Research Article

Analysis of Diversity and Distribution of Antibiotic Resistance Genes in Holstein Friesian Dairy Cattle Feces by Shotgun Metagenomic Approach

Azhar Ali[1], Faheem Ahmed Khan[2], Wu Di[3], Huang Chunjie[3], Muhammad Rizwan Yousaf[1], Bilal Ahmed[1], Farwa Shakeel[1], Nuruliarizki Shinta Pandupuspitasari[1], Windu Negara[2], Bambang Waluyo Hadi Eko Prasetiyono[1]*

[1]Department of Animal Science, Faculty of Animal and Agricultural Sciences, Universitas Diponegoro, Semarang, Indonesia; [2]Research Center for Animal Husbandry, National Research and Innovation Agency, Jakarta Pusat 10340, Indonesia; [3]Institute of Reproductive Medicine, School of Medicine, Nantong University, Nantong 226001, China.

Azhar Ali and Faheem Ahmed Khan are contributed equally to this work.

Abstract | Antibiotic resistance is a significant public health issue, and animals have a vital role in the development and preservation of antibiotic-resistant bacteria. This work employed shotgun metagenomics to examine antibiotic resistance genes (ARGs) present in the feces of dairy cattle. The presence of resistance genes in the feces indicates that certain microorganisms carry several resistance genes, which are often found in close proximity and originate from various species. Our findings indicates that the gastrointestinal system of dairy cows harbors a repertoire of 216 antimicrobial resistance genes, which are presumed to confer resistance to 23 distinct antibiotics. The identified ARGs were associated with tetracyclines, macrolides, bacitracin, quinolones, β-lactams, and aminoglycosides, which correspond to antibiotics often administered to both animals and humans. An important discovery in this study is the recognition of Enterobacteriaceae as the predominant family in the phylum Proteobacteria, together with enterococcus species from the phylum Firmicutes, which possess numerous antibiotic resistance genes associated with health risks. The presence of antibiotic-resistant Gram-negative and Gram-positive bacteria underscores the significance of using suitable antibiotic therapy to minimize the dissemination of resistance in both animals and humans.

Keywords | ARGs, Shotgun metagenomics, Dairy cows, Antibiotics

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***Correspondence** | Bambang Waluyo Hadi Eko Prasetiyono, Department of Animal Science, Faculty of Animal and Agricultural Sciences, Universitas Diponegoro, Semarang, Indonesia; **Email:** bambangwhep@ymail.com
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INTRODUCTION

Antibiotics, a medical breakthrough that has significantly impacted healthcare and prevented numerous fatalities, are widely employed for the prevention and treatment of bacterial infections. However, excessive utilization of antibiotics in healthcare, animal farming, and agriculture has led to the presence of antibiotic residues that affect plants, the environment, animals, and humans (Shen *et al.*, 2023). In 2013, the FDA disclosed that around

14.8 million kilograms of antibiotics were provided to animals. China annually produces more than 210,000 tons of antibiotics, with livestock contributing to around 46% of this amount (Su *et al*., 2014). Estimates indicate that antimicrobial resistance (AMR) leads to approximately 25,000 deaths annually in the European Union (EU) and 700,000 deaths globally. Moreover, it is projected that antimicrobial resistance (AMR) would surpass cancer in terms of lethality by the year 2050 (Zalewska *et al*., 2021). Regular antibiotic usage enhances the growth and results in the dissemination of antibiotic-resistant genes (ARGs) and bacteria in many environments. ARGs (Antibiotic Resistance Genes) and ARBs (Antibiotic-Resistant Bacteria) are prevalent in natural ecosystems, and human activities significantly contribute to their emergence and dissemination (Santos *et al*., 2022). Although pathogenic bacteria are becoming less susceptible to treatments, nosocomial infections that are currently manageable with antibiotics may develop into untreatable diseases in the future. Consequently, healthcare systems are now confronted with an increased financial strain. Nevertheless, this trend continues until the year 2050 and carries the possibility of being utilized during a time when antibiotics are no longer effective, known as the post-antibiotic age. In this era, even minor illnesses and accidents might contribute to a rise in human mortality (Gupta *et al*., 2018). The issue of antimicrobial resistance has been recognized as a significant danger to the well-being of both humans and animals, necessitating careful handling (Aarestrup, 2005). Antibiotics are categorized into various groups, including macrolides, penicillin, aminoglycosides, arsenicals, sulfonamides, and β-lactams (Sharma *et al*., 2016). Antibiotics are employed in agriculture as biocides to control pests on vegetable and fruit crops, for veterinary treatment, and as additions in poultry and cow feed (Sharma *et al*., 2016). According to Huang *et al*. (2019), previous studies indicate that livestock farming techniques can lead to higher levels of biomass and the presence of antibiotic resistance genes (ARGs) in the environment.

