



# Bacteria Isolation from Public Cemeteries Soil and Test for Resistance to Antibiotics

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## Abstract

**Background:** A public cemetery, also known as a public burial place (TPU) is an active decomposition of corpses in the soil, which produces soil nutrients and minerals that can support the growth of microorganisms in the ground, including pathogenic bacteria. Pathogenic bacteria have a more severe impact if they are resistant to antibiotics. **Methods:** Soil samples were taken in the Bonoloyo TPU area, Surakarta, Central Java, at 3 points each of 2 depths of 20 and 50 cm. Soil samples were inoculated on Nutrient agar (NA) using the spread plate method. After 48 hours, colony counting and morphology observations were carried out, followed by gram staining. Isolated bacterial isolates were tested for resistance to 3 types of antibiotics. **Results:** The average population of soil bacteria in blocks 12, 17, and 21 at a depth of 20 cm is  $4 \times 10^6$  CFU/g;  $8 \times 10^6$  CFU/g; and  $1 \times 10^6$  CFU/g, while at a depth of 50 cm, it is  $2.3 \times 10^6$  CFU/g;  $6 \times 10^6$  CFU/g; and  $4 \times 10^6$  CFU/g. The morphology of bacterial colonies is irregular (26 isolates), and flat elevation (19 isolates), and the color of isolates is predominantly white. The Gram staining results obtained 23 isolates are Gram-negative, and 22 are Gram-positive with a dominant cell form in cocci. Antibiotic resistance tests showed that the bacterial isolates were resistant to ampicillin (28.9 %), Bacitracin (64.4 %), and Cefepime (57.8 %). **Conclusions:** Burial soil bacterial populations at Bonoloyo TPU at different depths did not differ markedly, with numbers ranging from  $1 - 8 \times 10^6$  CFU/g.

**Keywords:** Bacteria; antibiotic resistance; public burial places.

## Introduction

A graveyard is an area reserved for the burial of the dead, regulated by the local government (Zanial & Irwansyah, 2016). After being declared dead, the human body undergoes decay, and a decomposition process begins. The decomposition process has five stages: fresh stage, bloated, active rot, dry putrefaction, and skeletonized (Sunniiyah, 2021). Simple compounds enrich soil nutrients and minerals (Laskow, 2018). Soil is one of the habitats of diverse microorganisms. The active decomposition of bodies in the ground varies between individuals and environmental conditions. The results of the disintegration of the corpse include organic and inorganic nitrogen compounds, potassium (K), and sodium (Na) (Sunniiyah, 2021). Various environmental factors can significantly affect the process of decomposition of corpses (Ciaffi et al., 2018). One study reported that the total rhizosphere soil bacteria in the TPU area was  $1.0 \times 10^7$  CFU/g (Putra et al., 2023). The number of rhizosphere bacteria in the TPU area is more significant than outside the TPU area, as in the total rhizosphere bacteria of rubber plants of  $1.06 \times 10^6$  (Wulandari et al., 2020). The abundance of rhizosphere bacteria in the TPU area proves that the process of decomposition of corpses can affect the diversity and function of soil microbes because the



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bodies release nutrients into the soil in large quantities (Singh et al., 2018).

TPU was also found in pathogenic bacteria and fungi; research in Poland reported that burial grounds contained *Bacillus spp.*, *Escherichia spp.*, *Enterococcus spp.*, *Klebsiella Enterobacter-Serratia*, *Staphylococcus spp.*, *Penicillium spp.*, and *Aspergillus spp.*, with the most common pathogens being *Enterococcus spp.* (80.6 %), *Bacillus spp.* (77.4 %), *Penicillium spp.* (51 %), and *Aspergillus spp.* (6.4 %) (Całkosiński et al., 2015). From microbiome analysis in South African burial grounds, it is predicted to contain pathogens that cause tuberculosis, Alzheimer's, Huntington's, influenza, vibrio cholera, toxoplasmosis, Parkinson's, amoebiasis, myocarditis, and cancer (Abia et al., 2019). Based on the results of this study, it turns out that TPU can be a severe problem for public health, especially for people living around TPU. One of the largest TPUs in Surakarta is TPU Bonoloyo, with an area of 124,253.80 m<sup>2</sup> (Pradana et al., 2021). Around the Bonoloyo TPU, some residential areas are located very close, even though most use groundwater for consumption. This TPU will be used as a sampling location.

The presence of pathogenic microbes, especially bacteria in TPU, will have a more severe impact if it is resistant to antibiotics. Antibiotic resistance is becoming a global public health problem. Reports on deaths caused by antibiotic resistance are estimated at 10 million per year, projected by 2050 (de Kraker et al., 2016). Indonesia forecasts that there will be the highest increase in antimicrobial consumption in 2030, including Indonesia (WHO Indonesia, 2022). This is due to improper use of antibiotics. A lack of understanding about antibiotics affects their use behavior (Yunita et al., 2021). Some bacteria that are resistant to antibiotics are *Pseudomonas aeruginosa* which is resistant to antibiotics ceftazidime and ciprofloxacin and *Haemophilus influenza* resistant to antibiotics imipenem and ampicillin (Pratiwi, 2017). Reduced sensitivity of antibiotics to a bacterium can lead to increased mortality due to complications and healthcare expenditure (Hayati et al., 2022).

