beta-lactamase (ESBL) in wastewater of dairy farms in East Java, Indonesia. Biodiversitas 24: 1143-1150. Poor wastewater treatment on dairy farms can potentially become environmental contaminants (hazardous chemicals, organic matter, and pathogenic bacteria). Bacteria from dairy cattle that have been resistant can be found in wastewater. It can migrate around the farm and cause resistance to environmental microbiota. Over the years, cases of antimicrobial resistance (AMR) in Escherichia coli bacteria are increasing due to the uncontrolled use of antibiotics in dairy farms. The present study aimed to identify the potential occurrence of dairy farm wastewater as a reservoir for Extended-Spectrum Beta-Lactamase (ESBL)producing E. coli in East Java. The number of research samples used was 342 and came from 6 cities/districts with the highest dairy cattle population in East Java (Pasuruan District, Malang District, Tulungagung District, Blitar District, Batu City, and Kediri District). The results showed that 69.30% (237/342) of dairy farm wastewater samples were positive for E. coli. The incidence of Escherichia coli AMR was 99.17% (235/237). The resistance conditions to each class of antibiotics were 76.4% ampicillin, 66.2% cefotaxime, 37.6% tetracycline, 15.6% ciprofloxacin, 96.2% streptomycin, 16.5% sulfamethoxazole-trimethoprim, and 84.0% chloramphenicol. The incidence of E. coli MDR was 84.25% (198/235), with the highest incidence (44.44%; 88/198) found in the four groups of antibiotics. The incidence of ESBL-producing E. coli from samples of dairy farm wastewater in East Java through DDST confirmation was 22.80% (78/342), with the highest incidence (20.51%; 16/78) in Pasuruan District and Batu City. In conclusion, dairy farm wastewater could be a reservoir for ESBL-producing E. coli which has the potential to impact human health in East Java Province.

Keywords: AMR-MDR, dairy farm, ESBL, Escherichia coli, human health, wastewater

#### **INTRODUCTION**

Dairy cattle in Indonesia use antibiotics as cattle need antibiotics during the dry and wet seasons while getting disease/sick to treat diseases, especially mastitis, endometritis, and diarrhea (Indriani et al. 2013). Uncontrolled use of antibiotics with inappropriate and effective concentrations can lead to antibiotic resistance (Astorga et al. 2019; Said et al. 2015). Traditional farms dominate dairy farming in Indonesia with small-scale ownership and disposal of the waste in rivers or the environment around farms. It leads to antibiotic resistance and harms public health (Pamungkasih and Febrianto 2021). Poor waste treatment causes resistant bacteria in livestock to migrate around the farm, so the environmental microbiota also experiences resistance (Sore 2020). The environment around the cage can store various resistant materials that can transfer between bacteria/mobile genetic elements (MGEs). Therefore, the environment is considered the primary source of resistance between humans and animals (Niasono et al. 2016; Woolhouse et al.

2015). Antibiotic resistance and environmental pollution in the last decade have increased and become a significant threat to global health (IMF 2014).

According to WHO (World Health Organization) (2019), the cases of death caused by AMR reach 700 thousands of people per year. The use of antibiotics globally in livestock is up to 80% higher than in humans on specific antibiotics. The use of antibiotics in the livestock sector in 2013 reached 131,109 tons and is expected to reach more than 200,000 tons in 2030 (WHO 2012; He et al. 2020). Beta-lactam antibiotics contribute to 50-70% of the antibiotics world's usage (Adekanmbi et al. 2020). The over and non-scientific use of antibiotics is unwise and causes the incidence of AMR in dairy cows. In general, the incidence of AMR in dairy cows is due to high doses of antibiotics to treat disease, increase milk and meat production and improve performance while raising dairy cows (Jayarao et al. 2019). Misuse of antibiotics causes difficulty in curing bacterial infections, increases the cost of animal health care, and even causes death (Overdevest et al. 2011). ESBL-producing Escherichia coli in dairy cattle causes various infectious diseases, especially mastitis, and transmits them to humans through direct contact with the food chain and the environment. In humans, ESBL-producing *E. coli* pathogenic strains are associated with urinary tract infections and bacteremia in communities and hospitals (Gelalcha and Dego 2022). Antimicrobial Resistance Collaborators reported cases of death associated with AMR in 2019, reaching 4.95 million and are predicted to continue to increase to 10 million per year in 2050, and 70% of these events are caused by fluoroquinolone and ESBL resistance (Antimicrobial Resistance Collaborators 2022).

Reports of incidents of ESBL-producing E. coli are of severe concern. Extended-spectrum beta-lactamase (ESBL) is an enzyme that can hydrolyze and deactivate beta-lactam antibiotics. penicillin, first-second-third generation cephalosporins, and aztreonam (Biutifasari 2018). Genes or types from the TEM, SHV, and CTX-M groups are the most frequently encountered ESBLs. The ESBL coding gene resides in MGEs and produces enzymes to hydrolyze beta-lactam antibiotics. ESBL genes are mediated mainly by plasmids making it easy to transmit to other bacteria (Adekanmbi et al. 2020; Huang et al. 2020). ESBL TEM and SHV have been reported for more than 100 variants, while CTX-M has 50 variants. That was probably caused by a mutation process- associated with bacterial infection, particularly E. coli in the community (Dhillon and Clark 2012). Escherichia coli is often used as an indicator of antibiotic resistance due to its high prevalence in the environment and healthy animals (Masruroh et al. 2016). Data on the incidence of ESBL-producing E. coli from rectal swab samples of dairy cows have been reported in various regions in Indonesia, namely, Surabaya at 72% and Sleman Yogyakarta at 25% (Imasari et al. 2018; Maulana et al. 2021). Livestock can transmit ESBL bacteria around the farm through a mixture of urine and feces with a liquid phase, commonly called liquid waste (Musruroh et al. 2016; Said et al. 2015). This situation can cause public health problems because livestock waste is the main reservoir of ESBL-producing E. coli in the environment, thus increasing the transmission of ESBL to humans (Normaliska et al. 2019). Although E. coli is considered a typical flora and rarely causes infection, it can transfer resistance genes horizontally to pathogenic bacteria, thereby contributing to the development of chronic infections and the spread of AMR (Weber et al. 2021).

