Research Article

Exploration of Anti-Microbial Resistant (AMR) Genes in the Gastrointestinal Microbiome of Birds of Paradise (Paradisaeidae) in Papua, Indonesia

Alif Rahman Rohim Puarada*, Safika, Agustin Indrawati

School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, Indonesia.

Abstract | In avian ecology, the gastrointestinal microbiome is pivotal in influencing host health, contributing to metabolic processes, and shaping ecological interactions. The escalating global significance of antimicrobial resistance (AMR) genes within diverse microbiota components has garnered increasing attention due to its profound consequences. Recent investigations have revealed the presence of antibiotic-resistant bacteria among wild bird species in remote habitats, suggesting the potential for these avian populations to serve as reservoirs and sources for emergent AMR profiles. Recognizing the critical role of wildlife in AMR dynamics this study focuses on birds of paradise, indigenous to Indonesia. Their gastrointestinal microbiome, intricately shaped by a unique ecological milieu, presents a compelling avenue for exploring AMR genes' reservoir and dissemination mechanisms. Fecal samples were collected from Papua, Indonesia. The DNA was extracted using the DNeasy Power Soil Pro Kit. A shotgun metagenomic sequencing process from the MGI DNBSEQ-G400 platform was carried out to obtain DNA sequence data from the genes in the sample. The resulting DNA sequence data were then analyzed using MEGAN6 software using the SEED subsystem. The investigation reveals a notable abundance of AMR genes, with bacitracin resistance standing out prominently, particularly associated with the prevalence of *Bacillus pumilus*. Metal and environmental resistance mechanisms underscore the complex adaptations within the birds' gastrointestinal. The study highlights concern regarding the elevated presence of antibiotic resistance genes, particularly those associated with synthetic antibiotics, pointing to potential environmental exposures, including agricultural antibiotic use.

Keywords | AMR genes, Avian gastrointestinal microbiome, Birds of paradise, Shotgun metagenomics

Received | December 08, 2023; **Accepted** | January 06, 2024; **Published** | February 12, 2024
***Correspondence** | Alif Rahman Rohim Puarada, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, Indonesia; **Email:** alif.rahman44@gmail.com
Citation | Puarada ARR, Safika, Indrawati A (2024). Exploration of anti-microbial resistant (AMR) genes in the gastrointestinal microbiome of birds of paradise (Paradisaeidae) in Papua, Indonesia. Adv. Anim. Vet. Sci., 12(3):539-545.
DOI | https://dx.doi.org/10.17582/journal.aavs/2024/13.2.539.545
ISSN (Online) | 2307-8316

INTRODUCTION

The avian gastrointestinal microbiome is a pivotal factor influencing host health, affecting metabolism and ecological interactions (Kohl, 2012). Within this intricate ecosystem, the prevalence and genomic characteristics of antimicrobial-resistant (AMR) genes within diverse microbiota components are gaining global attention (Grond *et al.*, 2018). The consequences of AMR, including treatment failures and increased mortality, underscore its significance (Cao *et al.*, 2020). Recent studies reveal the presence of antibiotic-resistant bacteria in wild bird species across the globe, even in remote habitats, suggesting the potential role of certain wild animal species as reservoirs for the emergence and spread of new AMR profiles (Martínez, 2008; Sjölund *et al.*, 2008).

Understanding Antimicrobial Resistance (AMR) in wildlife is imperative for global health security, as wildlife serves as natural reservoirs and disseminators of AMR, shedding light on how resistance naturally arises in ecosystems and transfers between species (Djuwanto, 2021; Elsohaby *et al*., 2021). The urgency of this research is emphasized by the potential transmission of antimicrobial resistance from wildlife to humans, either through meat consumption or direct contact with animals, particularly relevant in Indonesia. Investigating antibiotic use in wildlife habitats contributes to developing effective management strategies. Engaging in environmental prevention strategies, such as waste management and responsible antibiotic use in agriculture, mitigates the risk of AMR and provides insights essential for maintaining global food security (McKernan *et al*., 2021; Olaru *et al*., 2023). The escalating concern over the rise of AMR necessitates a comprehensive understanding of these dynamics in diverse ecosystems such as birds of paradise (Hernández *et al*., 2012).

Birds of paradise, classified under the family Paradisaeidae, is an endemic species to the Indonesian islands of Maluku and Papua (Firth and Firth, 2009). This taxonomic group has 45 species, each characterized by intricate and highly specialized morphological adaptations. These birds present a fascinating intersection of ecology and microbial dynamics. Notable among these adaptations are the elaborate plumage and vibrant coloration, features that play a pivotal role in the elaborate courtship displays observed in these species (Gregory, 2020). The intricate mating rituals and specialized dietary habits of birds of paradise suggest potential symbiotic relationships with specific microorganisms, influencing nutrient metabolism and immune function could serve as a reservoir and mechanism for the spread of AMR genes (Culp and Goodman, 2023). Investigating the microbiome of these avifauna could provide valuable insights into the coevolutionary processes shaping both the microbial communities and the birds themselves.