There has been no research on bacterial diversity in TPU and antibiotic resistance tests in Indonesia, so the study was conducted to obtain data on bacterial diversity in TPU and antibiotic resistance tests. Based on reference searches, in several countries, there have been studies on soil bacterial diversity in the TPU area and antibiotic resistance tests such as in South Africa, Australia (Zychowski & Bryndal, 2015), Brazil (Fineza et al., 2014), Latvia (Kazarina et al., 2019), and Poland (Całkosiński et al., 2015). The results presented through these references vary. This may be due to differences in climate in Poland and Australia which have summer, winter, spring, and autumn. The way of burial is also different. In Indonesia, Muslim bodies are buried wrapped in shrouds, and Christian and Catholic bodies are dressed in full clothes and put in coffins like in Australia. This study aimed to analyze the diversity of soil bacteria in TPU and their antibiotic resistance. Research data can be used as a reference to increase awareness of the risk of pathogenic microbial contamination from TPU and set policies for the use of antibiotics.

## Method

This research is conducted at the Biology Laboratory of the Biology Education Study Program, Universitas Muhammadiyah Surakarta from January 2023 to February 2023. Soil samples were taken from TPU Bonoloyo, Surakarta, Central Java, in 3 blocks, namely blocks 12, 17, and 21 (Figure 1). Soil samples were taken using a drill with a depth of 20 cm and 50 cm. Exploratory was used to determine the diversity of soil bacteria in TPU and their resistance to antibiotics. Tools used for media manufacturing, bacterial diversity analysis, and bacterial resistance to antibiotics are Erlenmeyer (Pyrex), sprayer, petri dish, shaker, autoclave (GEA LS-35LJ), oven incubator (Memmert), colony counter (Funke gerber), vortex (DLAB MX-S), digital analytical balance (Durascale DAB-E223), hot plate (Cimarec<sup>+</sup>), micropipette (Socorex 10-100), blue tip, Laminer Air Flow (LAF), microscope and glass object. And the materials used include nutrient agar (Merck), nutrient broth, burial soil samples, 70 % alcohol, Gram dyes, malachite green, molten paper, spiritus, antibiotic disc

ampicillin (Liofilchem), antibiotic disc bacitracin (Oxoid), and antibiotic disc cefepime (Liofilchem).



**Figure 1.** Sampling Floor Plan

**Isolation of soil bacteria**

Soil samples were diluted serially from 10<sup>-1</sup> to 10<sup>-6</sup> dilutions. Isolation of soil bacteria using the spread plate method at dilutions of 10<sup>-5</sup> and 10<sup>-6</sup> on top of NA (Himedia). Incubation at 30°C for 48 hours (Fitriana & Asri, 2021). The bacterial population is calculated based on the number of growing colonies. The isolate is stored in the refrigerator for further analysis in an oblique agar medium. Identification of bacterial isolates obtained by observing colony morphology and gram staining. Observations of colony morphology include colony shape, edge, elevation, and color while from Gram staining to get Gram grouping data, cell shape, and cell arrangement (Ayuti et al., 2023). If the cell is purple or violet, it belongs to the Gram-positive bacteria group, while if the cell is red, it is included in the Gram-negative group (Andayani et al., 2022).

**Antibiotic Resistance Test**

Antibiotic resistance test using three types of antibiotics, namely Ampicillin (Liofilchem), Bacitracin (Oxoid), and Cefepime (Liofilchem) with agar diffusion method (Khusuma et al., 2019). This test is carried out by inoculating a liquid culture of bacteria that has been incubated for 16 hours on the surface of NA with a swab method. Petridisk inoculated with bacteria is then placed on an antibiotic disk. Incubation at 37°C for 24 hours and antibiotic activity is shown by forming a clear zone (Mulyani et al., 2023). According to Amri & Wulandari (2022), antibiotic resistance is determined based on the Clinical and Laboratory Standard Institute (CLSI) with sensitive, intermediate, and resistant categories.

**Result**

**Soil bacteria population**

The mean burial soil bacteria population sequentially from block 12, block 17, and block 21 was 4 x 10<sup>6</sup> CFU/g; 2.3 x 10<sup>6</sup> CFU/g; 8 x 10<sup>6</sup> CFU/g; 6 x 10<sup>6</sup> CFU/g, 1 x 10<sup>6</sup> CFU/g, and 4 x 10<sup>6</sup> CFU/g at depths of 20 cm and 50 cm respectively (Table 1.). The soil bacteria population at TPU Bonoloyo, Surakarta, did not show significant differences among the blocks tested at different depths.

**Table 1.** Population Soil Bacteria

Burial Block	Dept (Cm)	Average Bacterial Population (Cfu/G)	Altitude of the Premises (Mdpl)
12	20	4 x 10 <sup>6</sup>	128,7
12	50	2,3 x 10 <sup>6</sup>	
17	20	8 x 10 <sup>6</sup>	126,2

17	50	6 x 10 <sup>6</sup>	
21	20	1 x 10 <sup>6</sup>	129,2
21	50	4 x 10 <sup>6</sup>	

**Identification of colony morphology and Gram staining results**

A total of 45 isolates were isolated based on differences in colony morphology. Furthermore, the isolates observed colony morphology and Gram staining results, as seen in Table 2.