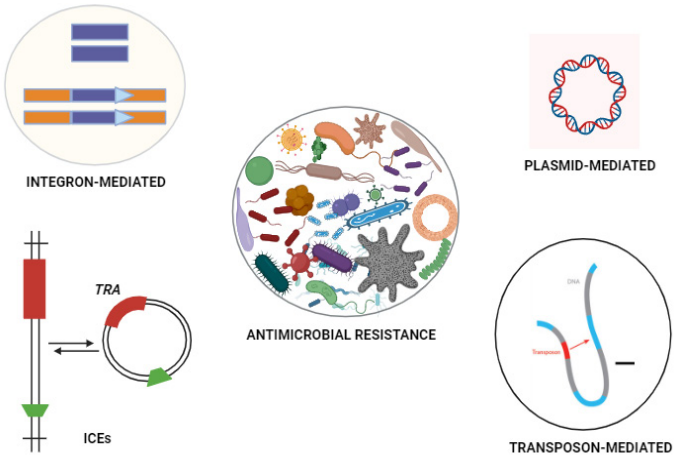

Figure 1: Heritable antimicrobial resistance.

Bacterial resistance to antibiotics is acquired by genetic alterations in the DNA. ARGs often spread by horizontal gene transfer. ARGs present in MGES (mobile genetic elements) (Inda-Díaz *et al*., 2023) facilitate the transfer of genetic material within the bacterial genome (Che *et al*., 2019), as depicted in Figure 1. These MGES, such as conjugative plasmids, integrative elements, integrons, and transposons, play a crucial role in the dissemination of genetic material among various bacterial cells, including both non-pathogenic and commensal bacteria, as well as pathogenic strains (Inda-Díaz *et al*., 2023).

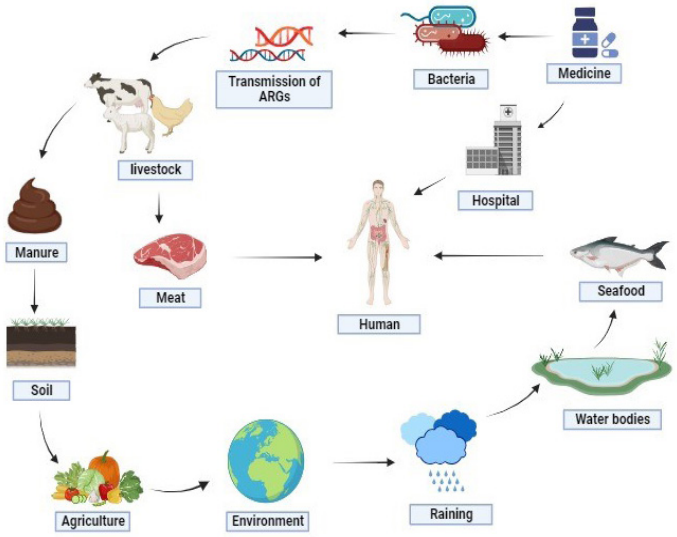

Figure 2: Illustrates the propagation of antibiotic resistance genes (ARGs). ARGs can be disseminated through diverse mediums, such as soil, water bodies, animals, and medications. This presents a substantial hazard to the health of both animals and humans.

