AL-KAUNIYAH: Jurnal Biologi, 18(1), 2025, 57-64
Website: http://journal.uinjkt.ac.id/index.php/kauniyah
P-ISSN: 1978-3736, E-ISSN: 2502-6720

IN SILICO EVALUATION REVEALS THE POTENTIAL RISK OF ANTIMICROBIAL RESISTANCE IN *Bifidobacterium*

EVALUASI IN SILICO MENGUNGKAPKAN POTENSI RISIKO RESISTENSI ANTIMIKROBA PADA *Bifidobacterium*

Anwar Rovik[1]*, Ahmad Reza Maulana[2], and Silmi Qurrotu Aini[3], Dyah Fitri Kushayarti[4]

[1]*Master Program in Biotechnology, The Graduate School of Universitas Gadjah Mada, Jl. Teknika Utara, Caturtunggal, Sleman 55281, Yogyakarta, Indonesia*
[2]*Master Program of Chemistry, Faculty of Mathematical and Natural Science, Universitas Gadjah Mada, Jl. Sains, Bulaksumur, Sleman 55281, Yogyakarta, Indonesia*
[3] *School of Life Sciences and Technology, Institute Technology of Bandung, Jl. Ganesa No.10, Coblong, Bandung 40132, Jawa Barat, Indonesia*
[4]*Department of Microbiology, Faculty of Biology, Universitas Jenderal Soedirman, Jl. Dr. Suparno No. 63, Purwokerto, Banyumas 53122, Jawa Tengah, Indonesia*
**Corresponding author:* anwarrovik@mail.ugm.ac.id

Submitted: 19 September 2023; Revised: 31 December 2023; Accepted: 4 March 2024

Abstract

Bifidobacteria have beneficial health effects for their hosts. However, they may acquire antibiotic-resistance genes. They may transfer antibiotic-resistance genes to pathogenic microbes in the human intestine, resulting in antibiotic-resistant pathogens. This study aimed to predict their resistance to antibiotics by analyzing the whole genome sequence. The entire genome data of *Bifidobacterium* spp. were obtained from the National Center for Biotechnology Information (NCBI). This study included five *Bifidobacterium* strains of human origin, five strains of animal origin, and three strains isolated from the environment. The genomic sequences were analyzed using ResFinder and CARD web service. Antibiotic-resistance genes were detected in *Bifidobacterium* spp. from all sample sources. *Bifidobacteria* were potentially resistant to various antibiotics, such as tetracycline, rifamycin, chloramphenicol, macrolide, lincosamide, streptogramin, and mupirocin-like antibiotics. This study suggests the safety of applying *Bifidobacterium* spp. as a potential probiotic.

Keywords: Antibiotic resistance; *Bifidobacterium*; Probiotic; Whole genome

Abstrak

Bifidobacteria memiliki efek kesehatan yang menguntungkan bagi inangnya. Namun, sel Bifidobacteria dapat memperoleh gen resistensi antibiotik. Hal ini memunculkan potensi transfer gen resistensi antibiotik ke mikroba patogen yang ditemukan di usus manusia yang mengakibatkan munculnya patogen yang resisten terhadap antibiotik. Penelitian ini bertujuan untuk memprediksi resistensi Bifidobacteria terhadap antibiotik dengan menganalisis seluruh urutan genomnya. Seluruh data genom Bifidobacterium spp. diperoleh dari National Center for Biotechnology Information (NCBI). Penelitian ini melibatkan lima strain Bifidobacterium yang diisolasi dari manusia, lima strain yang berasal dari hewan, dan tiga strain yang diisolasi dari lingkungan. Sekuens genom dianalisis menggunakan ResFinder dan layanan web CARD. Gen resistensi antibiotik terdeteksi pada Bifidobacterium spp. dari semua sumber sampel. Bifidobacteria berpotensi menjadi resisten terhadap berbagai antibiotik, seperti tetrasiklin, rifamisin, kloramfenikol, makrolida, linkosamida, streptogramin, dan mupirocin-like antibiotics. Penelitian ini menyarankan pertimbangan aspek keamanan dalam menggunakan Bifidobacterium spp. sebagai probiotik potensial.