**Table 2.** Colony morphology and gram staining results of soil bacterial isolate from TPU Bonoloyo, Surakarta

Code	Colony Morphology				Gram Staining			
	Shape	Edge	Elevation	Color	Color	Cell Shape	Cell Arrangement	Gram
BN1	Circular	Entire	Convex	Yellow	Yellow	Coccus	Diplococcus	Negative
BN2	Circular	Entire	Convex	White	White	Coccus	Diplococcus	Negative
BN3	Irregular	Lobate	Flat	White	White	Coccus	Monococcus	Negative
BN4	Circular	Entire	Convex	Pink	Pink	Basil	Monobasil	Negative
BN5	Irregular	Undulate	Flat	White	White	Coccus	Diplococcus	Negative
BN6	Irregular	Undulate	Convex	White	White	Coccus	Streptococcus	Negative
BN7	Rhizoid	Filamentous	Flat	White	White	Coccus	Diplococcus	Negative
BN8	Irregular	Undulate	Flat	White	White	Coccus	Monococcus	Negative
BN9	Irregular	Entire	Convex	White	White	Coccus	Monococcus	Negative
BN10	Punctiform	Entire	Flat	Yellow	Yellow	Coccus	Monococcus	Negative
BN11	Irregular	Entire	Convex	White	White	Coccus	Monococcus	Negative
BN12	Circular	Entire	Flat	Yellow	Yellow	Coccus	Staphylococcus	Negative
BN13	Irregular	Lobate	Flat	White	White	Coccus	Streptococcus	Negative
BN14	Circular	Entire	Raised	Yellow	Yellow	Coccus	Staphylococcus	Positive
BN15	Circular	Entire	Flat	Pink	Pink	Coccus	Diplococcus	Positive
BN16	Filamentous	Lobate	Raised	White	White	Coccus	Diplococcus	Positive
BN17	Irregular	Undulate	Umbonate	White	White	Coccus	Diplococcus	Positive
BN18	Circular	Entire	Convex	White	White	Coccus	Staphylococcus	Positive
BN19	Circular	Entire	Flat	White	White	Coccus	Diplococcus	Positive
BN20	Circular	Entire	Flat	White	White	Coccus	Monococcus	Positive
BN21	Irregular	Entire	Umbonate	White	White	Coccus	Diplococcus	Positive
BN22	Filamentous	Filamentous	Raised	White	White	Coccus	Streptococcus	Positive
BN23	Irregular	Undulate	Umbonate	White	White	Coccus	Diplococcus	Positive
BN24	Irregular	Undulate	Flat	White	White	Coccus	Streptococcus	Negative
BN25	Irregular	Undulate	Umbonate	White	White	Coccus	Streptococcus	Negative
BN26	Irregular	Undulate	Umbonate	White	White	Coccus	Streptococcus	Positive
BN27	Irregular	Undulate	Umbonate	White	White	Coccus	Staphylococcus	Negative
BN28	Irregular	Lobate	Flat	White	White	Coccus	Staphylococcus	Positive
BN29	Irregular	Undulate	Unbonate	White	White	Coccus	Staphylococcus	Positive
BN30	Circular	Entire	Convex	White	White	Basil	Diplobacilli	Positive
BN31	Irregular	Entire	Umbonate	Yellow	Yellow	Basil	Diplobacilli	Positive
BN32	Irregular	Filamentous	Flat	White	White	Coccus	Diplococcus	Positive
BN33	Irregular	Undulate	Flat	White	White	Coccus	Diplococcus	Negative
BN34	Punctiform	Entire	Umbonate	White	White	Coccus	Staphylococcus	Negative
BN35	Circular	Entire	Raised	Yellow	Yellow	Coccus	Staphylococcus	Negative
BN36	Irregular	Undulate	Umbonate	White	White	Coccus	Diplococcus	Negative
BN37	Irregular	Undulate	Umbonate	White	White	Coccus	Monococcus	Positive
BN38	Filamentous	Filamentous	Flat	White	White	Basil	Streptobacilli	Positive
BN39	Irregular	Entire	Flat	White	White	Coccus	Diplococcus	Negative
BN40	Irregular	Entire	Convex	White	White	Coccus	Monococcus	Positive
BN41	Irregular	Filamentous	Flat	White	White	Coccus	Monococcus	Negative
BN42	Circular	Entire	Flat	Pink	Pink	Coccus	Staphylococcus	Negative
BN43	Irregular	Entire	Flat	White	White	Coccus	Monococcus	Positive
BN44	Irregular	Entire	Convex	White	White	Coccus	Diplococcus	Positive
BN45	Circular	Entire	Convex	White	White	Coccus	Staphylococcus	Positive

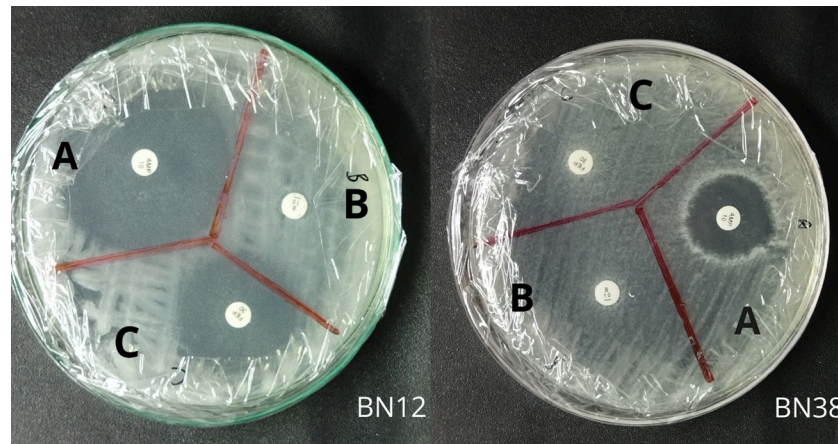
Based on observations, isolates of burial grounds at TPU Bonoloyo have different results. The colony's morphological form found as many as 26 irregular, 13 circular, three filamentous, two punctiform, and one rhizoid. The edges of the settlement are dominated by entire (23 isolates), undulate (13 isolates), filamentous (5 isolates), and lobate (4 isolates). The elevation was 19 flats, 11 convex, 11 umbonate, and four raised. The color that often appears in macroscopic observations is white, and there are other colors, namely yellow and



pink. In the Gram staining results, as many as 51% of isolates are included in Gram-negative, and 49 % are gram-positive. Cell forms of 45 isolates, found 91.1 % of coccus-shaped isolates and 8.9 % of bacilli-shaped isolates with diplooccal cell arrangement (33.3 %), monococci (22.2 %), streptococci (13.3 %), staphylococci (22.2 %), diplobacilli (4.4 %), monobacilli (2.2 %), and streptobacilli (2.2 %) (Table 2).