Using the state-of-the-art genomic technique 'Shotgun metagenomics, this study aims to unveil the genomic structure of AMR genes in the gastrointestinal microbiome of birds of paradise (Wang *et al*., 2022). This high-throughput sequencing method provides insights into complex interactions between the host and the microbiome.

Papua, dominated by tropical rainforests and divided into fifteen areas based on geographical differences, offers diverse habitats for birds of paradise. The lowland rainforest, a significant habitat, hosts species like the king bird-of-paradise, utilizing tall trees for various activities (Beehler and Pratt, 2016). The rich plant and wildlife diversity in these habitats provides a variety of food sources, including fruits, insects, flower nectar, seeds, and flowers (Frith *et al*., 1998). Understanding the genomic landscape of the gastrointestinal microbiome of birds of paradise in Papua is crucial to uncover the diversity of AMR genes in these unique microbial communities (Culp and Goodman, 2023).

MATERIALS AND METHODS

Sampling

Six fecal samples were collected in December 2022 from wild birds of paradise inhabiting the Rhepang Muaif Monitoring Site, Rhepang Muaif Village, Nimbokrang District, Jayapura Regency. The identification of the birds of paradise for sampling was conducted based on morphology. To ensure the samples truly represented a variety of individuals, a site survey was conducted to pinpoint the stopover locations most frequently visited by individual birds, facilitating the collection of fresh fecal samples. The fresh samples were obtained immediately after defecation to minimize surface contamination. Direct collection from wild avian specimens was implemented without human intervention or any form of invasive treatment. Each fecal sample was labeled at the time of collection to ensure accurate tracking of individual specimens.

To ensure the diversity of the sampled individuals, the collection process involved maintaining a minimum 1-meter radius between sampled feces. This precautionary measure aimed to guarantee that each sample originated from a distinct bird of paradise within their natural habitat. Following collection, all fecal samples were carefully placed in sterile containers within sterile tubes. The samples were transported to the laboratory using dry ice to preserve their integrity and were stored at -80°C until further processing. This approach ensures the reliability and representativeness of the collected samples from the wild birds of paradise in their native environment.

DNA extraction

Fecal samples were extracted using the DNeasy Power Soil Pro kit (Qiagen, Germany) according to the manufacturer's procedures for the best performance (Shaffer *et al*., 2022).

Product qualification and sequencing

A total of 25 µl of total DNA was taken and then the DNA concentration was measured at the Advanced Research Laboratory (Bogor) using a Nano Photometer® N60/N50 (Implen, Germany). The A260/A280 ratio was recorded and calculated to determine the purity of the DNA. Qubit fluorometric (Thermo Fisher Scientific, USA) quantitation was then used to assess the extracted DNA quality. Total DNA concentration ≥15 ng/µL will proceed to the sequencing stage. A shotgun metagenomic sequencing process from the MGI DNBSEQ-G400 platform was carried out to obtain DNA sequences.

Analysis

Merging raw paired-end reads and quality control simultaneously using FLASH 1.2.11 (Fast Length Adjustment of SHort reads) (Magoč and Salzberg, 2021). This facilitates accurate sequence reconstruction while filtering out low-quality reads. Subsequently, Qiime2 (3.5.3 phyton version) is employed for further quality filtering to enhance data integrity by removing noise and artifacts (Bolyen *et al*., 2019). The criteria for quality control were a sequence was removed once its average quality within the window fell below Q20; sequences containing any N-bases were filtered out; reads that were below 50 bp in length were dropped; only paired-end reads were retained. The dataset undergoes dereplication and chimera removal using UCHIME, contributing to reduced redundancy and elimination of artificial sequences (Edgar, 2017).

Taxonomic classification of the processed sequences is performed using Diamond followed by MEGAN6 (Bağcı *et al*., 2021). These tools provide insights into the relative abundance of bacteria, offering valuable information on microbial community composition. If assembly is required, Qiime2 is utilized for this purpose (Culp and Goodman, 2023). Using MEGAN6, functional annotations (hits to SEED subsystem) were extracted (e value less than 1×10^{-5} and sequence match length greater than 50 nucleotides) to compare functional antimicrobial resistance gene attributes across the birds of paradise gastrointestinal metagenome (Overbeek *et al*., 2014). Finally, RStudio is utilized for custom scripting to quantify gene abundance and visualization (Rstudio Team, 2020).