Research on dairy farm wastewater as a reservoir for ESBL-producing *E. coli* in East Java Province is essential to report. East Java Province has the highest population number of dairy cattle in Indonesia. According to data from the East Java Livestock Service (2020), the population of dairy cows in East Java reaches 293,556 heads. It is spread over six districts, with the largest populations in Pasuruan District (94,101 heads), Malang District (86,986 heads), Tulungagung District (25,139 heads), Blitar District (19,258 individuals), Batu City (12,579 individuals) and Kediri District (10,786 individuals). An increase in the dairy cattle population produces waste pollution in the environment (Widiastuti et al. 2015). Dairy cattle farms in Indonesia are generally small-scale ownership that throws waste into rivers without prior processing, polluting the environment (Hidayatullah

et al. 2005; Kasworo et al. 2013; Widiastuti et al. 2015). The data on the incidence of ESBL-producing *E. coli* from rectal swab samples of dairy cows have been reported in East Java like Tulungagung District at 6% and Pasuruan at 5.9% (Putra et al. 2020; Soekoyo et al. 2020). This study aimed to determine the potential for the occurrence of dairy cattle wastewater as a reservoir for *E. coli*-producing ESBL and monitoring AMR in East Java Province so that it can understand and overcome the global situation of ESBL-producing *E. coli*.

#### MATERIALS AND METHODS

#### Study area

This study used a purposive sampling method with inclusion criteria where samples came from six cities/districts with the largest dairy cattle population in East Java, and dairy farms had a record or history of antibiotic use. The sample was obtained from Pasuruan District, Malang District, Tulungagung District, Blitar District, Batu City, and Kediri District, with 342 samples of dairy farm wastewater in June 2022.

#### **Sampling collections**

A sample of 100 ml of wastewater was isolated from ditches/cages and stored in a centrifuge tube. The sample was stored in an icebox during sample transportation and brought to the laboratory for further testing (Yanestria et al. 2019).

### Isolation and identification of Escherichia coli

The isolation stage of Escherichia coli was carried out by adding 5 ml of dairy farm wastewater to Buffer Pepton Water (BPW) media with 2% of concentration (1:1) (Oxoid, UK) and incubated at 37°C for 24 hours. The isolates from BPW media were inoculated on Eosin Methylene Blue Agar (EMBA) media (Oxoid, UK) using a streak technique and incubated at 37°C for 24 hours (Maulana et al. 2021). Colonies that appear as metallic green were identified using gram stain and biochemical assays, IMViC (Indol-motility (HiMedia, India), Methyl Red & Voges Proskauer (HiMedia, India), Citrate (HiMedia, India)), Triple Sugar Iron Agar (TSIA) (HiMedia, India) and Urease (HiMedia, India) (Imasari et al. 2018) to identifying as E. coli bacteria. Procedure for gram staining with crystal violet for 2 minutes and rinse with water, followed by iodine, acetone alcohol for 1 minute, and safranin for 2 minutes. The gram-stained preparations were then observed under a 1000x magnification microscope (Tripathi and Sapra 2022).

#### Antibiotic susceptibility test

Isolates with confirmed *E. coli* were tested for antibiotic sensitivity using the Kirby-Bauer disc diffusion method on Mueller Hinton Agar (MHA) media (Oxoid, UK). The isolates were inoculated on Brain Hearth Infusion Broth (BHIB) medium (GranuCult, Germany) and incubated for 24 hours at 37°C. Isolates on BHIB media were suspended with turbidity equivalent to 0.5 McFarland. The bacterial

suspension was grown evenly on the MHA medium. Then types of antibiotic classes were placed seven (penicillin/ampicillin (AMP) (Oxoid, UK), cefotaxime (CTX) (Oxoid, UK), tetracycline (TE) (Oxoid, UK), ciprofloxacin (CIP) (Oxoid, UK), streptomycin (S) (Oxoid, UK), sulfamethoxazole-trimethoprim (SXT) (Oxoid, UK) and chloramphenicol (C) (Oxoid, UK)) on the surface of the media (Blaak et al. 2015) then incubated at 37°C for 24 hours. The class of antibiotics was selected based on the AMR testing literature and the use of antibiotics in dairy farms in Indonesia. In addition, the visual inhibition zone diameter-interpretation results were adjusted to the Clinical Laboratory Standard Institute (CLSI) standards (Table 1) (CLSI 2020). Escherichia coli isolates that show resistance to  $\geq$  3 groups of antibiotics are categorized as Multidrug Resistance Bacteria (MDR) (Yanestria et al. 2022).

# Confirmation of ESBL using Double Disc Synergy Test (DDST)

DDST testing was carried out on bacterial isolates with MDR properties. The DDST method was used to confirm the phenotype of ESBL-producing bacteria. DDST testing was carried out using the Kirby-Bauer disc diffusion method on MHA media with three types of antibiotics ceftazidime (CAZ; 30g) (Oxoid, UK), cefotaxime (CTX; 30g) (Oxoid, UK), and amoxicillin-clavulanic (AMC: 30/10ug) (Oxoid, UK) (Zhang et al. 2016). The bacterial suspension was reequated to 0.5 McFarland standard and grown evenly on MHA media. The AMC antibiotic disk was placed in the middle of the media and continued with CAZ and CTX disks at a 15 mm distance from AMC in parallel. The bacterial culture was incubated at 35-37°C for 18-24 hours (Imasari et al. 2018). The sample was confirmed phenotypically positive for ESBL when a synergistic/increased diameter of the inhibition zone  $\geq 5$  mm between the cephalosporin inhibition zone (cefotaxime and ceftazidime) and amoxicillinclavulanic acid (Chukwunwejim et al. 2018). ESBL confirmation was carried out with the control using E. coli ATCC 25922 as a negative control and Klebsiella pneumoniae ATCC 700603 as a positive control (Masruroh et al. 2016).