**Antibiotic Resistance Test**

Antibiotic resistance tests were carried out on three types of antibiotics, namely ampicillin, bacitracin, and cefepime. Antibiotic resistance test results are determined by measuring the clear zone formed around the antibiotic disc (Figure 2).



**Figure 2.** Test Results of Isolate Resistance with BN12 (Left) and BN38 (Right) Codes Against Ampicillin (A), Bacitracin (B), and Cefepime (C) Antibiotics

Based on the results of antibiotic resistance tests on burial soil bacteria, results were obtained that showed antibiotic multi-resistant properties. The percentage of antibiotic resistance tested, namely Ampicillin, Bacitracin, and Cefepime, respectively 28.9 %, 64.4 %, and 57.8 % (Table 3.).

**Table 3.** Percentage of bacterial isolates from TPU Bonoloyo that are already antibiotic-resistant.

Antibiotic	Percentage of Isolates by Category (%)		
	Susceptible	Intermediate	Resistance
Ampicillin	66,7	4,4	28,9
Bacitracin	35,6	0	64,4
Cefepime	24,4	20	57,8

**Discussion**

The lowest population of soil bacteria from TPU Bonoloyo was found in block 21 at a depth of 20 cm, namely  $1 \times 10^6$  CFU/g, while the highest population in block 17 at a depth of 20 cm was  $8 \times 10^6$  CFU/g. This may be due to the location of block 21 in a higher position than other blocks (Table 1.). In addition, the content of several elements such as  $P_2O_5$ , C-organic, and N-total required to support bacterial growth is lower than blocks 17 and 12 (Table 4 Supplement). With the highest soil position, the leachate water resulting from the corpse's decomposition process will flow to lower places (Block 12 and Block 17) to enrich soil minerals in blocks in a lower position. This is evident from the higher electrical conductivity (DHL) parameters in Block 17 (126.2 masl) (Table 4 Supplement). DHL shows the ability of an electrolyte solution to conduct electricity (Sofiah et al., 2016). More bacterial populations were detected at depths of 50 cm except block 21.

Soil bacteria in the TPU area are much higher when compared to soil bacteria populations outside the TPU, such as in land-use soils of annuals and secondary forests have the highest

number of soil bacteria populations of  $1.5 \times 10^5$  CFU/g with a depth of 0 - 30 cm (Zainudin & Kesumaningwati, 2021), mountain soils of  $7.36 \times 10^5$  CFU/g (Sahara et al., 2019), land in oil palm plantations of  $1.16 \times 10^5$  CFU/g with a depth of 0 cm (Irfan, 2014), and waste burnt land of  $5.5 \times 10^5$  CFU/g (Situmorang et al., 2022). The study's results (Putra et al., 2023) showed data on the population of rhizosphere bacteria in burial grounds of  $1.0 \times 10^7$  CFU/g. The bacterial population at TPU Bonoloyo Surakarta at a depth of 10 cm is around  $3.4 \times 10^6$  CFU/gram (Rahmawati et al., 2023). At a depth of 10 cm, the soil bacteria population is still strongly influenced by cover plant factors (Sriwulan et al., 2022). It may not have been affected by soil elements resulting from the corpse's decomposition because the body was buried at a depth of about 1.5 m. The growth rate and activity of bacteria can be influenced by several factors, namely pH, osmosis pressure, and so on (Fitria & Zulaika, 2019). In addition, climatic conditions, including temperature, humidity, rainfall, and wind speed, can affect the life and breeding of microorganisms (Utami & Windraswara, 2019). This corroborates the notion that many factors affect the bacterial population of burial soils.

The identification results of bacterial isolates showed the dominance of white to yellow colony color in irregular shapes with flat edges. Gram staining results were identified as 51% Gram-negative bacteria with a dominant diplococcal and staphylococcal cell arrangement (Table 2.). Most Gram-negative are pathogenic because they have endotoxins on the outer membrane (Prastio et al., 2022). This is corroborated by several morphological studies on soil bacteria, including research in Kampung Melayu, three out of four test soil samples obtained the results of coccus-shaped bacteria (Abna et al., 2020), at TPU Pracimoloyo, the majority of coccus cell forms were acquired and had gram-negative (Putra et al., 2023). In the soil at TPU Bonoloyo, it is possible to find coccus-shaped genus bacteria with Gram-negative, such as the genus *Streptococcus*, genus *Streptomyces* and genus *Klebsiella* (Sayuti et al., 2016). The morphology of soil bacteria in TPU is different from soil bacteria other than in the TPU area from the results of research on endophytic bacteria and rhizobacteria from clove plants, bacilli-shaped bacteria with Gram-positive (Dwimartina et al., 2021), in endophytic bacteria of mangrove plants in the form of bacilli with Gram-positive (Suryani & A'yun, 2022), and in soil endogenous bacteria used oil spills in the form of bacilli with Gram-positive (Welan et al., 2019).