Moreover, mobile genetic elements with additional genes possess the capacity to disseminate over numerous microbial taxonomic groups in soil. This phenomenon acts as a conduit for the transmission of genes that confer resistance to antibiotics, connecting the soil surface to the deeper layers of the soil. This process has the potential to affect human populations, as demonstrated in Figure 2. Wastewater treatment plants (WWTPs) are constructed with the objective of removing organic compounds, nutrients, and disease-causing agents (pathogens) found in wastewater. These contaminants can pollute the ecosystem and pose a substantial threat to human well-being. Nevertheless, as a result of the extensive utilization of antibiotics, wastewater has transformed into an ecological repository for antibiotic-resistant bacteria (ARBs) and antibiotic resistance genes (ARGs) (Cheng *et al*., 2021). Nevertheless, the biological processing of microorganisms leads to an increase in the reproduction and spread of antibiotic resistance genes (ARGs) in wastewater treatment plants (WWTPs), resulting in the accumulation of both ARGs and antibiotics (Shen *et al*.,

2023). Hospital wastewater (HWW) contains a diverse range of large and small pollutants, such as waste from clinical laboratories, surgical procedures, pharmaceuticals, nutritional solutions, and disinfectants. Bacterial toxins present in hospital wastewater can persist in aquatic environments for prolonged durations, hence presenting significant health hazards to both humans and animals (Manoharan *et al*., 2021). Moreover, wastewater treatment plants (WWTPs) routinely gather wastewater from several origins, each possessing its unique chemical makeup and bacterial population. The quantities of resistant bacteria in wastewater treatment may fluctuate in direct proportion as a consequence. However, there is a lack of extensive research on the temporal and geographical patterns of both the resistome (collection of antibiotic resistance genes) and the bacterial population during the wastewater treatment process, especially on a large-scale sampling basis (An et al., 2018).

Prior research employed diverse methodologies to investigate genetic arrangements, including Mobile Genetic Elements (MGEs) and Antibiotic Resistance Gene (ARG) hosts, such as high-throughput sequencing with isolation linked, concatenation PCR, and epicPCR. Utilizing whole-genome sequencing, together with the isolation of pure cultures, is still an essential method for comprehending the correlations between genotypes and phenotypes of antibiotic-resistant bacteria (ARB) and identifying associated mobile genetic elements (MGEs) (Che *et al*., 2019). Metagenomic approaches have revealed clear functional profiles of antibiotic resistance genes (ARGs), as well as patterns of interaction between host bacteria and ARGs (Manoharan *et al*., 2021). Metagenomics is presently employed to investigate undiscovered and uncharacterized antibiotic resistance genes (ARGs) from publicly accessible datasets in order to advance our comprehension of the recognized, emergent, and dormant resistome (Davis *et al*., 2023). The aim of this study was to conduct a thorough analysis of the antibiotic resistance gene (ARG) profile in dairy cow fecal samples using shotgun metagenomics. Additionally, we aimed to determine the diversity and prevalence of ARGs across various antibiotics. Furthermore, we sought to assess the taxonomic composition of multiple bacterial communities observed in our investigation.

MATERIALS AND METHODS

Animal selection and metagenomic analysis

Managed Animal farms from various areas in Pakistan, namely Salt Range, Chakwal, Patoki, and animals kept by local farmers, were selected for inclusion in the study. We selected five healthy Holstein Friesian cows for fecal collection from each region. Dairy cow fecal samples were collected in DNA /RNA shield tubes and kept in the refrigerator at -20 degrees Celsius. We pooled the samples of five dairy cows from each location, homogenized, and DNA was extracted using a QIAMP Fast DNA Mini Stool Extraction Kit. The quality of the extracted DNA was assessed. Quantification of the sample was conducted using a Qubit fluorometer, and its integrity was validated using agarose gel electrophoresis. Genomic DNA was subjected to approximately 40 min at the 100V with 1% agarose gel electrophoresis, whereas PCR products were run for approximately 50 mints at 80V with 2% agarose gel. Following the quality examination of DNA, end-repair was used to establish a DNA genomic library, in addition to PCR amplification and A tail. For measuring library concentrations 2.0 Qubit was utilized, those then diluted for insert size verification in 2 ng/ul with the use of Agilent-2100 system. qPCR was used to maintain library quality. Libraries that successfully met the quality criteria were sequenced on Novaseq 6000.

ARG identification

We employed Resistance Gene Identifier software to annotate antibiotic-resistant genes in non-redundant gene sets. This annotation process involved comparing the genes to the Comprehensive Antibiotic Resistance Database (CARD). The results of this annotation were used to evaluate the species associated with ARGs.