### **RESULTS AND DISCUSSION**

The results of isolation and identification of E. coli from a total of 342 samples of dairy farm wastewater collected after testing were 69.30% (237/342) positive for E. coli. These results divided into Kediri District 13.45% (46/342), Blitar District 9.07% (31/342), Malang District 12.87% (44/342), Batu City 12.57% (43/342), Pasuruan District 13.45% (46/237) and Tulungagung District 7.89% (27/342) as shown in (Table 1). The positive E. coli sample showed a metallic green metachromatic sheen (Figure 1 (a)), which was caused by the end product of lactose fermentation (acid), resulting in the absorption of methylene blue color (Lal and Cheeptham 2007). The isolates from EMBA media were confirmed by gram staining and biochemical tests. The gram staining test results show a rod shape and pink-red (Figure 1 (b)) (Ulfah et al. 2017).

Confirmation of the IMViC, TSIA, and Urease biochemical tests (Figure 2) showed the results following the literature, where the TSIA media turns yellow and produces gas, indole, and several motile and non-motile. In addition, *E. coli* isolates were positive on the MR test and negative on VP, Citrate, and Urease tests (Cappucino and Welsh 2020; Chu et al. 2012; Leboffe and Pierce 2011).

# Antimicrobial resistance (AMR) and multidrug resistance (MDR)

All *E. coli* isolates were continued to the antibiotic resistance test according to the CLSI standards. The antibiotics used in this test were based on the literature on antibiotic testing in wastewater (Blaak et al. 2015) and the types of antibiotics that are often used in dairy farms in Indonesia. This test showed that 99.17% of *E. coli* isolates (235/237) have AMR properties. They were resistant to each class of antibiotics; ampicillin (76.4%), cefotaxime (66.2%), tetracycline (37.6%), ciprofloxacin (15.6%), streptomycin (96.2%), sulfamethoxazole-trimethoprim (16.5%) and chloramphenicol (84.0%). The detailed AMR results can be seen in (Table 3).

*Escherichia coli* isolates that show resistance to  $\geq$  3 are categorized as multidrug resistance bacteria (MDR). The results of the MDR incidence for 235 isolates of *E. coli* were 84.25% (198/235), presented in (Table 4). The MDR incidence showed 17.17% (34/198) for three groups of antibiotics, 44.44% (88/198) for four groups of antibiotics, 25.25% (50/198) for five antibiotic classes, 10.61% (21/198) for six antibiotic classes and 2.53% (5/198) for seven antibiotic classes.

 
 Table 1. The standard interpretation of antibiotic sensitivity (CLSI 2020)

Antibiotics	Disc content	In	hibition z diameter	rone r
		S	Ι	R
Ampicillin	10µg	≥17	14-16	≤13
Cefotaxime	30µg	≥26	23-25	≤22
Tetracyiclin	30µg	≥15	12-14	≤11
Ciprofloxacin	5µg	≥31	21-30	≤20
Streptomycin	10µg	≥15	12-14	≤11
Trimethoprim-	1.15/23.75µg	≥16	11-15	≤10
sulfamethoxazole				
Cloramphenicol	30µg	$\geq 18$	13-17	≤12

**Table 2.** Results of the isolation and identification of *Escherichia coli* in the waste of dairy farms in East Java, Indonesia

Data isolation &	Escher	richia coli	Other bacteria		
identification	n	%	n	%	
Kediri	46	13.45	4	1.17	
Blitar	31	9.07	24	7.01	
Malang	44	12.87	19	5.56	
Batu City	43	12.57	16	4.68	
Pasuruan	46	13.45	15	4.39	
Tulungangung	27	7.89	27	7.89	
Total	237	69.30	105	30.70	



**Figure 1.** Results of isolation and identification of *Escherichia coli*. A. *Escherichia coli* on EMBA medium, B. *Escherichia coli* on gram staining in 1000x magnification



**Figure 3.** Results of *E. coli* antibiotic resistance to 7 classes of antibiotics. (CTX) cefotaxime, (C) chloramphenicol, (AMP) ampicillin, (TE) tetracycline, streptomycin (S), (SXT) sulfamethoxazole-trimethoprim and (CIP) ciprofloxacin, R: Resistant, I: Intermediate, S: Sensitive



Figure 2. Identification results of *Escherichia coli* in the TSIA, IMViC, and Urease Biochemical tests. A. TSIA, B. SIM, C. Citrate, D. MR, E. VP, F. Urease

Table 3. Results of Escherichia coli antibiotic resistance in the wastewater of dairy farms in East Java, Indonesia

Data A	MD -	Ke	diri	M	alang	B	litar	Bat	u City	Pas	uruan	Tulu	ngagung	Sub	total
Data A		n	%	n	%	n	%	n	%	n	%	n	%	n	%
CTX	R	24	10.1	15	6.3	19	8.0	32	13.5	41	17.3	26	11.0	157	66.2
	Ι	19	8.0	18	7.6	6	2.5	10	4.2	4	1.7	1	0.4	58	24.5
	S	3	1.3	11	4.6	6	2.5	1	0.4	1	0.4	0	0.0	22	9.3
С	R	38	16.0	33	13.9	25	10.5	37	15.6	41	17.3	25	10.5	199	84.0
	Ι	8	3.4	11	4.6	6	2.5	6	2.5	5	2.1	2	0.8	38	16.0
	S	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
AMP	R	39	16.5	30	12.7	20	8.4	32	13.5	36	15.2	24	10.1	181	76.4
	Ι	7	3.0	11	4.6	11	4.6	8	3.4	5	2.1	3	1.3	45	19.0
	S	0	0.0	3	1.3	0	0.0	3	1.3	5	2.1	0	0.0	11	4.6
TE	R	16	6.8	16	6.8	8	3.4	21	8.9	21	8.9	7	3.0	89	37.6
	Ι	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	S	30	12.7	28	11.8	23	9.7	22	9.3	25	10.5	20	8.4	148	62.4
S	R	45	19.0	42	17.7	31	13.1	43	18.1	43	18.1	24	10.1	228	96.2
	Ι	0	0.0	2	0.8	0	0.0	0	0.0	2	0.8	1	0.4	5	2.1
	S	1	0.4	0	0.0	0	0.0	0	0.0	1	0.4	2	0.8	4	1.7
SXT	R	11	4.6	9	3.8	4	1.7	8	3.4	3	1.3	4	1.7	39	16.5
	Ι	2	0.8	0	0.0	1	0.4	5	2.1	1	0.4	1	0.4	10	4.2
	S	33	13.9	35	14.8	26	11.0	30	12.7	42	17.7	22	9.3	188	79.3
CIP	R	5	2.1	2	0.8	5	2.1	9	3.8	11	4.6	5	2.1	37	15.6
	Ι	40	16.9	38	16.0	24	10.1	34	14.3	33	13.9	22	9.3	191	80.6
	S	1	0.4	4	1.7	2	0.8	0	0.0	2	0.8	0	0.0	9	3.8