The results of antibiotic resistance tests on bacterial isolates in burial grounds showed that the isolates were already resistant to the three antibiotics tested. In this study, the most significant percentage of bacterial resistance was found in bacitracin, while ampicillin resistance was smaller than the percentage of other antibiotic resistance (Table 3.) (Table 2 Supplement). Antibiotic resistance occurs due to various factors, one of which is the use of inappropriate antibiotics without a doctor's prescription (Simamora et al., 2021). The sensitivity chart shows ampicillin is used to cure Gram-positive and Gram-negative bacterial infections but is more effective against Gram-positive bacteria. Bacitracin is effective for curing infectious diseases by Gram-positive bacteria and cefepime for diseases caused by Gram-positive and negative bacteria. Denpasar Hospital in the period 2019-2020 uses ampicillin to cure pneumonia (Sukertiasih et al., 2021). 41.67% of ampicillin antibiotics were also prescribed to hospitalized patients affected by bacterial infections (Nugraheni et al., 2021). The large percentage of resistance to bacitracin antibiotics is possible because bacitracin has a complex chemical structure and high molecular weight and cannot pass through the outer membrane of Gram-negative bacteria, so bacitracin is intrinsically resistant to Gram-negative bacteria (Hong et al., 2020). Bacitracin is a polypeptide antibiotic applied to animals and humans. If used for a long time, it can increase resistance genes in microorganisms (Ma et al., 2019). Resistance to bacitracin is supported by the potential spread of resistance genes in Gram-positive bacteria, especially pathogenic bacteria derived from humans and animals, such as enterococci, staphylococci, and streptococci (Huang et al., 2019). Bacterial resistance to antibiotics will cause problems in the treatment of disease, although many potent *Streptomyces* genera produce antibiotics (Ambarwati et al., 2019). Based on the results of antibiotic resistance tests on burial grounds, the community needs to maintain cleanliness when making pilgrimages to cemeteries.

## Conclusions

The population of soil bacteria at TPU Bonoloyo, Surakarta, Central Java, in several blocks with depths of 20 cm and 50 cm ranged from  $1 \times 10^6$  -  $8 \times 10^6$  CFU/g but showed no noticeable difference. The bacterial isolates were dominated by irregular colonies, flat colony edges, flat elevation, convex, umbonate, and raised. In contrast, cocci-shaped gram-negative groups dominated the gram-staining results; the results of the antibiotic resistance test obtained data that the isolate was multi-resistant to antibiotics.

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## Declaration statement

The authors reported no potential conflict of interest.

## References

- Abia, A. L. K., Alisoltani, A., Ubomba-Jaswa, E., & Dippenaar, M. A. (2019). Microbial life beyond the grave: 16S rRNA gene-based metagenomic analysis of bacteria diversity and their functional profiles in cemetery environments. *Science of the Total Environment*, 655, 831–841. <https://doi.org/10.1016/j.scitotenv.2018.11.302>
- Abna, I. M., Mahayasih, P. G. M. W., & Amir, M. (2020). Isolasi dan Karakterisasi Bakteri Tanah Di Kelurahan Kampung Melayu Jakarta Timur. *Archives Pharmacia*, 2(2), 102–111. <https://doi.org/10.47007/ap.v2i2.3393>
- Ambarwati, A., Wahyuono, S., Moeljopawiro, S., Sembiring, L., & Yuwono, T. (2019). Antimicrobial activities and phylogenetic study of bacteria associated with *Cyperus rotundus* rhizosphere from Cemoro Sewu plateau, Indonesia. *Biodiversitas*, 20(8), 2206–2212. <https://doi.org/10.13057/biodiv/d200814>
- Amri, I. A., & Wulandari, E. (2022). Identification of Colibacillosis and Antibiotic Resistance Test in Free-range Chicken: Identifikasi Kolibasilosis dan Uji Resistensi Antibiotik pada Ayam Kampung. *Veterinary Biomedical and Clinical Journal*, 4(1), 22–29. <https://doi.org/10.21776/ub.VetBioClinJ.2022.004.01.4>
- Andayani, N., Nurhayati, D., & Saing, M. D. (2022). Optimasilisasi Pertumbuhan Bakteri *E. Coli* dan *Bacillus Subtilis* pada Media Edamame Agar. *Jurnal Pengembangan Potensi Laboratorium*, 1(1), 45–53. <https://doi.org/10.25047/plp.v1i1.3095>
- Ayuti, S., Hidayah, W., Admi, M., Rosmaidar, Zainuddin, Hennivanda, & Makmur, A. (2023). Isolasi dan Identifikasi Bakteri Gram Positif. *Jurnal Perternakan Indonesia*, 25(1), 98–109. <https://doi.org/10.25077/jpi.25.1.98-109.2023>
- Całkosiński, I., Płoneczka-Janeczko, K., Ostapska, M., Dudek, K., Gamian, A., & Rypuła, K. (2015). Microbiological analysis of necrosols collected from urban cemeteries in Poland. *BioMed Research International*, 2015. <https://doi.org/10.1155/2015/169573>
- Ciaffi, R., Feola, A., Perfetti, E., Manciocchi, S., Potenza, S., & Marella, G. L. (2018). Overview on the estimation of post mortem interval in forensic anthropology: Review of the literature and practical experience. *Romanian Journal of Legal Medicine*, 26(4), 403–411. <https://doi.org/10.4323/rjlm.2018.403>
- de Kraker, M. E. A., Stewardson, A. J., & Harbarth, S. (2016). Will 10 Million People Die a Year due to Antimicrobial Resistance by 2050? *PLoS Medicine*, 13(11), 1–6. <https://doi.org/10.1371/journal.pmed.1002184>
- Dwimartina, F., Joko, T., & Arwiyanto, T. (2021). Karakteristik Morfologi Dan Fisiologi Bakteri Endofit Dan Rizobakteri Dari Tanaman Cengkeh Sehat. *Agro Wiralodra*, 4(1), 1–8. <https://doi.org/10.31943/agrowiralodra.v4i1.58>
- Fineza, A. G., Marques, E. A. G., Bastos, R. K. X., & Betim, L. S. (2014). Impacts on the groundwater quality within a cemetery area in southeast Brazil. *Soils and Rocks*, 37(2), 161–169. <https://doi.org/10.28927/sr.372161>
- Fitria, A. N., & Zulaika, E. (2019). Aklimatisasi pH dan Pola Pertumbuhan *Bacillus cereus* S1 pada Medium MSM Modifikasi. *Jurnal Sains Dan Seni ITS*, 7(2), 3–5. <https://doi.org/10.12962/j23373520.v7i2.36788>
- Fitriana, N., & Asri, M. T. (2021). Aktivitas Proteolitik pada Enzim Protease dari Bakteri Rhizosphere Tanaman Kedelai (*Glycine max* L.) di Trenggalek. *LenteraBio : Berkala Ilmiah Biologi*, 11(1), 144–152. <https://doi.org/10.26740/lenterabio.v11n1.p144-152>
- Hayati, Z., Widyastuti, E., Hayati, Z., Nurjannah, N., Mudatsir, M., & Saputra, I. (2022). Hubungan Kualitas Penggunaan Antibiotik dengan Luaran Klinis Pasien Bakteriemia yang Disebabkan Methicillin Resistant *Staphylococcus aureus* (MRSA). *Jurnal Kedokteran Syiah Kuala*, 22(1), 37–43. <https://doi.org/10.24815/jks.v22i1.25400>