RESULT AND DISCUSSION

Samples characteristic

Six of the initial samples failed to meet the required DNA concentration standard, which stipulated a minimum of 15.0 ng/µL. Two samples with the highest concentrations of 14.0 ng/µL and 14.6 ng/µL, with A260/280 ratios of 1.59 and 1.75, respectively, were used to continue the sequencing process using the shotgun metagenomics method of the MGI DNBSEQ-G400 platform. Although these concentrations are below the standard threshold, the A260/280 ratio and total DNA concentration being close to the optimal value indicates a fairly good relative purity of the DNA. This decision was based on the reliability of the MGI DNBSEQ-G400 platform in coping with samples with low DNA quantity and still producing accurate sequences. We believe that by selecting these two best samples, we can still obtain informative metagenomic data, despite the relatively low concentrations of the samples. The two samples were obtained from distinct bird of paradise species with varying dietary habits and display behaviors, as detailed in Table 1.

Gastrointestinal microbiome: Species abundance

The results of metagenomic analysis using the shotgun metagenomics platform on birds of paradise samples and feces found diverse species. A total of 104 species were successfully sequenced from the samples, 53 species from sample CDW2 (5 unspecified species) and 51 species from CDW6 (18 unspecified species) where 26 of the same species were obtained from both samples. Based on Figure 1, it can be seen that most of the microbiome abundance in the gastrointestinal of birds of paradise from both samples are *Delftia* sp., *Pedobacter* sp. (ASV17, ASV12, ASV8, ASV2), *Klebsiella pneumoniae*, *Achromobacter* sp., *Bacillus pumilus*, *Rhizobium* sp., and *Brevundimonas* sp. with some differences in the species *Flavobacterium* sp., *Aquitaiea* sp., and *Acidobacterium* sp., respectively.

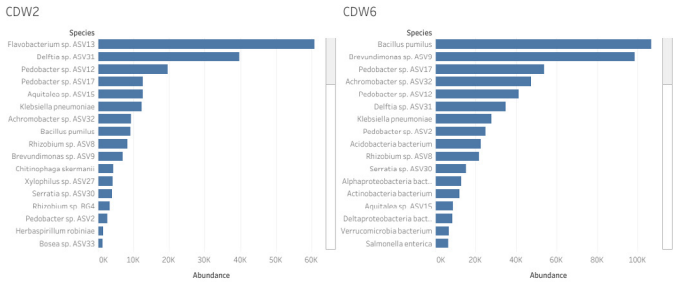

Figure 1: Highest abundance of species from bird of paradise gastrointestinal microbiome.

SEED subsystems functional annotation

A total of 100,057 genes in the CDW2 sample and 225,830 from the CDW6 sample were successfully annotated into the SEED subsystem functional annotation. Annotation was carried out up to level 3 to determine the composition of AMR genes in the gastrointestinal of birds of paradise (Figure 2). Only 2379 genes from CDW2 and 8744 genes from CDW6 have functional genes of AMR (Figure 3).

Table 1: Birds of paradise sample information.

Sample ID	Date collected (dd/mm/yyyy)	Bird species	Diet	Display behavior	A2600/A280 ratio	DNA concentration (ng/µL)
CDW2	28/12/2022	Twelve-wired bird-of-paradise (*Seleucidis melanoleucus*)	50% fruits	Arboreal, solitary, dispersed, not territorial	1.59	14.0
CDW6	30/12/2022	Magnificent bird-of-paradise (*Cicinnurus magnificus*)	70% fruits	Terrestrial, solitary, dispersed, not territorial	1.75	14.6

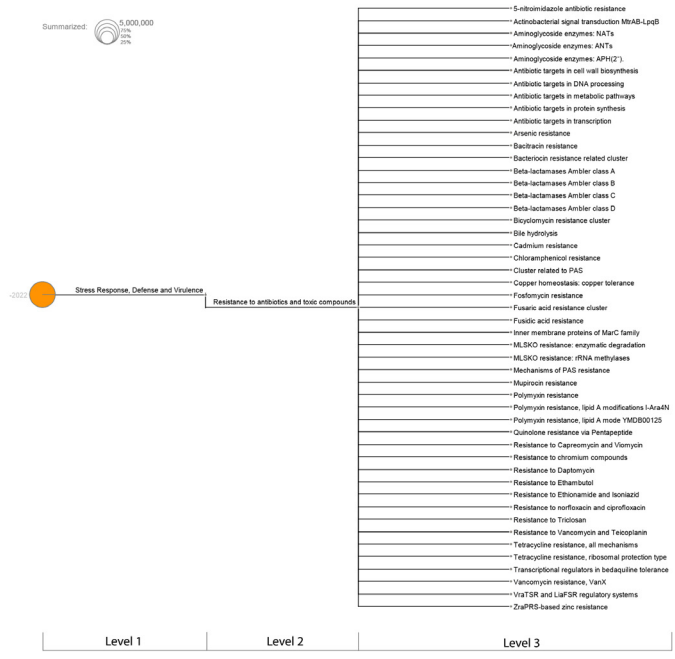

Figure 2: SEED Subsystem tree structure.