***Kata Kunci**: Bifidobacterium; Probiotik; Resistensi antibiotik; Whole genome*

Permalink/DOI: http://dx.doi.org/10.15408/kauniyah.v18i1.34873

INTRODUCTION

Lactic acid bacteria (LAB) are known as safe bacteria that have beneficial health effects for their hosts, including *Bifidobacterium*. Various *Bifidobacterium* species reside in the gastrointestinal tract, mouth, non-human animal intestines, and dairy products (Hendrati et al., 2017; Kusharyati et al., 2020; Sakanaka et al., 2020; Sirilun et al., 2015). *Bifidobacterium* is one of the most commonly used probiotics because it produces various metabolites, such as lactic acid, H_2O_2, short-chain fatty acids, and bacteriocin. Consuming probiotics regularly as part of a healthy diet can help promote a healthy microbiome. A healthy microbiome is essential for many aspects of health, including digestion, immune function, cardiovascular health, and even mental health (Liu et al., 2016; Shreiner et al., 2015; Yan et al., 2017; World Health Organization, 2002).

Some LAB strains may acquire antibiotic-resistance genes through natural evolution or antibiotic exposure in the environment and food production chain. LAB can potentially develop and spread antibiotic-resistant genes (Duranti et al., 2017; Gueimonde et al., 2013; Zarzecka et al., 2022). Fermented foods can be a significant conduit for the spread of antibiotic-resistance genes from non-pathogenic microorganisms to humans. It results in the emergence of antibiotic-resistant pathogens. Therefore, EFSA suggested that only strains lacking acquired antibiotic-resistance genes (ARGs) can be used as starter cultures, probiotics, and feed additives (EFSA-FEEDAP Panel, 2018).

Bifidobacteria are intrinsically resistant to many antibiotics. Nunziata et al. (2022) reviewed that *Bifidobacteria* are typically regarded as susceptible to β-lactams, Gram-positive spectrum antibiotics, and broad-spectrum antibiotics at low doses. However, some *Bifidobacterium* were reportedly resistant to narrow and broad-spectrum antibiotics (Erginkaya et al., 2018; Rozman et al., 2020; Yasmin et al., 2020). This present study concerned the safety of applying *Bifidobacterium* spp. as a potential probiotic.

Genotypic methods have been explored as an alternative to phenotypic antimicrobial susceptibility testing (AST). However, phenotypic AST using disc diffusion and broth microdilution has many limitations. Some errors may arise during the culturing stage, inoculum preparations, and result interpretation (Florensa et al., 2022; Pedersen et al., 2018; Su et al., 2019). The findings between these two methods are not always equivalent, which may impede the results' repeatability (Rozman et al., 2020). Molecular-based resistance testing to antibiotics is usually done through PCR detection but with a limited number of genes. Whole genome sequences (WGS) based screening enhances the likelihood of discovering genes involved in antibiotic resistance. Understanding the resistance gene profiles of *Bifidobacterium* strains used in probiotics is crucial to ensure their safety. This study aimed to evaluate the genes associated with the probiotic properties of *Bifidobacterium* spp.

MATERIALS AND METHODS

The importance of studying antibiotic-resistance genes within *Bifidobacterium* strains from environmental, animal, and human samples cannot be understated. One of the critical reasons for studying ARGs in *Bifidobacterium* across diverse samples is to track the transfer of resistance. *Bifidobacterium* is known to engage in horizontal gene transfer, allowing resistance genes to move between different strains and species. The whole genome data of *Bifidobacterium* spp. was collected from The National Center for Biotechnology Information (NCBI, https://www.ncbi.nlm.nih.gov/). There are 107 available genomic data sets for *Bifidobacterium* spp. Only view data reported the source of *Bifidobacterium* isolates. Genome sequences are randomly selected from *Bifidobacterium* strains that have complete data and represent the source of the sample (Table 1). The data is downloaded in a fasta format (.fna).

This present study used five *Bifidobacterium* strains of human origin, five strains of animal origin, and three strains isolated from the environment. Thirteen whole genome sequences were analyzed for their resistance genes using ResFinder 4.1. It is available for free at the Center for Genomic Epidemiology's online platform at https://cge.food.dtu.dk/services/ResFinder/ (Babatunde et al., 2022; McArthur et al., 2013). ResFinder detects acquired genes and chromosomal alterations that mediate antimicrobial resistance in the bacteria's complete or partial DNA sequence. The second

analysis used the Comprehensive Antibiotic Resistance Database (CARD) web service at https://card.mcmaster.ca/analyze/rgi. It is a primary bacterial antimicrobial resistance knowledge resource and database that provides genotype analysis and phenotype prediction from curated publications and sequences (Alcock et al., 2023; Zankari et al., 2017). Using homology and SNP models, the Resistance Gene Identifier (RGI) can predict resistomes from protein or nucleotide data.