RESULTS AND DISCUSSION

Resistance genes identification

A total of 23 ARG classes and 216 ARGs were identified in our samples are aminoglycoside, multidrug resistance, peptide antibiotic, rifamycin, bacitracin, chloramphenicol antibiotic, beta-lactamase, tetracyclines, fluoroquinolones, erythromycin, macrodile, lincosamide, glycopeptide, aminocoumarin, cephalosporin, carbapenem, and sulfonamide are more abundant and broadly distributed in these samples, but phosphonic acid fusidane was detected at very low levels. The predominant mechanisms of antibiotic resistance include antibiotic efflux (50%), alteration of antibiotic targets (11.3%), and antibiotic inactivation (33%) (Santos *et al*., 2022).

Sub-type profile of ARGs

Abundant summary of sub type of antibiotic resistance genes within dairy cows fecal samples 216 (ARGs) were identified in which 17 classes of ARGs are aminoglycoside (AAC_6'-Iad AAC_6'-Ie-APH_2"-Ia, aadA, ANT_9-Ia, ANT_9-Ia, APH_2"-IIa, APH_3'-IIIa, APH_6-Id, baeR, aad 1) , multidrug (abeM, abeS, acrD, AcrE, AcrF, AcrS, adeI, arlR), Peptide (arnA, eptA, rosB, ugd), rifamycin (arr-1, rpoB2), bacitracin (bacA, bcrA), chloramphenicol (catB8, optrA), Carbapenem (golS), fluoroquinilones

(efrA, efrB, emrA, emrB, emrR, pmrA), cephalosporin (OXA-2, TEM-1), class A beta lactamase (CfxA3,), Phenicol antibiotic (floR) tetracycline (tet_D, tetO, tetB_P, tet_W/N/W, tet32,), erythromycin (ErmF),Macrolide (macB mefA, mefC), lincosamide (lnuC, lnuD, lsaB, lsaE), aminocoumarin (mdtB mdtB , mdtC, mdEt, mdtF mdtH, mdtM, mdtN, mdtO) , sulfonamide (sul 4) and glycopeptide (vanRF, vanRG, vanSA, vanUG, vanYG1) are predominated distributed in all samples as shown in Figure 3A.

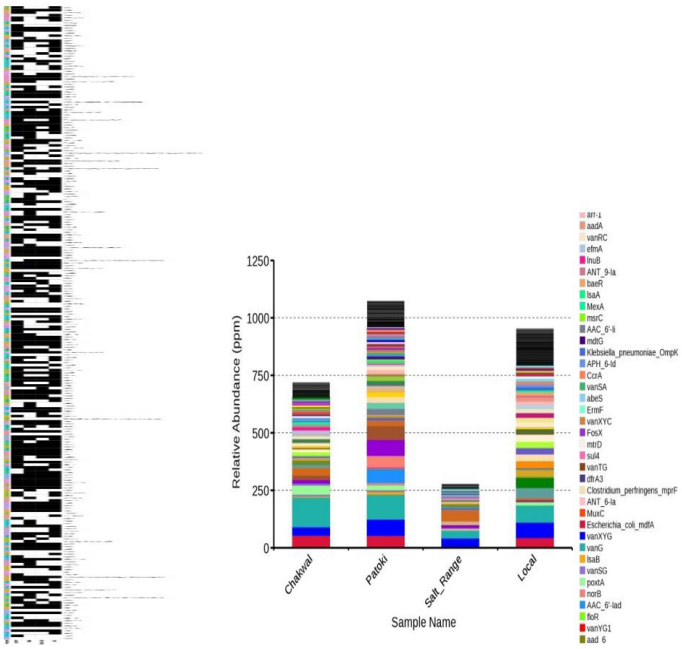

Figure 3: (**A**) Prevalence of ARGs. The presence of a specific ARG is represented by black box corresponding to each of the samples, while the white boxes for each sample indicate the absence of corresponding ARGs. (**B**) The Relative abundance of ARG. The distribution of ARGs within the samples.