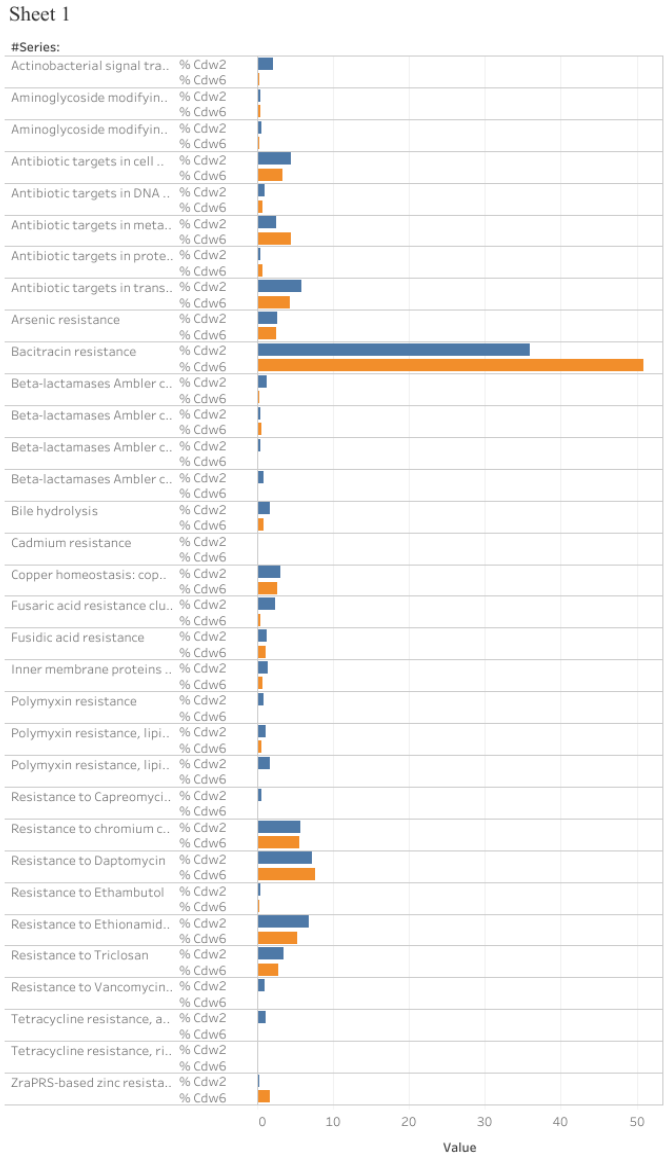

Figure 3: Abundance of AMR genes in bird of paradise gastrointestinal microbiome.

The study reveals significant challenges in combating bacterial infections, particularly in light of the high resistance observed in specific mechanisms. Bacitracin resistance exhibits a formidable resistance level of 43.4%. Similarly, resistance to Daptomycin showcases a notable resistance level of 7.4%. In addition to antibiotic resistance, the diversity of antibiotic targets across various cellular processes is evident. Antibiotic targets encompass cell wall biosynthesis (3.9%), DNA processing (0.8%), metabolic pathways (3.5%), protein synthesis (0.6%), and transcription (5.1%). This diversity highlights the multifaceted nature of bacterial resistance. Bacteria exhibit not only antibiotic resistance but also resilience to environmental stressors. Mechanisms such as Resistance to chromium compounds (5.6%), Copper homeostasis: Copper tolerance (2.9%), and Arsenic resistance (2.5%) underscore the adaptability of bacteria to diverse challenges.

The study delves into the intricate world of signal transduction systems, exemplified by the Actinobacterial signal transduction system MtrAB-LpqB. These systems suggest complex regulatory networks influencing bacterial responses. While some resistance mechanisms exhibit lower values, such as tetracycline resistance, ribosomal protection type (0.005%), and macrolides, lincosamides, streptogramins, ketolides, oxazolidinones (MLSKO) resistance: Ribosomal protection (0.03%). The study also sheds light on regulatory systems contributing to bacterial tolerance, including VraTSR and LiaFSR three-component regulatory systems (0.1%) and Transcriptional regulators implicated in bedaquiline tolerance (0.005%).