Table 1. Retrieved data for *Bifidobacterium* genome

Isolates	Host/Source	NCBI taxonomy id
Bifidobacterium longum subsp. *longum* JCM1217	Human	565042
Bifidobacterium breve	Human	1685
Bifidobacterium bifidum NCIMB 41171	Human	398513
Bifidobacterium dentium ATCC 27678	Human	473819
Bifidobacterium adolescentis	Human	1680
Bifidobacterium asteroides PRL2011	Animal	1147128
Bifidobacterium porcinum DSM 17755	Animal	1435463
Bifidobacterium pullorum	Animal	78448
Bifidobacterium ruminantium	Animal	78346
Bifidobacterium choerinum	Animal	35760
Bifidobacterium tibiigranuli	Environment	2172043
Bifidobacterium indicum LMG 11587	Environment	1341694
Bifidobacterium aquikefiri	Environment	1653207

RESULTS

Scientists have significantly advanced in utilizing whole genome sequencing to screen probiotic properties. By analyzing the WGS, this present study reported that *Bifidobacterium* spp. contains antibiotic-resistant genes (86.97% ≤ ID ≤ 100%). For example, those against the classes of tetracycline, rifamycin, macrolide, lincosamide, streptogramin A, streptogramin B, and mupirocin-like antibiotics (Table 2). The CARD analysis showed that antibiotic-resistant genes were found in *Bifidobacterium* spp. from all sample sources, i.e., humans, animals, and the environment.

Table 2. Analysis of antimicrobial resistance of *Bifidobacterium* spp. using a comprehensive antibiotic resistance database (CARD)

Isolates	ARO term	AMR gene family	Drug class	Resistance mechanism	% identity of matching region
Bifidobacterium longum	*Bifidobacterium adolescentis* rpoB mutants conferring resistance to rifampicin	Rifamycin-resistant beta-subunit of RNA polymerases (rpoB)	Rifamycin antibiotic	Antibiotic target alteration, antibiotic target replacement	92.65
Bifidobacterium breve	*Bifidobacterium adolescentis* rpoB mutants conferring resistance to rifampicin	Rifamycin-resistant beta-subunit of RNA polymerases (rpoB)	Rifamycin antibiotic	Antibiotic target alteration, antibiotic target replacement	91.55
	Bifidobacterium bifidum ileS conferring resistance to mupirocin	Antibiotic-resistant isoleucyl-tRNA synthetase (ileS)	Mupirocin-like antibiotic	Antibiotic target alteration	99.46
Bifidobacterium bifidum	ErmX	Erm 23S ribosomal RNA methyltransferase	Macrolide, lincosamide, streptogramin, streptogramin A, and streptogramin B antibiotics	Antibiotic target alteration	88.03
	Bifidobacterium adolescentis rpoB mutants conferring resistance to rifampicin	Rifamycin-resistant beta-subunit of RNA polymerases (rpoB)	Rifamycin antibiotic	Antibiotic target alteration, antibiotic target replacement	92.48
Bifidobacterium dentium	*Bifidobacterium adolescentis* rpoB	Rifamycin-resistant Beta-subunit of RNA polymerases (rpoB)	Rifamycin antibiotic	Antibiotic target Alteration,	90.56