- Hong, W., Gao, X., & Qiu, P. (2020). Corrigendum: Synthesis, construction, and evaluation of self-assembled nano-bacitracin as an efficient antibacterial agent in vitro and in vivo. *International Journal of Nanomedicine*, 15, 4753. <https://doi.org/10.2147/IJN.S267460>
- Huang, J., Sun, J., Wu, Y., Chen, L., Duan, D., Lv, X., & Wang, L. (2019). Identification and pathogenicity of an XDR *Streptococcus suis* isolate that harbours the phenicol-oxazolidinone resistance genes *oprA* and *cfr*, and the bacitracin resistance locus *bcrABDR*. *International Journal of Antimicrobial Agents*, 54(1), 43–48. <https://doi.org/10.1016/j.ijantimicag.2019.04.003>
- Irfan, M. (2014). Isolasi dan enumerasi bakteri tanah gambut di perkebunan kelapa sawit PT. Tambang Hijau Kecamatan Tambang Kabupaten Kampar. *Jurnal Agroteknologi*, 5(1), 1–8.
- Kazarina, A., Gerhards, G., Petersone-Gordina, E., Kimsis, J., Pole, I., Zole, E., Leonova, V., & Ranka, R. (2019). Analysis of the bacterial communities in ancient human bones and burial soil samples: Tracing the impact of environmental bacteria. *Journal of Archaeological Science*, 109(March), 104989. <https://doi.org/10.1016/j.jas.2019.104989>
- Khusuma, A., Safitri, Y., Yuniarni, A., & Rizki, K. (2019). Uji Teknik Difusi Menggunakan Kertas Saring Media Tampung Antibiotik dengan *Escherichia Coli* Sebagai Bakteri Uji. *Jurnal Kesehatan Prima*, 13(2), 151. <https://doi.org/10.32807/jkp.v13i2.257>
- Laskow, S. (2018). *There's a bunch of gross stuff, besides human bodies, hiding under graveyards.*
- Ma, J., Liu, J., Zhang, Y., Wang, D., Liu, R., Liu, G., Yao, H., & Pan, Z. (2019). Bacitracin resistance and enhanced virulence of *Streptococcus suis* via a novel efflux pump. *BMC Veterinary Research*, 15(1), 1–11. <https://doi.org/10.1186/s12917-019-2115-2>
- Mulyani, A., Bahar, M., Pasiak, T., & Fauziah, C. (2023). Pengaruh Optimasi Waktu Fermentasi dan Kontrol pH pada Aktivitas Antimikroba Actinomycetes terhadap *Salmonella typhi*. *Jurnal Sains Farmasi & Klinis*, 10(1), 120–128. <https://doi.org/10.25077/jfsk.10.1.120-128.2023>
- Nugraheni, A. Y., Putri, M. S., & Saputro, A. Y. (2021). Evaluasi Ketepatan Antibiotik pada Pasien Sepsis. *Pharmacon: Jurnal Farmasi Indonesia*, 18(2), 194–207. <https://doi.org/10.23917/pharmacon.v18i2.16635>
- Pradana, B., Priambudi, B. N., & Wijaya, M. I. H. (2021). Ketersediaan Lahan Pemakaman Saat Pandemi Covid-19 (Studi Kasus: Kota Surakarta Dengan Pemanfaatan Open Data). *SPECTA Journal of Technology*, 5(3), 298–307. <https://doi.org/10.35718/specta.v5i3.378>
- Prastio, R. A., Isnawati, I., & Rahayu, D. A. (2022). Isolasi, Karakterisasi, dan Identifikasi Bakteri Patogen pada Tumbuhan Kantong Semar (*Nepenthes gracilllis*). *LenteraBio: Berkala Ilmiah Biologi*, 11(2), 255–262. <https://doi.org/10.26740/lenterabio.v11n2.p255-262>
- Pratiwi, R. H. (2017). Mekanisme Pertahanan Bakteri Patogen Terhadap Antibiotik. *Jurnal Pro-Life*, 4(3), 418–429. <https://doi.org/10.33541/jpvol6Iss2pp102>
- Putra, S. S., Rahayu, T., & Tyastuti, E. M. (2023). Isolation and Characterization of Cambodian Tree Rhizosphere Bacteria (*Plumeria acuminata*) at TPU Pracimaloyo as a producer of IAA. *BIOEDUSCIENCE*, 7(1), 15–23. <https://doi.org/10.22236/jbes/7111375>
- Rahmawati, Y., Tyastuti, E. M., Sidiq, Y., & Rahayu, T. (2023). Keragaman dan Resistensi Antibiotik Isolat Bakteri Tanah di Dalam dan Luar TPU Bonoloyo, Surakarta Jawa Tengah Diversity and Resistance to Antibiotic Isolates of Soil Bacteria Inside and Outside Bonoloyo Cemetery, Surakarta, Central Java. *Jurnal Farmasi Indonesia*, 20(1), 87–95. <https://doi.org/10.23917/pharmacon.v20i1.22572>
- Sahara, N., Wardah, & Rahmawati. (2019). Populasi Fungi Dan Bakteri Tanah Di Hutan Pegunungan Dan Dataran Rendah Di Kawasan Taman Nasional Lore Lindu Sulawesi Tengah. *J. ForestSains*, 16(2), 85–93.
- Sayuti, I., Yustina, & Hardianti, N. (2016). Identifikasi Bakteri Pada Sampah Organik Pasar Kota Pekanbaru Dan Potensinya Sebagai Rancangan Lembar Kerja Siswa (LKS) Biologi Sma. *Jurnal Biogenesis*, 13(1), 51–60. <https://doi.org/10.31258/biogenesis.13.1.51-60>
- Simamora, S., Sarmadi, Rulianti, M. R., & Suzalin, F. (2021). Pengendalian Resistensi Bakteri Terhadap Antibiotik Melalui Pemberdayaan Perempuan Dalam Kelompok Masyarakat ( Bacterial Resistance Control Of Antibiotics Through Empowerment Of Women In Community Groups ). *Jurnal Abdikemas*, 3(1), 12–20. <https://doi.org/10.36086/j.abdikemas.v3i1.642>
- Singh, B., Minick, K. J., Strickland, M. S., Wickings, K. G., Crippen, T. L., Tarone, A. M., Eric Benbow, M., Sufrin, N., Tomberlin, J. K., & Pechal, J. L. (2018). Temporal and spatial impact of human cadaver decomposition on soil bacterial and arthropod community structure and function. *Frontiers in Microbiology*, 8(JAN), 1–12. <https://doi.org/10.3389/fmicb.2017.02616>
- Situmorang, T. S., Simanjuntak, H. A., Lingkungan, S. T., Teknik, F., Efarina, U., Biologi, S., Sains, F., Area, U. M., Farmasi, P. S., Tinggi, S., Kesehatan, I., & Medan, S. (2022). Isolasi Dan Karakterisasi Bakteri Dari Tanah Bakaran Sampah Isolation And Characterization of From Fire Soils. *Journal of Natural Science*, 3(3), 162–167. <https://doi.org/10.34007/jonas.v3i3.306>
- Sofiah, V., Chamid, C., & Sriyanti. (2016). *Kajian TDS dan DHL Untuk Menentukan Tingkat Pencemaran Air Tanah Dangkal di Sekitar Lokasi TPA Leuwigajah Kabupaten Bandung Provinsi Jawa Barat.* 297–306.
- Sriwulan, Andriani, R., Anggraini, S. D., Kurniahu, H., & Rahmawati, A. (2022). Bakteri Tanah Di Sekitar Rhizosfer Tumbuhan Pioner