This study revealed interesting findings regarding the high level of resistance to bacitracin in the gastrointestinal of birds of paradise. This resistance is most likely attributed to the abundance of *Bacillus pumilus* bacteria in the birds' gastrointestinal. *Bacillus pumilus,* as a Gram-positive bacterial species, can produce a variety of bioactive compounds, including antibiotics, which may provide a competitive advantage in such environments (Iqbal *et al*., 2021).

High bacitracin resistance in the gastrointestinal of birds of paradise suggests that *Bacillus pumilus*, as a potential producer of bacitracin, may play a key role in developing bacterial resistance within the digestive system. Resistance to bacitracin is the result of evolutionary selection pressures that bacteria may face in competing for survival in an environment rich in antimicrobial compounds (Awais *et al*., 2007).

It has been discovered through recent research that bacitracin, a type of polypeptide antibiotic, has a positive role in supporting the growth of certain microorganisms, including *Bacillus* sp., and also promotes the intake of ion

in the soil (Haavik, 1975, 1976). This discovery suggests that bacitracin not only has antimicrobial properties but can also act as a growth factor that could potentially benefit producer organisms during their growth process. This can also answer the higher abundance of *Bacillus pumilus* in terrestrial compared to arboreal birds of paradise.

Furthermore, the abundance of *Bacillus pumilus* may create a unique microecosystem within the gastrointestinal of birds of paradise. Factors such as the production of antimicrobial compounds, the capacity to form biofilms, and interactions with other bacteria may influence the dynamics of antimicrobial resistance in this environment (Uruén *et al*., 2020).

The metal and environmental resistance mechanisms observed in the gastrointestinal of birds of paradise reflect complex bacterial adaptations to specific ecological conditions. Arsenic resistance indicates the ability of bacteria to resist the toxic effects of arsenic, a metalloid found in soil and water. Cadmium resistance and chromium resistance indicate the ability of the bacteria to tolerate high concentrations of these metals. Copper homeostasis and tolerance indicate the ability of bacteria to maintain a balance of copper ions. This adaptation may be particularly important for birds of paradise as this resistance potentially originating from the bird of paradise environment, such as soil (especially in wetland) or food sources (Sánchez-Virosta *et al*., 2015; Łanocha-Arendarczyk and Kosik-Bogacka, 2019).

Resistance to chromium compounds further underscores the ability of bacteria to withstand certain chromium compounds present in the environment of paradise. Papua in Indonesia has chromium concentrations of stream sediment samples ranging from 3 ppm to 74,600 ppm, with a median value of 145 ppm, which was higher than the upper crustal abundance of chromium and the chromium geochemical baselines of Europe, Australia, North America, and China (Zhao *et al*., 2023). However, the sedimentation concentrations of chromium and arsenic did not exceed the Threshold Effect Level (TEL) (Hamuna and Wanimbo, 2021). Some possible factors that could influence this phenomenon involve ecological and biochemical aspects of the ecosystem. There may be genetic variation in bacterial populations on the island of Papua that accounts for the high abundance of chromium resistance genes. Bacterial populations in a given area can have different genetic profiles that develop in response to local environmental conditions. In addition, natural selection can promote the development of genetic resistance in bacterial populations (Santos-Lopez *et al*., 2021). Bacteria that have higher resistance genes have adaptation advantages and can reproduce better in certain environments. Also, certain microbiological conditions, such as soil pH, moisture, or certain types of vegetation, can favor the growth and proliferation of bacteria with chromium resistance genes (Abdul-Rahman *et al*., 2021).

Many AMR genes have been found in the gastrointestinal of birds of paradise. Most of these genes can appear naturally because antibiotic compounds can naturally exist in nature as a protective mechanism from other organisms (Andersson *et al*., 2020). However, some genes such as resistance to ethionamide and isoniazid (antibiotic to treat tuberculosis), as well as some resistance genes to floroquinolone mechanisms which are synthetic antibiotics can be found in this microbiome (Wolff and Nguyen, 2012; Gegia *et al*., 2017).

This is a concern because one of the possible causes of this resistance is continuous exposure to chemical compounds in their environment or even the possible transfer of genetic resistance from other microorganisms in their ecosystem (Larsson and Flach, 2022). The resistance gene can also come from genetic mutations in *Mycobacterium tuberculosis* (i.e. inhA double mutations) or even possibly from other microbes in the microbiome that have some similarity with this gen (Machado *et al*., 2013; Sandoval *et al*., 2019).

Of particular consideration is the role of antibiotics in agriculture and animal husbandry, which may result in antibiotic residues in the habitats of birds of paradise. This contamination can be a direct cause or contributor to antibiotic resistance in bird populations (Jechalke *et al*., 2014). It is crucial to note that, despite their primary diet consisting of fruits, birds of paradise can inadvertently be exposed to antibiotics through certain human activities in their habitat, such as agricultural runoff, disposal of pharmaceutical waste, or the use of antibiotics in nearby livestock or directly to these birds (Larsson and Flach, 2022). However, it is pertinent to highlight that these birds have not been actively administered any antibiotics and have limited interaction with humans, primarily occurring during monitoring purposes. Consequently, a comprehensive understanding of the factors contributing to antibiotic resistance in birds of paradise is imperative, with environmental pollution being one potential source among various factors.