Isolates	ARO term	AMR gene family	Drug class	Resistance mechanism	% identity of matching region
	mutants conferring resistance to rifampicin			antibiotic target replacement	
Bifidobacterium adolescentis	*Bifidobacterium adolescentis* rpoB mutants conferring resistance to rifampicin	Rifamycin-resistant beta-subunit of RNA polymerases (rpoB)	Rifamycin antibiotic	Antibiotic target alteration, antibiotic target replacement	100
Bifidobacterium asteroides	*Bifidobacterium adolescentis* rpoB mutants conferring resistance to rifampicin	Rifamycin-resistant beta-subunit of RNA polymerases (rpoB)	Rifamycin antibiotic	Antibiotic target alteration, antibiotic target replacement	93.17
	ErmX	Erm 23S ribosomal RNA methyltransferase	Macrolide, lincosamide, streptogramin, streptogramin A, and streptogramin B antibiotics	Antibiotic target alteration	90.14
Bifidobacterium porcinum	*Bifidobacterium adolescentis* rpoB mutants conferring resistance to rifampicin	Rifamycin-resistant beta-subunit of RNA polymerases (rpoB)	Rifamycin antibiotic	Antibiotic target alteration, antibiotic target replacement	89.19
Bifidobacterium pullorum	*Bifidobacterium adolescentis* rpoB mutants conferring resistance to rifampicin	Rifamycin-resistant beta-subunit of RNA polymerases (rpoB)	Rifamycin antibiotic	Antibiotic target alteration, antibiotic target replacement	93.5
	Tet(W)	Tetracycline-resistant ribosomal protection protein	Tetracycline antibiotic	Antibiotic target protection	97.03
Bifidobacterium ruminantium	*Bifidobacterium adolescentis* rpoB mutants conferring resistance to rifampicin	Rifamycin-resistant beta-subunit of RNA polymerases (rpoB)	Rifamycin antibiotic	Antibiotic target alteration, antibiotic target replacement	91.98
Bifidobacterium choerinum	*Bifidobacterium adolescentis* rpoB mutants conferring resistance to rifampicin	Rifamycin-resistant beta-subunit of RNA polymerases (rpoB)	Rifamycin antibiotic	Antibiotic target alteration, antibiotic target replacement	98.57
	Tet(W)	Tetracycline-resistant ribosomal protection protein	Tetracycline antibiotic	Antibiotic target protection	98.44
Bifidobacterium tibiigranuli	*Bifidobacterium adolescentis* rpoB mutants conferring resistance to rifampicin	Rifamycin-resistant beta-subunit of RNA Polymerases (rpoB)	Rifamycin antibiotic	Antibiotic target alteration, Antibiotic target replacement	90.27
Bifidobacterium indicum	*Bifidobacterium adolescentis* rpoB mutants conferring resistance to rifampicin	Rifamycin-resistant beta-subunit of RNA polymerases (rpoB)	Rifamycin antibiotic	Antibiotic target alteration, antibiotic target replacement	88.77
Bifidobacterium aquikefiri	*Bifidobacterium adolescentis* rpoB mutants conferring resistance to rifampicin	Rifamycin-resistant beta-subunit of RNA polymerases (rpoB)	Rifamycin antibiotic	Antibiotic target alteration, antibiotic target replacement	88.09

Note: The antibiotic resistance ontology (ARO) describes antibiotic resistance genes and mutations, their products, mechanisms, and associated phenotypes, as well as antibiotics and their molecular targets (Alcock et al., 2023)

Table 2 shows that only one genetic background was detected in *Bifidobacterium* spp. isolated from humans and the environment, i.e., the resistant gene of erythromycin (ErmX). Four genetic backgrounds associated with antibiotic resistance were detected in *Bifidobacterium* spp. from animal

origin. They were resistant genes for erythromycin (ermX), lincomycin (lnuC), chloramphenicol (cmX), and tetracycline (tetW). Surprisingly, four animal-derived *Bifidobacterium* strains have developed a resistance gene that expresses a tetracycline-resistant ribosome-protecting protein (tetW).

Another analysis showed that *Bifidobacterium* spp. is also potentially resistant to various antibiotic classes (Table 3). For example, those strains resist streptogramin B, lincosamide, macrolide, amphenicol, and tetracycline antibiotics. This present study detected ARGs of the amphenicol (i.e., chloramphenicol) and tetracycline classes in the *Bifidobacterium* strains of animal origin, which were broad-spectrum antibiotics. Most *Bifidobacterium* strains used in this study were resistant to broad (e.g., tetracycline) and narrow-spectrum (e.g., erythromycin) antibiotics. The Tet (tetracycline) and Erm (rifamycin) genetic background were found in *Bifidobacterium* spp. from all sample sources, i.e., animals, humans, and the environment.