Antibiotic	K	lediri	Μ	alang	E	Blitar	Ba	tu City	Pas	suruan	Tulu	ngangung	Su	b total
classes	n	%	n	%	n	%	n	%	n	%	n	%	n	%
3	10	29.41	10	29.41	5	14.71	4	11.76	3	8.82	2	5.88	34	17.17
4	16	18.18	12	13.64	11	12.50	16	18.18	17	19.32	16	18.18	88	44.44
5	8	16.00	5	10.00	3	6.00	12	24.00	16	32.00	6	12.00	50	25.25
6	4	19.05	3	14.29	3	14.29	6	28.57	5	23.81	0	0.00	21	10.61
7	1	20.00	1	20.00	1	20.00	0	0.00	0	0.00	2	40.00	5	2.53

Table 4. Results of MDR Escherichia coli occurrence in dairy farm wastewater in East Java, Indonesia

 
 Table 5. The results of ESBL-producing Escherichia coli using the DDST test on the waste of dairy farms in East Java, Indonesia

Location	Positive ESBL	Negative ESBL
Location	n	n
Kediri	11	35
Blitar	12	19
Malang	9	35
Batu City	16	27
Pasuruan	16	30
Tulungagung	14	13
Total	78	159



**Figure 4.** ESBL confirmation results for *Escherichia coli* using the DDST test. (AMC) amoxicillin-clavulanic acid, (CTX) Cefotaxime, (CAZ) Ceftazidime, arrows are the synergies formed

# Confirmation of ESBL using *Double Disc Synergy Test* (DDST)

The DDST result was positive based on the synergy between cephalosporin antibiotics and amoxicillinclavulanic acid. This synergy was observed with an increase in the diameter of the inhibition zone observed (Figure 4) (Chukwunwejim et al. 2018). The results of ESBL-producing *E. coli* from samples of dairy farm wastewater in East Java with DDST confirmation were 22.80% (78/342), presented in (Table 4). The confirmation results of ESBL-producing *E. coli* in Kediri District 14.10% (11/78), Blitar District 15.38% (12/78), Malang District 11.54% (9/78), Batu City and Pasuruan District 20.51% (16/78), 17.95% (14/78) Tulungangung District. The highest incidence occurred in Pasuruan District and Batu City at 20.51% (16/78).

#### Discussion

According to data from the East Java Livestock Service (2020), the population of dairy cows in East Java reaches 293,556 heads. It is spread over six districts, with the largest populations in Pasuruan District, Malang District,

Tulungagung District, Blitar District, Batu City, and Kediri District. An increase in the dairy cattle population resulted in increasing environmental waste pollution (Widiastuti et al. 2015). Dairy cattle farms in Indonesia are generally small-scale ownership that throws waste into rivers without prior processing, polluting the environment (Hidayatullah et al. 2005; Kasworo et al. 2013; Widiastuti et al. 2015). In addition, waste from animals treated with antibiotics can contain antibiotic residues and resistant bacteria (FAO 2018; Loo et al. 2019). A study by Marshall and Levy (2011) stated that 75-90% of animal antibiotics are not metabolized in the intestine and are then excreted through waste. This incident can cause a problem because animal waste containing resistant bacteria and antibiotics can enter the sewage system and water sources, thus, helping the emergence of antimicrobial-resistant bacteria in the environment (Loo et al. 2019).

The results of this study indicated that of the 69.30% (237/342) samples that were positive for E. coli, the other 30.7% were for other pathogenic bacteria, like Klebsiella sp., Proteus sp., Pseudomonas sp., and Enterobacter sp. based on the results of identification on EMBA media. Not only pathogenic, but these bacteria also resist most antibiotics and show a high Minimal Inhibitory Concentration (MIC) of 30 to 100 µg/ml (Atef and Idriss 2013). The E. coli isolates (99.17%; 235/237) had AMR properties. Based on Baker et al. (2022), the waste generated from dairy farms could be a way of discovering the presence of AMR bacteria. The prevalence value of AMR in this study was higher than the study conducted by Massé et al. (2021), which stated that 70% of the samples were resistant to the antibiotics tested. Bacteria with AMR properties that pollute the environment can indirectly affect humans (Landers 2012). AMR causes an increase in morbidity and mortality due to microorganisms in humans. The side effects of AMR are increased disease severity, increased risk of complications, increased case fatality rate, increased mortality, delayed treatment, treatment failure, use of expensive drugs, increased economic burden or costs, and more extended stay in hospital and intensive care units. Medical treatment to the higher costs associated with implementing AMR infection control measures in healthcare units (Friedman et al. 2015). The global cost of handling AMR is very high and varies in each country. The US Center for Disease Control and Prevention (CDC) estimates that the cost of losses due to antimicrobial resistance is \$55 billion annually in the United States, of which \$20 billion is spent on healthcare in the AMR sector and \$35 billion due to decreased productivity (Prestinaci 2015).

The AMR *E. coli* showed significant antibiotic resistance, 76.7% ampicillin, 96.2% streptomycin, 66.2% cefotaxime, and 84.0% chloramphenicol. The antibiotics ampicillin (penicillin) and streptomycin show high resistance because these antibiotics are often used to treat mastitis and endometritis in dairy cows. This combination of antibiotics is used because it is a broad-spectrum antibiotic (Riyanto et al. 2016; Negasee 2020). The results of resistance to ampicillin and cefotaxime can indicate the ESBL enzyme's activity. Thus, the use of these antibiotics needs awareness of their side effects (Dhillon and Clark 2012). In addition, second and third-generation cephalosporin antibiotics have been reported to be a risk factor for infection with ESBL-producing bacteria (Amin et al. 2020; Wibisono et al. 2020).