While the relative abundance showed that VanYG1 was present in equal abudance in Chakwal and patoki, as compared to local but was absent in salt range. In addition, vanXYG exibit equal amounts in patoki and local followed by lower amount in Chakwal and salt range. The relative abundance of vanG is greater in Chakwal, followed by patoki and local while the abundance is low in salt range. The vanXYC frequency present only in the salt range but absent in in all other samples. The vanTG relative abundance is higher in Patoki, with minimal presence in Chakwal and the local region, and it is absent in the Salt Range. The relative abundance of poxtA gene is high in Chakwal as compared to Patoki and local region while absent in salt range. The relative abundance of FosX is higher in Patoki whereas less abundance in Chakwal and salt range but absent in local. The relative abundance of msrc is high in local followed by Chakwal while absent in patoki and salt range. The APH_6-lad showed higher abundance only in Patoki region but absent in all other samples. The relative abundance of add 6 shown only in local region while absent in all other samples. The relative abundance of AAC_6-lad present only in patoki while absent in all other samples as shown in Figure 3B.

Table 1: Different mechanism related to antibiotics and their encoding genes.

Class of antibiotic	Encoding resistance genes	Mechanism of resistance
Aminoglycoside	aadA,	Antibiotic inactivation
	AAC_6'-Iad	Antibiotic inactivation
	AAC_6'-Ie-APH_2''-Ia	Antibiotic inactivation
Multidrug resistance	abeM	Antibiotic efflux
	abeS	Antibiotic efflux
	arlR	Antibiotic efflux
Peptides	arnA	Antibiotic target *al*teration
	eptA	Antibiotic target *al*teration
	rosB	Antibiotic efflux
Rifamycin	arr-1	Antibiotic inactivation
	rpoB2	Antibiotic target *al*teration
Bacitracin	bacA	Antibiotic target *al*teration
	bcrA	Antibiotic efflux
Chloramphenicol	optrA	Antibiotic target *al*teration
	catB8	Antibiotic inactivation
Tetracycline	tet_D	Antibiotic efflux
	tet_O	Antibiotic target *al*teration
	tet_32	Antibiotic target *al*teration
Sulfonamide	sul 4	Antibiotic target *al*teration
Cephalosporin	OXA-2	Antibiotic inactivation
	TEM-1	Antibiotic inactivation
Class A beta lactamase	CfxA3	Antibiotic inactivation
Macrolide	macB	Antibiotic efflux
	mefA,	Antibiotic target *al*teration
Glycopeptide	vanRG,	Antibiotic target *al*teration
	vanSA	Antibiotic target *al*teration

The shotgun metagenomic approach was used to investigate fecal resistomes and bacterial populations, as well as the impact of antibiotic selection pressure on dairy cows. We hypothesized that the diversity of drug resistance in the gut microbiota of dairy cows could be linked to various antibiotic selection pressures (Xiong *et al*., 2018). The animals involved in this study were not administered antibiotics, hence reinforcing the notion of a widespread occurrence of antimicrobial resistance (AMR). However, the intestinal microbiota continues to serve as a natural source for antibiotic resistance genes (ARGs) (Liu *et al*.,