Table 3. Analysis of antimicrobial resistance of *Bifidobacterium* spp. using ResFinder 4.1

Isolates	Antimicrobial	Class	Genetic background
Bifidobacterium longum	No resistance	No resistance	
Bifidobacterium breve	No resistance	No resistance	
Bifidobacterium bifidum	No resistance	No resistance	
Bifidobacterium dentium	Virginiamycin s	Streptogramin b	Erm(X) (erm(X)_X51472)
	Clindamycin	Lincosamide	Erm(X) (erm(X)_X51472)
	Quinupristin	Streptogramin b	Erm(X) (erm(X)_X51472)
	Erythromycin	Macrolide	Erm(X) (erm(X)_X51472)
	Pristinamycin ia	Streptogramin b	Erm(X) (erm(X)_X51472)
	Lincomycin	Lincosamide	Erm(X) (erm(X)_X51472)
Bifidobacterium adolescentis	No resistance	No resistance	
	Virginiamycin s	Streptogramin b	Erm(X) (erm(X)_U21300), erm(X) (erm(X)_X51472)
Bifidobacterium asteroids			
	Clindamycin	Lincosamide	Erm(X) (erm(X)_U21300), erm(X) (erm(X)_X51472)
	Lincomycin	Lincosamide	Erm(X) (erm(X)_U21300), lnu(C) (lnu(C)_AY928180), Erm(X) (erm(X)_X51472)
	Erythromycin	Macrolide	Erm(X) (erm(X)_U21300), erm(X) (erm(X)_X51472)
	Pristinamycin ia	Streptogramin b	Erm(X) (erm(X)_U21300), erm(X) (erm(X)_X51472)
	Chloramphenicol	Amphenicol	Cmx (cmx_U85507)
	Quinupristin	Streptogramin b	Erm(X) (erm(X)_U21300), erm(X) (erm(X)_X51472)
Bifidobacterium porcinum	No resistance	No resistance	
Bifidobacterium pullorum	Doxycycline	Tetracycline	Tet(W) (tet(W)_FN396364)
	Tetracycline	Tetracycline	Tet(W) (tet(W)_FN396364)
	Minocycline	Tetracycline	Tet(W) (tet(W)_FN396364)
Bifidobacterium ruminantium	No resistance	No resistance	
Bifidobacterium choerinum	Tetracycline	Tetracycline	Tet(W) (tet(W)_FN396364)
	Doxycycline	Tetracycline	Tet(W) (tet(W)_FN396364)
	Minocycline	Tetracycline	Tet(W) (tet(W)_FN396364)
Bifidobacterium tibiigranuli	No resistance	No resistance	
Bifidobacterium indicum	No resistance	No resistance	
Bifidobacterium aquikefiri	No resistance	No resistance	

DISCUSSION

Bifidobacteria are a group of beneficial bacteria commonly found in the human gut, particularly abundant in infants. They play a crucial role in maintaining a healthy gut microbiome by promoting digestion, inhibiting the growth of harmful bacteria, and boosting the immune system (Liu et al., 2016; Shreiner et al., 2015). Due to their health benefits, *Bifidobacterium* strains are increasingly used as probiotics in food products and dietary supplements. However, a concerning trend has emerged - the prevalence of antibiotic-resistance genes in commercially available *Bifidobacterium* strains. Among these, erythromycin and tetracycline-resistant genes were reported as the most prevalent in

various commercial *Bifidobacterium* strains (Cao et al., 2020; Rozman et al., 2020). This raises concerns about the potential impact on human health and the effectiveness of antibiotic treatment when these resistant bacteria are introduced into the gut.

The most common erythromycin resistance gene in *Bifidobacteria* is erm(X) (Table 2). This gene encodes an enzyme that modifies the antibiotic's target site, rendering it ineffective (Cao et al., 2020). Other less frequent genes include erm(B) and erm(A). Meanwhile, several tetracycline resistance genes have been identified in *Bifidobacteria*, including tet(W), tet(M), and tet(S). These genes encode different mechanisms for tetracycline resistance, such as ribosomal protection or drug efflux pumps that remove the antibiotic from the cell (Duranti et al., 2017; Gueimonde et al., 2013).