CONCLUSIONS AND RECOMMENDATIONS

This study shows the abundance of AMR genes in the gastrointestinal of birds of paradise. the highest abundance is shown in bacitracin resistance. This significant resistance is linked to the prevalence of *Bacillus pumilus*. This resistance is indicative of evolutionary selection pressures and suggests a potential role of *Bacillus pumilus* in bacterial

resistance development. The presence of metal and environmental resistance mechanisms further underscores complex bacterial adaptations in the unique micro-ecosystem within the birds' gastrointestinal. The abundance of antibiotic resistance genes, especially synthetic antibiotics, raises concerns about continuous exposure to environmental chemicals, including antibiotics used in agriculture, emphasizing the need for a comprehensive understanding of the factors influencing resistance in birds of paradise.

ACKNOWLEDGEMENT

The author is grateful to IPB University and PT. Saraswanti Gene Indotech for all the facilities to achieve this study. The author is also grateful to BKSDA Jayapura Mrs. Lusi, Mr. Danial, and other colleagues for their input in this research, especially in field monitoring and bird of paradise behavior, for Mr. Kholiq for the time and knowledge for this research and other people who contributed to this research.

NOVELTY STATEMENT

This study uses shogun metagenomics sequencing tool to explore AMR gene of bird of paradise and functional annotation is performed using SEED subsystems.

AUTHOR'S CONTRIBUTION

ARRP, S, and AI: Study conception and design, contributed to writing, revising, and editing the manuscript, review, editing, and visualization of the manuscript. ARRP and S: Conducted the experiments and analyzed the data, acquisition data, review, and editing. All authors have read, reviewed, and approved the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Abdul-Rahman NS, Abdul Hamid NW, Nadarajah K (2021). Effects of abiotic stress on soil microbiome. Int. J. Mol. Sci., 22: 9001-9036. https://doi.org/10.3390/ijms22169036

Andersson DI, Balaban NQ, Baquero F, Courvalin P, Glaser P, Gophna U, Tønjum T (2020). Antibiotic resistance: turning evolutionary principles into clinical reality. FEMS Microb. Rev., 44: 171-188. https://doi.org/10.1093/femsre/fuaa001

Awais M, Shah AA, Hameed A, Hasan F (2007). Isolation, identification and optimization of bacitracin produced by *Bacillus* sp. Pak. J. Bot., 39: 1303-1312.

Bağcı C, Patz S, Huson DH (2021). Diamond+ Megan: Fast and easy taxonomic and functional analysis of short and long microbiome sequences. Curr. Protoc., 1: 5901-5930. https://doi.org/10.1002/cpz1.59

Beehler BM, Pratt TK (2016). Birds of New Guinea: Distribution, taxonomy, and systematics. Princeton University Press, Princeton, New Jersey, USA. pp. 672. https://doi.org/10.1515/9781400880713

Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, Bai Y, Bisanz JE, Bittinger K, Brejnrod A, Brislawn CJ, Brown CT, Callahan BJ, Caraballo-Rodríguez AM, Chase J, Cope EK, Da Silva R, Diener C, Dorrestein PC, Douglas GM, Durall DM, Duvallet C, Edwardson CF, Ernst M, Estaki M, Fouquier J, Gauglitz JM, Gibbons SM, Gibson DL, Gonzalez A, Gorlick K, Guo J, Hillmann B, Holmes S, Holste H, Huttenhower C, Huttley GA, Janssen S, Jarmusch AK, Jiang L, Kaehler BD, Kang KB, Keefe CR, Keim P, Kelley ST, Knights D, Koester I, Kosciolek T, Kreps J, Langille MGI, Lee J, Ley R, Liu Y, Loftfield E, Lozupone C, Maher M, Marotz C, Martin BD, McDonald D, McIver LJ, Melnik AV, Metcalf JL, Morgan SC, Morton JT, Naimey AT, Navas-Molina JA, Nothias LF, Orchanian SB, Pearson T, Peoples SL, Petras D, Preuss ML, Pruesse E, Rasmussen LB, Rivers A, Robeson-II MS, Rosenthal P, Segata N, Shaffer M, Shiffer A, Sinha R, Song SJ, Spear JR, Swafford AD, Thompson LR, Torres PJ, Trinh P, Tripathi A, Turnbaugh PJ, Ul-Hasan S, van der Hooft JJJ, Vargas F, Vázquez-Baeza Y, Vogtmann E, von Hippel M, Walters W, Wan Y, Wang M, Warren J, Weber KC, Williamson CHD, Willis AD, Xu ZZ, Zaneveld JR, Zhang Y, Zhu Q, Knight R, Caporaso JG. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol., 37: 852-857.