- Pada Lahan Bekas Tambang Kapur. *Bioeksperimen*, 8. <https://doi.org/10.23917/bioeksperimen.v8i1.14414>
- Sukertiasih, N. K., Megawati, F., Meriyani, H., & Sanjaya, D. A. (2021). Studi Retrospektif Gambaran Resistensi Bakteri terhadap Antibiotik. *Jurnal Ilmiah Medicamento*, 7(2), 108–111. <https://doi.org/10.36733/medicamento.v7i2.2177>
- Sunniyyah, D. (2021). Perubahan Kadar Nitrogen Total Pada Tanah Sebagai Alternatif Estimasi Post-Mortem Interval. *Jurnal Biosains Pascasarjana*, 23(1), 1. <https://doi.org/10.20473/jbp.v23i1.2021.1-5>
- Suryani, S., & A'yun, Q. (2022). Isolasi Bakteri Endofit Dari Mangrove Sonneratia Alba Asal Pondok 2 Pantai Harapan Jaya Muara Gembong, Bekasi. *BIO-SAINS : Jurnal Ilmiah Biologi*, 1(2), 12–18. <https://doi.org/10.6084/m9.figshare.23564679>
- Utami, H. T., & Windraswara, R. (2019). Korelasi Meteorologi dan Kualitas Udara dengan Pneumonia Balita di Kota Semarang Tahun 2013-2018. *HIGEIA (Higeia Journal of Public Health Research and Development)*, 3(4), 588–598. <https://doi.org/10.15294/higeia.v3i4.31063>
- Welan, Y. S. L., Refli, & Mauboy, R. S. (2019). Isolasi dan uji biodegradasi bakteri endogen tanah tumpahan oli bekas di kota kupang. *Jurnal Biotropikal Sains*, 16(1), 61–72.
- WHO Indonesia. (2022). *Sekarang Saatnya Beraksi Menangkal Resistensi Antimikroba*.
- Wulandari, N., Irfan, M., & Saragih, R. (2020). Isolasi Dan Karakterisasi Plant Growth Promoting Rhizobacteria Dari Rizosfer Kebun Karet Rakyat. *Dinamika Pertanian*, 35(3), 57–64. [https://doi.org/10.25299/dp.2019.vol35\(3\).4565](https://doi.org/10.25299/dp.2019.vol35(3).4565)
- Yunita, S. L., Novia Atmadani, R., & Titani, M. (2021). Faktor-faktor Yang Mempengaruhi Pengetahuan Dan Perilaku Penggunaan Antibiotika Pada Mahasiswa Farmasi UMM. *Pharmaceutical Journal of Indonesia*, 6(2), 119–123. <https://doi.org/10.21776/ub.pji.2021.006.02.7>
- Zainudin, Z., & Kesumaningwati, R. (2021). Identifikasi Jamur Dan Bakteri Pada Beberapa Penggunaan Lahan Di Kota Samarinda. *Ziraa'Ah Majalah Ilmiah Pertanian*, 46(2), 165. <https://doi.org/10.31602/zmip.v46i2.4352>
- Zanial, & Irwansyah. (2016). Sistem Informasi Geografis Tempat Pemakaman Umum (TPU) Di Wilayah Kota Palembang. *Jurnal Ilmiah MATRIK*, 18(3), 271–280. <https://doi.org/10.33557/jurnalmatrik.v18i3.417>
- Zychowski, J., & Bryndal, T. (2015). Impact of cemeteries on groundwater contamination by bacteria and viruses - A review. *Journal of Water and Health*, 13(2), 285–301. <https://doi.org/10.2166/wh.2014.119>