Tetracycline is one of the most extensively utilized antibiotics in veterinary and clinical settings (Indrawati et al., 2021; Peiris et al., 2017). Tetracycline resistance genes can be transferred horizontally between different bacterial species through conjugation, transduction, and transformation (Ding et al., 2023). Although conjugative plasmids are uncommon in *Bifidobacterium* spp., other mobile genetic elements (e.g., transposons) must be considered because the tet gene appears to be positioned on the chromosome. Tetracycline resistance genes can be co-located on the same mobile genetic elements (e.g., plasmids) as genes conferring resistance to other antibiotics or stressors (Gueimonde et al., 2013). It is surrounded by transposase-coding genes or transposase target sequences in some *Bifidobacterium* strains (Duranti et al., 2017; Gueimonde et al., 2013). Therefore, tetracycline resistance in *Bifidobacterium* spp. warrants special consideration.

Bifidobacterium is not limited to the human gut but also inhabits various environmental niches, such as soil and water. Antibiotic resistance in environmental *Bifidobacterium* strains may contribute to the overall environmental resistors. It potentially affects the persistence and spread of tetracycline resistance genes in natural ecosystems (Larsson & Flach, 2022). ARGs within *animal Bifidobacterium strains* can directly impact food safety and public health. In humans, *Bifidobacterium* species are essential members of the gut microbiome, contributing to overall health and well-being. ARGs within human-associated Bifidobacterium strains raise concerns about the potential transfer of resistance to pathogenic bacteria.

The resistance of *Bifidobacterium* strains to various antibiotics complicates their potential use as probiotics. Tetracycline resistance in *Bifidobacterium* can also lead to failure to treat bacterial infections. Monitoring tetracycline resistance in *Bifidobacterium* strains is essential to prevent failure to treat bacterial infections and maintain digestive health. Industry can select strains with low or no ARGs, minimizing the risk of transferring resistance to the host or other gut bacteria. This discovery also emphasizes the need for prudent and responsible antibiotic use in clinical settings and agriculture. The spread of antibiotic resistance among bacteria, including beneficial ones like *Bifidobacterium*, underscores the urgency of minimizing unnecessary antibiotic use in healthcare and agriculture.

Bifidobacteria can acquire new genes from other bacteria in their environment through horizontal gene transfer (Larsson & Flach, 2022). This allows them to gain resistance genes not originally part of their genome. The widespread use of antibiotics in agriculture and human medicine creates a selective pressure, favoring the survival and propagation of bacteria with resistance genes. These genes can then be transferred to *Bifidobacteria* in the gut. If individuals consuming these probiotics become infected with bacteria susceptible to antibiotics, e.g., erythromycin or tetracycline, the presence of resistant *Bifidobacteria* in their gut could hinder the effectiveness of the antibiotics. The resistant *Bifidobacteria* could transfer their resistance genes to pathogenic bacteria in the gut, further complicating antibiotic treatment.

CONCLUSION

Antibiotic-resistance genes were detected in *Bifidobacterium* spp. used in this study from different sample sources, i.e., humans, animals, and the environment. *Bifidobacterium* spp. is potentially resistant to various antibiotic classes, including narrow- and broad-spectrum antibiotics. Tetracycline and erythromycin resistance warrants special attention since *Bifidobacterium* strains are increasingly used as probiotics in food products and dietary supplements.

In silico evaluation can be used to assess the potential risk associated with using *Bifidobacteria* strains isolated from various sources, such as food products or environmental samples. Combining in silico evaluation with traditional laboratory methods and ongoing research can develop strategies to mitigate the risks associated with antibiotic resistance in *Bifidobacterium* and harness the full potential of these beneficial bacteria for promoting gut health.

ACKNOWLEDGEMENTS

We acknowledge all researchers and institutions that submitted the *Bifidobacterium* genomic sequence. We also acknowledge the developer of Resfinder and CARD.

REFERENCES

Alcock, B. P., Huynh, W., Chalil, R., Smith, K. W., Raphenya, A. R., Wlodarski, M. A., … McArthur, A. G. (2023). CARD 2023: Expanded curation, support for machine learning, and resistome prediction at the comprehensive antibiotic resistance database. *Nucleic Acids Research, 51*(D1), D690-D699. doi: 10.1093/nar/gkac920.