2019). The utilization of antimicrobials in animal feed has experienced a significant surge due to the reduction in manufacturing and production expenses. Administering subtherapeutic doses has led to a notable enhancement in animal growth rates and feed efficiency, together with a reduction in mortality rates (Xiong *et al*., 2018). Moreover, researchers have found that the improper use of antibiotics in agricultural practices can apply selection force, potentially promoting the development of drug resistance and the continued existence of resistance genes in the gastrointestinal system (Wang *et al*., 2021). Consistently, these numerous antibiotic-resistance genes are often associated with antibiotics that are routinely utilized in veterinary and human healthcare (Li *et al*., 2015). In a previous investigation of cattle feces, scientists found a significant presence of bacitracin, multidrug, and MLS resistance genes. The detection of antibiotic resistance genes (ARGs) in chicken feces suggests the misuse of antibiotics and the specific effects of these drugs (Manoharan *et al*., 2021). Gaeta *et al*. (2020) study examined the frequency of resistance mechanisms to beta-lactams, aminoglycosides, glycopeptides, tetracyclines, multi-drug resistance, macrodile-lincosamide-streptogramin B, and mupirocin. In our investigation, the most prevalent antibiotics were tetracycline, glycopeptide, aminoglycosides, fluoroquinolones, and beta-lactams. The most common forms of antibiotic resistance genes (ARGs) found in dairy and meat cattle, as determined by metagenomic analysis, were β-lactam, quinolone, and tetracycline resistance genes. The resistance function of these genes has been identified in several environments, including soils, animals, and humans (Wang *et al*., 2021). ErmF genes are detected in Chakwal, patoki, salt range and local region, while Sul-1 is present in Chakwal Salt Range, dairy cows of local farmers and absent in Patoki. Waseem *et al*. (2019) reported these genes in the poultry fecal gut microbiota previously in Pakistan. Tetracycline resistance was more prevalent, especially among Bacteroidetes species, Enterobacteriaceae, and staphylococci (Call *et al*., 2008). The tetA_48 gene was previously identified in animal feces samples, and it had identical genetic characteristics to the genes found in the Chakwal and Salt Range regions. Nevertheless, it was not detected in Patoki or its neighboring regions (Chakraborty *et al*., 2020). Moreover, the tetO (Table 1) gene exhibited a widespread distribution in our samples, and these genes are accountable for the production of ribosomal protective proteins (Zhuang *et al*., 2021). The tetO (Table 1) gene is the most commonly found resistance gene, responsible for conferring resistance to tetracycline. Moreover, previous studies have identified tetO (Table 1) as the sole resistance-associated element linked to E. The reference is "hirae" as mentioned in the study by Beukers *et al*. (2017). We have identified optrA (Table 1), a newly found gene that confers resistance to multiple essential medications. The optrA gene confers resistance to both phenicols and oxazolidinones (Liu *et al*., 2019). In addition, optrA exhibited resistance to essential therapies such as linezolid, a frequently employed treatment for gram-positive infections such as VRE (vancomycin-resistant enterococci) and MRSA (methicillin-resistant Staphylococcus aureus). The study conducted by Wang *et al*. (2015). The presence of the ermB gene is quite prevalent in our samples. ErmB confers resistance to macrolides (Caudle *et al*., 2014). Moreover, a study conducted by Zalewska *et al*. (2021) revealed that lincosamides and macrolides are frequently employed in the treatment of prevalent bovine ailments such as mastitis. The bcrA gene (Table 1), renowned for its elevated prevalence, had a notably widespread occurrence in our collected samples. Ninety-nine percent of this gene, which imparts resistance to bacitracin, was discovered in human excrement, indicating substantial human impact on the surrounding ecosystem (Chakraborty *et al*., 2020). Prior studies have demonstrated that bacA (Table 1) is among the most notable genes associated with targ*et al*teration antimicrobial resistance genes (ARGs). BacA (Table 1) efficiently neutralizes the inhibitory effects of bacitracin on the dephosphorylation of isoprenyl pyrophosphate (Santos *et al*., 2022). In the context of mechanisms for modifying the intended targets, previous research reveals that bacA stands out as one of the predominant genes associated with target alteration antimicrobial resistance genes (ARGs). bacA effectively counteracts the inhibitory effect of bacitracin on the dephosphorylation of isoprenyl pyrophosphate (Santos *et al*., 2022). Significantly, this form of resistance has been associated with the most common subtype in each study sample, namely the ARG subtype macB (Table 1). Additionally, influent samples contained arlR (Table 1), CpxR, and genes, while effluent samples contained golS and evgS genes. It is worth noting that all of these genes were present in high quantities in all four samples (Gupta *et al*., 2018). Dairy samples exhibit a higher prevalence of aminoglycoside acetyltransferases (Noyes *et al*., 2016). Wastewater included a high abundance of genes that exhibited resistance to specific aminoglycosides, specifically ANT_3', ANT_6-la, and APH_3'–IIIa. The effluent discharge into the river had a substantial impact on augmenting the relative prevalence of these genes in the river water. The presence of these resistance genes has also been identified in our samples. Moreover, the occurrence of mobile genetic elements (MGEs) such as insertion sequences, integrons, and plasmids exhibited notable differences between samples of wastewater and river water (Jia *et al*., 2017). However, the disinfection process found the presence of multidrug, bacitracin, and beta-lactam, which may have the capacity to decrease antibiotic resistance genes in drinking water (Jia *et al*., 2020). Nevertheless multidrug, bacitracin and beta-lactam were identified after disinfection and this could potentially

control antibiotic resistance genes within consumption water (Jia *et al*., 2020).