Supplements Attachment

Table 4. Soil Sample Analysis Results

Test parameters	Unit	Block and Depth (cm)			Method
		12 – 20 cm	17 – 20 cm	21 – 20 cm	
pH (H <sub>2</sub> O)		7.16	7.29	7.84	pH meter 1:5 IK. 5.4.c
pH (KCl)		6.21	6.69	6.54	pH meter 1:5 IK. 5.4.c
DHL	( $\mu$ s/cm)	28	87	13	Konduktometer 1 : 5
C-organik	%	2.23	1.23	1.13	Walkly & Black IK 5.4.d
N-total	%	0.12	0.08	0.04	Kjeldahl IK 5.4.e
K tersedia	Ppm	132	106	129	Morgan-Wolf
P <sub>2</sub> O <sub>5</sub>	ppm	7	2	ttd	Olsen IK.5.4.h

Table 5. Antibiotic Test Data

Isolat Name	Types of Antibiotics								
	Ampicillin			Bacitracin			Cefepime		
	S	I	R	S	I	R	S	I	R
BN1	40 mm	-	-	27 mm	-	-	-	20 mm	-
BN2	24 mm	-	-	21 mm	-	-	-	20 mm	-
BN3	-	-	7 mm	-	-	7 mm	-	-	0 mm
BN4	18 mm	-	-	-	-	9 mm	-	-	8 mm
BN5	-	-	0 mm	-	-	9 mm	-	-	0 mm
BN6	-	-	0 mm	-	-	8 mm	-	-	0 mm
BN7	20 mm	-	-	-	-	0 mm	-	-	8 mm
BN8	-	-	0 mm	-	-	0 mm	-	-	0 mm
BN9	25 mm	-	-	44 mm	-	-	-	20 mm	-
BN10	-	-	0 mm	-	-	0 mm	-	-	0 mm
BN11	-	16 mm	-	-	-	8 mm	-	20 mm	-
BN12	43 mm	-	-	-	-	0 mm	30 mm	-	-
BN13	-	-	0 mm	-	-	0 mm	-	-	0 mm
BN14	43 mm	-	-	-	-	0 mm	30 mm	-	-
BN15	40 mm	-	-	26 mm	-	-	-	-	16 mm
BN16	25 mm	-	-	-	-	8 mm	-	22 mm	-
BN17	20 mm	-	-	32 mm	-	-	-	-	17 mm
BN18	19 mm	-	-	25 mm	-	-	-	24 mm	-
BN19	25 mm	-	-	36 mm	-	-	-	-	0 mm
BN20	18 mm	-	-	-	-	0 mm	30 mm	-	-
BN21	30 mm	-	-	17 mm	-	-	-	20 mm	-
BN22	32 mm	-	-	-	-	0 mm	-	-	0 mm
BN23	-	-	0 mm	-	-	8 mm	-	-	0 mm
BN24	23 mm	-	-	-	-	7 mm	-	-	15 mm
BN25	20 mm	-	-	-	-	10 mm	35 mm	-	-
BN26	25 mm	-	-	-	-	0 mm	25 mm	-	-
BN27	27 mm	-	-	-	-	0 mm	25 mm	-	-
BN28	25 mm	-	-	-	-	0 mm	25 mm	-	-
BN29	-	15 mm	-	20 mm	-	-	-	20 mm	-
BN30	17 mm	-	-	23 mm	-	-	28 mm	-	-
BN31	35 mm	-	-	40 mm	-	-	35 mm	-	-
BN32	32 mm	-	-	-	-	12 mm	-	-	10 mm
BN33	30 mm	-	-	-	-	11 mm	-	-	0 mm
BN34	-	-	11 mm	-	-	11 mm	-	-	0 mm
BN35	-	-	13 mm	20 mm	-	-	-	-	15 mm
BN36	-	-	0 mm	-	-	7 mm	-	-	0 mm
BN37	30 mm	-	-	15 mm	-	-	30 mm	-	-
BN38	20 mm	-	-	-	-	0 mm	-	-	0 mm
BN39	-	-	9 mm	13 mm	-	-	-	-	0 mm
BN40	-	-	8 mm	15 mm	-	-	-	-	0 mm
BN41	32 mm	-	-	-	-	10 mm	-	-	10 mm
BN42	33 mm	-	-	-	-	11 mm	-	-	8 mm
BN43	-	-	0 mm	-	-	0 mm	-	-	0 mm
BN44	40 mm	-	-	-	-	10 mm	-	20 mm	-
BN45	35 mm	-	-	25 mm	-	-	25 mm	-	-

Information:  
 S = Sensitive  
 I = Intermediate  
 R = Resistance