Apart from penicillin and streptomycin, chloramphenicol, beta-lactam antibiotics, and the antibiotic sulfonamide group are often used in dairy cows (Ansharieta et al. 2021). Chloramphenicol also showed resistance results that were significantly different from the research conducted by Maulana et al. (2021) on samples on dairy farms in Sleman, Jogjakarta. Resistance to chloramphenicol is caused by pressure on bacteria through the co-selection of mobile resistance elements or unknown substrates against chloramphenicol (Tadesse et al. 2012). In different circummures, sulfamethoxazole-trimethoprim and tetracycline showed significant sensitivity in 79.3% and 62.4%. These results can be used as a basis for the recommended use of antibiotics to treat infectious diseases in dairy cattle in East Java.

The highest multidrug-resistant (MDR) incidence was 44.44% for four groups of antibiotics. In addition, 2.53% of isolates are resistant to 7 antibiotics that need awareness. Multidrug resistance is common in ESBL-producing bacteria (Gregova et al. 2012). Furthermore, MDR shows microbial resistance to three or more classes of antibiotics (Effendi et al. 2021). These results impact the selection of antibiotics for treating limited bacterial infections. Resistant bacterial infections cause prolonged illness, increase the risk of death, increase the length of stay in the hospital, slow treatment response failure, causing patients to be infectious for a long time (carriers) and significant opportunities for resistant bacteria to spread to other organisms in a population (Deshpande et al. 2011). In addition, MDR is caused by the uncontrolled use of antibiotics, causing selective pressure from resistant bacteria to the evolutionary process through genetic mutations, exchange of genetic material, and proliferation to defend themselves against various types of antibiotics (Yanestria et al. 2022).

The results of ESBL-producing *E. coli* from samples of dairy farm wastewater in East Java with a DDST confirmation of 22.80% (78/342). This result should be noticed because there is an increase in the incidence compared to the results of other studies in Sleman (Yogyakarta, Indonesia), which is 16% (15/93). Data on the incidence of ESBL-producing *E. coli* in dairy farm wastewater were also reported by several countries, like Chiang Mai (Thailand) 88.7% and Tunisia (North Africa) 15.8% (Maulana et al. 2021; Saekhow and Sriphannam

2021; Said et al. 2015). The incidence of ESBL-producing *E. coli* in Indonesia is also increasing, as evidenced by the data on the incidence of ESBL-producing *E. coli* bacteria in various samples. For example, pneumonia patients at Sanglah General Hospital (Bali) reached 93.3%; at Wahidin General Hospital (Makassar) 80%; Blitar from swabs broiler cloaca 28.75% and layer 7.03%; broiler stool samples in Bogor 25%; rectal swabs of dairy cows 72% and companion dogs 9.41% in Surabaya; environmental samples of broiler farms in Pasauruan 9.14% and samples of Sleman dairy farms (Yogyakarta) reached 54% (Manuaba et al. 2021; Pratama et al. 2019; Widhi and Saputra 2020; Masruroh et al. 2016; Wibisono et al. 2020; Kristianingtyas et al. 2021; Yanestria et al. 2022; Imasari et al. 2018; Maulana et al. 2021).

Some evidence shows increased ESBL-producing E. coli, especially in dairy cows. The increased ESBL organisms in dairy farms can be a public health risk because E. coli can be zoonotic and transmitted to humans by various routes. Environments contaminated with ESBLproducing E. coli bacteria can potentially spread these bacteria to humans. An environment polluted by ESBLproducing E. coli allows amino acid substitution or genetic gene mutations to horizontal transfer through transformation, transduction, and conjugation processes to the environmental microbiota. Environmental microbiota can be a reservoir of ESBL resistance genes (Amine, 2013). As a result, variants from the TEM, SHV, and CTX-M groups, through gradual mutations, which are identified, continue to increase (Gelalcha and Dego 2022). ESBL coding genes are mainly located on plasmids and chromosomes, transferred from one organism to another, impacting a higher prevalence epidemiologically (Biutifasari 2018; Canton et al. 2012). ESBL-producing E. coli has the risk of spreading resistant genes, especially to susceptible individuals such as pregnant women, infants, children, the elderly, and people with immunosuppression, as well as postoperative and chemotherapy patients (Franz et al. 2015). ESBL-producing E. coli causes mortality, morbidity, and increasing death incidences (Nóbrega and bronchi 2014).

This study concludes that ESBL-producing *E. coli* in the wastewater of dairy farms in East Java has the potential to become a reservoir in the livestock environment. These results are significant for creating public awareness of antibiotics and wastewater treatment used to create better public health and welfare. Research and collaboration within the scope of One health are needed to deal with the incidence of ESBL-producing *E. coli* from wastewater, and the government needs to supervise the treatment of dairy farm wastewater.

#### ACKNOWLEDGEMENTS

The authors would like to thank *Penelitian Pasca Sarjana-Penelitian Disertasi Doktor* funding from DRTPM Kemendikbudristek 2022, with grant from Universitas Airlangga No. 905/UN3.15/PT/2022, the management of KUD Medowo (Kediri District), KUD Jabung, KUD Princi and KUD Poncokusumo (Malang District), KUD Semen (Blitar District), KUD Batu and KUD Junrejo (Batu City), KUD KPSP Setia Kawan (Pasuruan District), KUD Sendang (Tulungangung District) and Laboratory of Veterinary Microbiology and Immunology, Faculty of Veterinary Medicine, Universitas Brawijaya, Malang, Indonesia for providing the facilities for the study.