Cao J, Hu Y, Liu F, Wang Y, Bi Y, Lv N, Li J, Zhu B, Gao GF (2020). Metagenomic analysis reveals the microbiome and resistome in migratory birds. Microbiome, 8: 1-8. https://doi.org/10.1186/s40168-019-0781-8

Culp EJ, Goodman AL (2023). Cross-feeding in the gut microbiome: Ecology and mechanisms. Cell Host Microb., 31: 485-499. https://doi.org/10.1016/j.chom.2023.03.016

Djuwanto BA (2021). Wild birds as a reservoir of antimicrobial resistance (AMR) in the environment. Masters thesis. Edinburgh Napier University. Edinburgh, GBR.

Edgar RC (2017). Accuracy of microbial community diversity estimated by closed-and open-reference OTUs. PeerJ., 5: 1-17. https://doi.org/10.7717/peerj.3889

Elsohaby I, Samy A, Elmoslemany A, Alorabi M, Alkafafy M, Aldoweriej A, Al-Marri T, Elbehiry A, Fayez M (2021). Migratory wild birds as a potential disseminator of antimicrobial-resistant bacteria around Al-Asfar Lake, Eastern Saudi Arabia. Antibiotics, 10: 1-16. https://doi.org/10.3390/antibiotics10030260

Firth CB, Firth DW (2009). Family Paradisaeidae (Birds-of-paradise). in del Hoyo J, Elliott A, Christie D (eds), Handbook of the birds of the world. Volume 14: Bush-shrikes to old world sparrows. Lynx Edicions, Barcelona, ESP. pp. 404–459.

Frith CB, Beehler BM, Cooper WT, McGuire M (1998). The birds of paradise: Paradisaeidae. Oxford University Press, Oxford, GBR. https://doi.org/10.1093/oso/9780198548539.001.0001

Gegia M, Winters N, Benedetti A, van Soolingen D, Menzies D (2017). Treatment of isoniazid-resistant tuberculosis with first-line drugs: A systematic review and meta-analysis. Lancet Infect. Dis., 17: 223-234. https://doi.org/10.1016/S1473-3099(16)30407-8

Gregory P (2020). Birds of paradise and bowerbirds. Bloomsbury Publishing, London, GBR. pp. 6–8.

Grond K, Sandercock BK, Jumpponen A, Zeglin LH (2018). The avian gut microbiota: Community, physiology, and function in wild birds. J. Avian Biol., 49: 1-19. https://doi.org/10.1111/jav.01788

Haavik HI (1975). On the function of the polypeptide antibiotic bacitracin in the producer strain *Bacillus licheniformis*. Acta Pathol. Microbiol. Scand. B, 83: 519-524. https://doi.org/10.1111/j.1699-0463.1975.tb00133.x

Haavik HI (1976). Possible functions of peptide antibiotics during growth of producer organisms: bacitracin and metal (II) ion transport. Acta Pathol. Microbiol. Scand. B, 84: 117-124. https://doi.org/10.1111/j.1699-0463.1976.tb01913.x

Hamuna B, Wanimbo E (2021). Heavy metal contamination in sediments and its potential ecological risks in Youtefa Bay, Papua Province, Indonesia. J. Ecol. Eng., 22: 209-222. https://doi.org/10.12911/22998993/139116

Hernández J, Stedt J, Bonnedahl J, Molin Y, Drobni M, Calisto-Ulloa N, Gomez-Fuentes C, Astorga-España MS, González-Acuña D, Waldenström J, Blomqvist M (2012). Human-associated extended-spectrum β-lactamase in the Antarctic. Appl. Environ. Microbiol., 78: 2056-2058. https://doi.org/10.1128/AEM.07320-11

Iqbal S, Vollmers J, Janjua HA (2021). Genome mining and comparative genome analysis revealed niche-specific genome expansion in antibacterial Bacillus pumilus strain SF-4. Genes, 12: 1-19. https://doi.org/10.3390/genes12071060

Jechalke S, Heuer H, Siemens J, Amelung W, Smalla K (2014). Fate and effects of veterinary antibiotics in soil. Trends. Microbiol., 22: 536-545. https://doi.org/10.1016/j.tim.2014.05.005

Kohl KD (2012). Diversity and function of the avian gut microbiota. J. Comp. Physiol., 182: 591-602. https://doi.org/10.1007/s00360-012-0645-z