Babatunde, O. J., Okiti, A. F., Bayode, M. T., Babatunde, S. O., & Olaniran, A. M. (2022). Antibiogram profile prediction of selected bacterial strains by in silico determination of acquired antimicrobial resistance genes from their whole-genome sequence. *Bulletin of the National Research Centre, 46*(230). doi: 10.1186/s42269-022-00922-w.

Cao, L., Chen, H., Wang, Q., Li, B., Hu, Y., Zhao, C., … Yin, Y. (2020). Literature-based phenotype survey and in silico genotype investigation of antibiotic resistance in the genus Bifidobacterium. *Current Microbiology, 77*, 4104–4113. doi: 10.1007/s00284-020-02230-w.

Ding, D., Wang, B., Zhang, X., Zhang, J., Zhang, H., Liu, X., … Yu, Z. (2023). The spread of antibiotic resistance to humans and potential protection strategies. *Ecotoxicology and Environmental Safety, 254*, 114734. doi: 10.1016/j.ecoenv.2023.114734.

Duranti, S., Lugli, G. A., Mancabelli, L., Turroni, F., Milani, C., Mangifesta, M., … Ventura, M. (2017). Prevalence of antibiotic resistance genes among human gut-derived *Bifidobacteria*. *Applied Environmental Microbiology, 83*, e02894-16. doi: 10.1128/AEM.02894-16.

EFSA-FEEDAP Panel. (2018). Guidance on the characterization of microorganisms used as feed additives or as production organisms. *EFSA Journal, 16*(3), 5206. doi: 10.2903/j.efsa.2018.5206.

Erginkaya, Z., Turhan, E. U., & Tatli, D. (2018). Determination of antibiotic resistance of lactic acid bacteria isolated from traditional Turkish fermented dairy products. *Iran Journal of Veterinary Research, 19*, 53-56. doi: 10.22099/ijvr.2018.4769.

Florensa, A. F., Kaas, R. S., Clausen, P. T. L. C., Aytan-Aktug, D., & Aarestrup, F. M. (2022). ResFinder - an open online resource for identification of antimicrobial resistance genes in next-generation sequencing data and prediction of phenotypes from genotypes. *Microbial Genome, 8*(1), 000748. doi: 10.1099/mgen.0.000748.

Gueimonde, M., Sanchez, B., de los Reyes-Gavilan, C. G., & Margolles, A. (2013). Antibiotic resistance in probiotic bacteria. *Frontiers in Microbiology, 4*(202). doi: 10.3389/fmich.2013.00202.

Hendrati, P. M., Kusharyati, D. F., Ryandini, D., & Oedjijono. (2017). Characterization of *Bifidobacteria* from infant feces with different modes of birth at Purwokerto, Indonesia. *Biodiversitas, 18*(3), 1265-1269. doi: 10.13057/biodiv/d180352.

Indrawati, A., Khoirani, K., Setiyaningsih, S., Safika, U. A., & Ningrum, S. G. (2021). Detection of tetracycline resistance genes among Escherichia coli isolated from layer and broiler breeders in West Java, Indonesia. *Tropical Animal Science Journal, 44*(3), 267-272. doi: 10.5398/tasj.2021.44.3.267

Kusharyati, D. F., Pramono, H., Ryandini, D., Manshur, T. A., Dewi, M. A., Khatimah, K., & Rovik, A. (2020). *Bifidobacterium* from infant stool: The diversity and potential screening. *Biodiversitas, 21*(6), 2506-2513. doi: 10.13057/biodiv/d210623.

Larsson, D. G. K., & Flach, C. F. (2022). Antibiotic resistance in the environment. *Nature Reviews, 20*, 257. doi: 10.1038/s41579-021-00649-x.

Liu, Z., Roy, N. C., Guo, Y., Jia, H., Ryan, L., Samuelsson, L., … Young, W. (2016). Human breast milk and infant formulas differentially modify the intestinal microbiota in human infants and host physiology in rats. *Nutrients, 146*, 191-199. doi: 10.3945/jn.115.223552.

McArthur, A. G., Waglechner, N., Nizam, F., Yan, A., Azad, M. A., Baylay, A. J., … Wright, G. D. (2013). The comprehensive antibiotic resistance database. *Antimicrobial Agents and Chemotherapy, 57*(7), 3348–3357. doi: 10.1128/AAC.00419-13.