#### REFERENCES

- Adekanmbi AO, Akinpelu MO, Olaposi AV, Oyelade AA. 2020. Extended spectrum beta-lactamase encoding gene-fingerprints in multidrug resistant *Escherichia coli* isolated from wastewater and sludge of a hospital treatment plant in Nigeria. Intl J Environ Stud 78 (1): 140-150. DOI: 10.1080/00207233.2020. 177827.
- Amin M, Wasito EB, Triyono EA. 2020. Comparison between exposure of ciprofloxacin and cefotaxime on developing of *Escherichia coli* ESBL. Fol Med Indones 56 (2): 86-90. DOI: 10.20473/fmi.v56i2.21203.
- Amine AEK. 2013. Extended spectrum beta-lactamase producing bacteria in waste water Alexandria, Egypt. Intl J Biosci Biochem Bioinformatics 3 (6): 605-609. DOI: 10.7763/IJBBB.2013.V3.285.
- Ansharieta R, Effendi MH, Plumeriastuti H. 2021. Genetic identification of shiga toxin encoding gene from cases of Multidrug Resistance (MDR) *Escherichia coli* isolated from raw milk. Trop Anim Sci J 44 (1): 10-15. DOI: 10.5398/tasj.2021.44.1.10.
- Antimicrobial Resistance Collaborators. 2022. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. The Lancet 399 (55): 629-655.
- Astorga F, Talloni MJN, Miró MP, Bravo M, Toro M, Blondel CJ, Claude LPH. 2019. Antimicrobial resistance in *E. coli* isolated from dairy calves and bedding material. Heliyon 5 (11): 1-6. DOI: 10.1016/j.heliyon.2019.e02773.
- Atef D, Idriss A. 2013. Bacterial contaminating the haemodialysis dialysate-water exhaust-origin and fate at Al-Madinah Al-Mounwwarah. Life Sci J 10 (1): 1030-1035.
- Baker M, Williams AD, Hooton SPT, Helliwell R, King E, Dodsworth T, Baena-Nogueras RM, Warry A, Ortori CA, Todman H. 2022. Antimicrobial resistance in dairy slurry tanks: a critical point for measurement and control. Environ Intl 169: 107516. DOI: 10.1016/j.envint.2022.107516.
- Biutifasari W. 2018. Extended Spectrum Beta-Lactamase (ESBL). Oceana Biomed J 1 (1): 1-11. DOI: 10.30649/obj.v1i1.3 . [Indonesian]
- Blaak H, Lynch G, Italiaander R, Hamidjaja RA, Schets FM, de Roda Husman AM. 2015. Multidrug-Resistant and Extended Spectrum Beta-Lactamase Producing *Escherichia coli* in Dutch Surface Water and Wastewater. PLoS One 10 (6): e0127752. DOI: 10.1371/journal.pone.0127752.
- Canton R, Gonzalez-Alba JM, Galan JC. 2012. CTX-M Enzymes: Origin and Diffusion. Front Microbiol Rev 3 (110): 1-19. DOI: 10.3389/fmicb.2012.00110.
- Cappucino JG, Welsh C. 2020. Microbiology A Laboratory 12th Edition Global Edition. Pearson, England.
- Chu W, Zere TR, Weber MM, Wood TK, Whiteley M, McLean RJC. 2012. Indole production promotes *Escherichia coli* mixed-culture growth with *Pseudomonas aeruginosa* by inhibiting quorum signaling. Appl Environ Microbiol 78 (2): 411-419. DOI: 10.1128/AEM.06396-11.
- Chukwunwejim CR, Eze PM, Ujam NT, Abonyi IC, Ejikeugwu CP. 2018. Incidence of Community Acquired ESBL-producing Bacteria among Asymptomatic University Students in Anambra State, Nigeria. Eur J Biol Res 8 (3): 138-147. DOI: 10.5281/zenodo.1314719.
- Clinical and Laboratory Standards Institute. 2020. Performance standards for antimicrobial susceptibility testing in CLSI supplement M100. In. Wayne, PA: CLSI.
- Deshpande PR, Rajan S, Sudeepthi BL, Nasir CPA. 2011. Patient-Reported Outcomes: A New Era in Clinical Research. Perspect Clin Res 2 (4): 137-144. DOI: 10.4103/2229-3485.86879.
- Dhillon RHP, Clark J. 2012. Review Article ESBLs: A Clear and Present Danger? Crit Care Res Pract 2012 (11): 1-11. DOI: 10.1155/2012/625170.