Łanocha-Arendarczyk N, Kosik-Bogacka DI (2019). Copper, Cu. In: Kalisińska E (eds) mammals and birds as bioindicators of trace element contaminations in terrestrial environments. Springer, Berlin, DEU. pp. 125-161. https://doi.org/10.1007/978-3-030-00121-6_4

Larsson DJ, Flach CF (2022). Antibiotic resistance in the environment. Nat. Rev. Microbiol., 20: 257-269. https://doi.org/10.1038/s41579-021-00649-x

Machado D, Perdigao J, Ramos J, Couto I, Portugal I, Ritter C, Boettger EC, Viveiros M (2013). High-level resistance to isoniazid and ethionamide in multidrug-resistant Mycobacterium tuberculosis of the Lisboa family is associated with inhA double mutations. J. Antimicrob. Chemother., 68: 1728-1732. https://doi.org/10.1093/jac/dkt090

Magoč T, Salzberg SL (2021). FLASH: Fast length adjustment of short reads to improve genome assemblies. Bioinformatics, 27: 2957-2963. https://doi.org/10.1093/bioinformatics/btr507

Martínez JL (2008). Antibiotics and antibiotic resistance genes in natural environments. Science, 321: 365-367. https://doi.org/10.1126/science.1159483

McKernan C, Benson T, Farrell S, Dean M (2021). Antimicrobial use in agriculture: Critical review of the factors influencing behaviour. JAC. Antimicrob. Resist., 3: 1-15. https://doi.org/10.1093/jacamr/dlab178

Olaru ID, Walther B, Schaumburg F (2023). Zoonotic sources and the spread of antimicrobial resistance from the perspective of low and middle-income countries. Infect. Dis. Poverty, 12: 1-5. https://doi.org/10.1186/s40249-023-01113-z

Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, Edwards RA, Gerdes S, Parrello B, Shukla M, Vonstein V (2014). The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). Nucleic. Acids. Res., 42: 206-214. https://doi.org/10.1093/nar/gkt1226

RStudio Team (2020). RStudio: Integrated development for R [Internet]. PBC, Boston, MA; [cited 2022]. Available at: http://www.rstudio.com

Sánchez-Virosta P, Espín S, García-Fernández AJ, Eeva T (2015). A review on exposure and effects of arsenic in passerine birds. Sci. Total Environ., 512: 506-525. https://doi.org/10.1016/j.scitotenv.2015.01.069

Sandoval R, Monteghirfo M, Salazar O, Galarza M (2019). Cross-resistance between isoniazid and ethionamide and its strong association with mutation C-15T in *Mycobacterium tuberculosis* isolates from Peru. Rev. Argent. Microbiol., 52: 36-42. https://doi.org/10.1016/j.ram.2019.03.005

Santos-Lopez A, Marshall CW, Haas AL, Turner C, Rasero J, Cooper VS (2021). The roles of history, chance, and natural selection in the evolution of antibiotic resistance. Elife, 10: 1-19. https://doi.org/10.7554/eLife.70676

Shaffer JP, Carpenter CS, Martino C, Salido RA, Minich JJ, Bryant M, Sanders K, Schwartz T, Humphrey G, Swafford AD, Knight R (2022). A comparison of six DNA extraction protocols for 16S, ITS and shotgun metagenomic sequencing of microbial communities. Biotechniques, 73: 34-46. https://doi.org/10.2144/btn-2022-0032

Sjölund M, Bonnedahl J, Hernandez J, Bengtsson S, Cederbrant G, Pinhassi J, Kahlmeter G, Olsen B (2008). Dissemination of multidrug-resistant bacteria into the Arctic. Emerg. Infect. Dis., 14: 70-72. https://doi.org/10.3201/eid1401.070704

Uruén C, Chopo-Escuin G, Tommassen J, Mainar-Jaime RC, Arenas J (2020). Biofilms as promoters of bacterial antibiotic resistance and tolerance. Antibiotics, 10: 3. https://doi.org/10.3390/antibiotics10010003

Wang J, Hong M, Long J, Yin Y, Xie J (2022). Differences in intestinal microflora of birds among different ecological types. Front. Ecol. Evol., 10: 1-13. https://doi.org/10.3389/fevo.2022.920869

Wolff KA, Nguyen L (2012). Strategies for potentiation of ethionamide and folate antagonists against Mycobacterium tuberculosis. Expert. Rev. Anti. Infect. Ther., 10: 971-981. https://doi.org/10.1586/eri.12.87

Zhao Y, Kumul C, Wang T, Mosusu N, Yao Z, Zhu Y, Zhang B, Wang X (2023). National-scale geochemical baseline and anomalies of chromium in Papua New Guinea. Minerals, 13: 1-14. https://doi.org/10.3390/min13020205