Nunziata, L., Brasca, M., Morandi, S., & Silvetti, T. (2022). Antibiotic resistance in wild and commercial non-enterococcal lactic acid bacteria and *Bifidobacteria* strains of dairy origin: An update. *Food Microbiology, 104*, 103999. doi: 10.1016/j.fm.2022.103999.

Pedersen, S. K., Wagenaar, J. A., Vigre, H., Roer, L., Mikoleit, M., Aidara-Kane, A., … Hendriksen, R. S. (2018). Proficiency of WHO Global Foodborne Infections Network external quality assurance system participants in the identification and susceptibility testing of thermotolerant *Campylobacter* spp. from 2003 to 2012. *Journal of Clinical Microbiology, 56*(11), e01066-18. doi: 10.1128/JCM.01066-18.

Peiris, C., Gunatilake, S. R., Mlsna, T. E., Mohan, D., & Vithanage, M. (2017). Biochar based removal of antibiotic sulfonamides and tetracyclines in aquatic environments: A critical review. *Bioresource Technology, 246*, 150-159. doi: 10.1016/j.biortech.2017.07.150.

Rozman, V., Lorbeg, P. M., Acetto, T., & Matijasic, B. B. (2020). Characterization of antimicrobial resistance in lactobacilli and *Bifidobacteria* used as probiotics or starter cultures based on integration of phenotypic and in silico data. *International Journal of Food Microbiology, 314*, 108388. doi: 10.1016/j.ijfoodmicro.2019.108388.

Sakanaka, M., Gotoh, A., Yoshida, K., Odamaki, T., Koguchi, H., Xiao, J-Z., … Katayama, T. (2020). Varied pathways of infant gut-associated Bifidobacterium to assimilate human milk oligosaccharides: prevalence of the gene set and its correlation with *Bifidobacteria*-rich microbiota formation. *Nutrients, 12*(71). doi: 10.3390/nu12010071.

Shreiner, A. B., Kao, J. Y., & Young, V. B. (2015). The gut microbiome in health and disease. *Current Opinion on Gastroenterology, 31*(1), 69-75. doi: 10.1097/MOG.0000000000000139.

Sirilun, S., Takahashi, H., Boonyaritichaikij, S., Chaiyasut, C., Lertruangpanya, P., Koga, Y., & Mikami, K. (2015). Impact of maternal *Bifidobacteria* and the mode of delivery on *Bifidobacterium* microbiota in infants. *Beneficial Microbes, 6*, 767-774. doi: 10.3920/BM2014.0124.

Su, M., Satola, S. W., & Read, T. D. (2019). Genome-based prediction of bacterial antibiotic resistance. *Journal of Clinical Microbiology, 57*(3), e01405-18. doi: 10.1128/JCM.01405-18.

World Health Organization. (2002). *Guidelines for the evaluation of probiotics in food*. London: FAO/WHO Working Group.

Yan, S., Zhao, G., Liu, X., Zhao, J., Zhang, H., & Chen, W. (2017). Production of exopolysaccharide by *Bifidobacterium longum* isolated from elderly and infant feces and analysis of priming glycosyltransferase genes. *RSC Advance, 7*, 31736–31744. doi: 10.1039/C7RA03925E.

Yasmin, I., Saeed, M., Khan, W. A., Khaliq, A., Chughtai, M. F. J., Iqbal, R., … Tanweer, S. (2020). In vitro probiotic potential and safety evaluation (Hemolytic, cytotoxic activity) of bifidobacterium strains isolated from raw camel milk. *Microorganisms, 8*(3), 354. doi: 10.3390/microorganisms8030354.

Zankari, E., Allesoe, R., Joensen, K. G., Cavaco, L. M., Lund, O., & Aarestrup, F M. (2017). PointFinder: A novel web tool for WGS-based detection of antimicrobial resistance associated with chromosomal point mutations in bacterial pathogens. *Journal of Antimicrobial Chemotherapy, 72*, 2764-2768. doi:10.1093/jac/dkx217.

Zarzecka, U., Chajęcka-Wierzchowska, W., & Zadernowska, A. (2022). Microorganisms from starter and protective cultures-occurrence of antibiotic resistance and conjugal transfer of tet genes in vitro and during food fermentation. *LWT-Food Science and Technology, 153*, 112490. doi: 10.1016/j.lwt.2021.112490.