- Dinas Peternakan Provinsi Jawa Timur. 2020. Statistika Populasi Ternak 2020. Disnak jatimprov. [Indonesian]
- Effendi MH, Tyasningsih W, Yurianti YA, Rahmahani J, Harijani N, Plumeriastuti H. 2021. Presence of multidrug resistance (MDR) and extended-spectrum beta-lactamase (ESBL) of *Escherichia coli* isolated from cloacal swab of broilers in several wet markets in Surabaya, Indonesia. Biodiversitas 22 (1): 304-310. DOI: 10.13057/biodiv/d220137.
- Food and Agriculture Organization (FAO). 2018. Antimicrobial Resistance in the Environment. http://www.fao.org.
- Franz E, Veenman C, van HA, Husman AR, Blaak H. 2015. Pathogenic *Escherichia coli* producing extended spectrum β-lactamases isoated from surface water and wastewater. Sci Rep 5: 19. DOI: 10.1038/srep14372.
- Friedman DB, Kanwat CP, Headrick ML et al. 2015. Importance of prudent antibiotic use on dairy farms in South Carolina: a pilot project on farmers' knowledge, attitudes and practices. Zoonoses Public Health 2007 54: 366-375. DOI: 10.1111/j.1863-2378.2007.01077.x.
- Gelalcha BD, Dego OK. 2022. Extended-Spectrum Beta-Lactamases Producing Enterobacteriaceae in the USA Dairy Cattle Farms and Implications for Public Health. Antibiotics 11 (1313): 1-4. DOI: 10.3390/antibiotics11101313.
- Gregova G, Kmetova M, Kmet V, Venglovsky J, Feher A. 2012. Antibiotic resistance of *Escherichia coli* isolated from poultry a Slaughterhouse. Ann Agric Environ Med 19 (1): 75-77.
- He Y, Yuan Q, Mathieu J, Stadler L, Senehi N, Sun R, Alvarez PJJ. 2020. Antibiotic resistance genes from livestock waste: occurrence, dissemination, and treatment. NPJ Clean Water 3 (4): 1-4. DOI: 10.1038/s41545-020-0051-0.
- Hidayatullah, Gunawan, Mudikdjo K, Erliza N. 2005. Pengelolaan limbah cair usaha peternakan sapi perah melalui penerapan konsep produksi bersih. J Pengkajian dan Pengembangan Teknologi Pertanian 8 (1): 124-136. DOI: 10.21082/jpptp.v8n1.2005.p%25p. [Indonesian]
- Huang YH, Kuan NL, Yeh KS .2020. Characteristics of Extended-Spectrum β-Lactamase Producing *Escherichia coli* From Dogs and Cats Admitted to a Veterinary Teaching Hospital in Taipei, Taiwan From 2014 to 2017. Front Vet Sci 7: 395. DOI: 10.3389/fvets.2020.00395.
- Imasari T, Tyasningsih W, Wasito EB, Kuntaman K. 2018. Prevalensi dan Pola Gen Extended Spectrum β-lactamase Bakteri Usus Sapi Perah dan Penduduk Sekitar Peternakan di Surabaya. Jurnal Veteriner 19 (3): 313 - 320. DOI: 10.19087/jveteriner.2018.19.313. [Indonesian]
- Indriani AP, Muktiani A, Pangestu E. 2013. Konsumsi dan produksi protein susu sapi perah laktasi yang diberi suplemen temulawak (*Curcuma xanthorrhiza*) dan seng proteinat. Anim Agric J 2 (1): 128-135. [Indonesia]
- International Monetary Fund. 2014. Global Health Threats of the 21st Century. International Monetary Fund (IMF) 51 (4).
- Kristianingtyas L, Effendi MH, Witaningrum AM, Wardhana DK, Ugbo EN. 2021. Prevalence of extended-spectrum ß-lactamase-producing *Escherichia coli* in companion dogs in animal clinics, Surabaya, Indonesia. Intl J One Health 7 (2): 232-236. DOI: 10.14202/IJOH.2021.232-236.
- Jayarao B, Almeida R, Oliver SP. 2019. Antimicrobial Resistance on Dairy Farms. Foodborne Pathog Dis 16 (1): 1-4. DOI: 10.1089/fpd.2019.29011.edi.
- Lal A, Cheeptham N. 2017. Eosin Methylene Blue Agar Protocol. ML Library. American Society for Microbiology.
- Loo E, Lai KS, Mansor R. 2019. Antimicrobial Usage and Resistance in Dairy Cattle Production. In Bekoe SO, Saravanan M, Adosraku RK, Ramkumar P (eds.). Veterinary Medicine and Pharmaceuticals. IntechOpen. DOI: 10.5772/intechopen.81365.
- Marshall BM, Levy SB. 2011. Food animals and antimicrobials: impacts on human health. Clin Microbiol Rev 24 (4): 718-733. DOI: 10.1128/CMR.00002-11.
- Masruroh CA, Sudarwanto MB, Latif H. 2016. Tingkat Kejadian *Escherichia coli* Penghasil *Extended Spectrum-Lactamase* yang Diisolasi dari Feses Broiler di Kota Bogor. Jurnal Sain Veteriner 34 (1): 42-49. DOI: 10.22146/jsv.22813. [Indonesian]
- Manuaba IASP, Iswari IS, Pinatih KJP. 2021. Prevalensi Bakteri Escherichia coli dan Klebsiella pneumoniae Penghasil Extended Spectrum Beta Lactamase (ESBL) Yang Diisolasi Dari Pasien Pneumonia Di RSUP Sanglah Periode Tahun 2019-2020. J Medika Udayana 10 (12): 51- 57. DOI: 10.24843.MU.2021.V10.i12.P10. [Indonesian]

- Maulana KT, Pichpol D, Farhani NR, Widiasih DA, Unger F, Punyapornwithaya V, Meeyam T. 2021. Antimicrobial resistance characteristics of Extended Spectrum Beta Lactamase (ESBL) producing *Escherichia coli* from dairy farms in the Sleman district of Yogyakarta province, Indonesia. Vet Integr Sci 19 (3): 525-535. DOI: 10.12982/VIS.2021.041.
- Massé J, Lardé H, Fairbrother JM, Roy JP, Francoz D, Dufour S, Archambault M. 2021. Prevalence of antimicrobial resistance and characteristics of *Escherichia coli* isolates from fecal and manure pit samples on dairy farms in the Province of Québec, Canada. Front Vet Sci 8: 654125. DOI: 10.3389/fvets.2021.654125.
- Negasee KA. 2020. Clinical Metritis and Endometritis in Diary Cattle: A Review. Openventio 5 (2): 51-56. DOI: 10.17140/VMOJ-5-149.
- Niasono AB, Latif H, Purnawarman, T. 2019. Resistensi Antibiotik terhadap Bakteri *Escherichia coli* yang Diisolasi dari Peternakan Ayam Pedaging di Kabupaten Subang, Jawa Barat. J Veteriner 20 (2): 187-195. DOI: 10.19087/jveteriner.2019.20.2.187. [Indonesian]
- Normaliska R, Sudarwanto BM, Latif H. 2019. Pola resistensi antibiotik pada *Escherichia coli* Penghasil ESBL dari Sampel Lingkungan di RPH-R Kota Bogor. J Acta Veterinaria Indonesia 7 (2): 42-48. DOI: 10.29244/avi.7.2.42-48. [Indonesian]
- Overdevest I, Willemsen I, Rijnsburger M, Eustace A. 2011. Extended spectrum β-lactamase genes of *Escherichia coli* in chicken meat and humans, the Netherlands. Emerg Infect Dis 17 (12): 16-22. DOI: 10.3201/eid1707.110209.
- Pamungkasih E, Febrianto N. 2021. Profil peternak sapi perah di dataran rendah Kabupaten Malang. Karta Raharja 3 (2): 29-35. [Indonesian]
- Prestinaci F, Pezzotti P, Pantosti A. 2015. Antimicrobial resistance: a global multifaceted phenomenon. Pathog Glob Health 109 (7): 309-318. DOI: 10.1179/2047773215Y.0000000030.
- Putra AR, Effendi MH, Koesdarto S, Suwarno S, Tyasningsih W, Estoepangestie AT. 2020. Detection of the extended spectrum β lactamase produced by *Escherichia coli* from dairy cows by using the Vitek-2 method in Tulungagung district, Indonesia. Iraqi J Vet Sci 34 (1): 203-207. DOI: 10.33899/ijvs.2019.125707.1134
- Pratama A, Djide M, Nasrum M. 2019. Identifikasi Genotip CTX-M pada Escherichia coli Penghasil Extended Spectrum Beta Lactamase (ESBL) Yang Resisten Pada Cephalosporin Generasi III di RSUP Wahidin Sudirohusodo Makassar. Majalah Farmasi dan Farmakologi 23 (5): 1-7. DOI: 10.20956/mff.v23i1.6458. [Indonesian]
- Riyanto J, Sunarto BS, Hertanto M, Cahyadi R, Hidayah, Sejati W. 2016. Produksi dan kualitas susu sapi perah penderita mastitis yang mendapat pengobatan antibiotik. Sains Peternak 14: 30-41. DOI: 10.20961/sainspet.14.2.30-41. [Indonesian]
- Saekhow P, Sriphannam X. 2021. Prevalence of Extended-Spectrum Beta-Lactamase-Producing *Escherichia coli* Strains in Dairy Farm Wastewater in Chiang Mai. Vet Integr Sci 19 (3): 349-362. DOI: 10.12982/VIS.2021.030.
- Said LB, Jouini A, Klibi N, Dziri N, Alonso CA, Boudabous A, Ben SK, Torres C. 2015. Detection of Extended-Spectrum BetaLactamase (ESBL) producing enterobacteriaceae in vegetables, soil and water of the farm environment in Tunisia. Intl J Food Microbiol 203: 86-92. DOI: 10.1016/j.ijfoodmicro.2015.02.023.
- Soekoyo AR, Sulistiawati, Setyorini W, Kuntaman K. 2020. The epidemiological pattern and risk factor of ESBL (Extended Spectrum B-Lactamase) producing *Enterobacteriaceae* in gut bacterial flora of dairy cows and people surrounding in Rural Area, Indonesia. Indones J Trop Infect Dis 8 (3): 144-151. DOI: 10.20473/ijtid.v8i3.17553.
- Soré S, Sawadogo Y, Bonkoungou JI, Kaboré SP, Béogo S. 2020. Detection, identification and characterization of extended-spectrum beta-lactamases producing Enterobacteriaceae in wastewater and

salads marketed in Ouagadougou, Burkina Faso. Intl J Biol Chem Sci 14 (8): 2746-2757. DOI: 10.4314/ijbcs.v14i8.8.

- Tadesse DA, Zhao S, Tong E, Ayers S, Singh A, Bartholomew MJ, McDermott PF. 2012. Antimicrobial drug resistance in *Escherichia coli* from humans and food animals, United States, 1950-2002. Emerg Infect Dis 18: 741-749. DOI: 10.3201/eid1805.111153.
- Tripathi N, Sapra A. 2022. Gram Staining. In: StatPearls. StatPearls Publishing, Treasure Island (FL).
- Ulfah NF, Erina, Darniati. 2017. Isolation and identification *Escherichia coli* in roasted chicken from Restaurant in Syiah Kuala, Banda Aceh. J Ilmu Medik Veteriner 01 (3): 383-390. [Indonesian]
- Weber LP, Dreyer S, Heppelmann M, Schaufler K, Homeier-Bachmann T, Bachmann L. 2021. Prevalence and Risk Factors for ESBL/AmpC-*E. coli* in Pre-Weaned Dairy Calves on Dairy Farms in Germany. Microorganisms 9 (2135): 1-4. DOI: 10.3390/ microorganisms9102135.
- Wibisono FJ, Sumiarto B, Untari T, Effendi MH, Permatasari DA, Witaningrum M. 2020. CTX Gene of Extended Spectrum Beta-Lactamase (ESBL) Producing *Escherichia coli* on Broilers in Blitar, Indonesia. Syst Rev Pharm 11 (7): 396-403. DOI: 10.31838/srp.2020.7.59.
- Wibisono FJ, Sumiarto B, Untari T, Effendi MH, Permatasari DA, Witaningrum M. 2020. Short Communication: The presence of extended spectrum beta-lactamase (ESBL) producing *Escherichia coli* on layer chicken farms in Blitar Area, Indonesia. Biodiversitas 21 (6): 2667-2671. DOI: 10.13057/biodiv/d210638.
- Widhi APKN, Saputra IY. 2021. Residu antibiotik serta keberadaan *Escherichia Coli* penghasil ESBL pada daging ayam broiler di Pasar Kota Purwokerto. J Kesehatan Lingkungan Indonesia 20 (2): 137-142. DOI: 10.14710/jkli.20.2.137-142. [Indonesian]
- Widiastuti E, Kustono, Adiarto, Nurliyani. 2015. The impact of the local dairy cattle farm toward the river water quality in Gunungpati Subdistrict Central Java. Intl J Sci Eng 8 (1): 15-21. DOI: 10.12777/ijse.8.1.15-21.
- Woolhouse M, Ward M, van Bunnik B, Farrar J. 2015. Antimicrobial Retance in Humans, Livestock and The Wider Environment. Phil Trans R Soc B 370: 1-7. DOI: 10.1098/rstb.2014.0083.
- World Health Organization. 2012. The Evolving Threat of Antimicrobial Resistance: Options for Action. Geneva: World Health Organisation.
- World Health Organization. 2019. New report calls for urgent action to avert antimicrobial resistance crisis. Joint News Release. https://www.who.int.
- World Health Organization. 2021. WHO integrated Global Surveillance on ESBL-Producing *E. coli* using a "One Health" Approach: Implementation and Opportunities. https://www.who.int.
- Yanestria SM, Dameanti FNAEP, Musayannah BG, Pratama JWA, Witaningrum AM, Effendi MH, Ugbo EN. 2022. Antibiotic resistance pattern of Extended-Spectrum β-Lactamase (ESBL) producing *Escherichia coli* isolated from broiler farm environment in Pasuruan district, Indonesia. Biodiversitas 23 (9): 4460-4465. DOI: 10.13057/biodiv/d230911.
- Yanestria SM, Rahmaniar RP, Wibisono FJ, Effendi MH. 2019. Detection of invA gene of *Salmonella* from Milkfish (*Chanos chanos*) at Sidoarjo Wet Fish Market, Indonesia, using Polymerase Chain Reaction Technique. Vet World 12 (1): 170-175. DOI: 10.14202/vetworld.2019.170-175.
- Zhang H, Gao Y, Chang W. 2016. Comparison of *Extended-Spectrum β-Lactamase* producing *Escherichia coli* isolates from drinking well water and pit latrine wastewater in a rural area of China. BioMed Res Intl 1-7. DOI: 10.1155/2016/4343564.