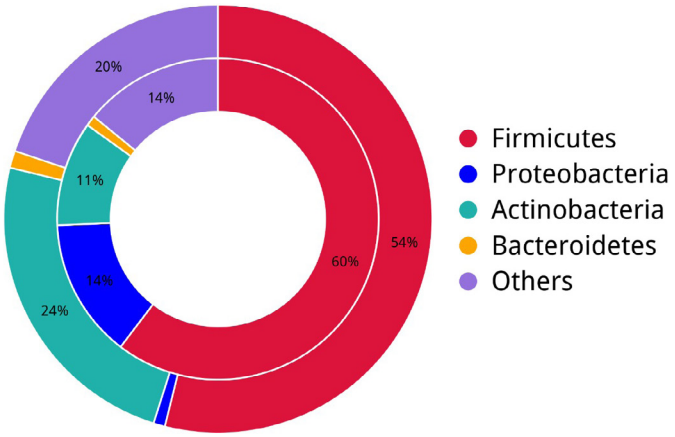

Figure 4: The figure presents a collective representation and diversity of bacterial phylum across all samples by Donut chart.

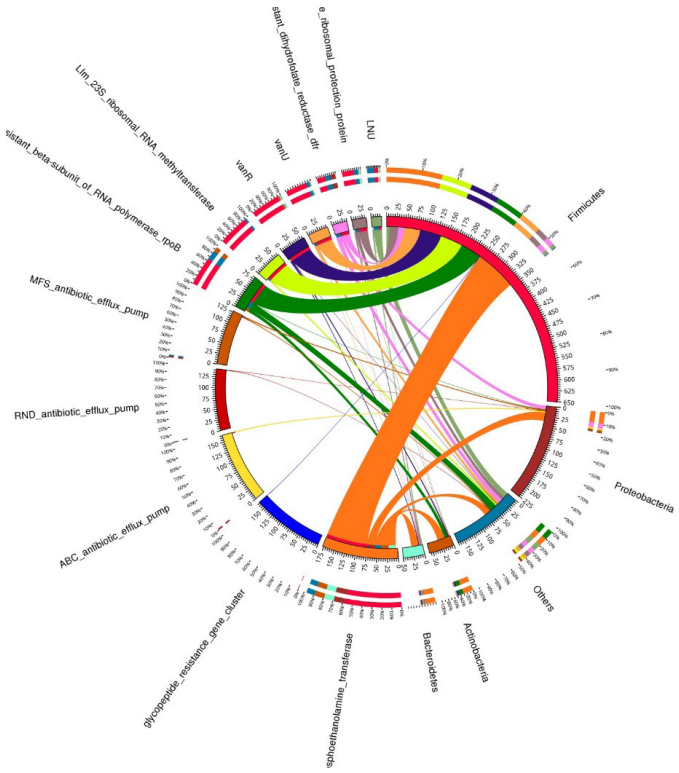

Figure 5: A circular diagram shows antibiotic resistance genes and metabolic processes at the bacterial phylum level in different sample of fecal gut microbiota of dairy cows. The left side focuses on metabolic activities while the right side of the representation highlights bacterial phyla. The widths of the inner and outer circles signify relative abundances, where inner circle colors indicate cumulative abundances by bacterial phylum at right and metabolic process at left side. Outer circle colors represent the relative abundances of processes of bacterial phylum at right side and metabolic process phyla at left side.

Recent research has demonstrated that mobile genetic elements (MGEs) are significant contributors to the development of resistance to tetracycline and quinolone antibiotics. Moreover, a correlation has been identified among these antibiotic resistance genes (ARGs), potentially leading to an escalation in multidrug resistance (Wang *et al*., 2021). The presence of the resistome and metabolic process has been observed in various organisms, including Actinobacteria, Firmicutes, Bacteroidetes, and Proteobacteria as shown in Figure 4 (Faden, 2014). The metabolic process signifies to dominant resistance mechanisms of antibiotic such as antibiotic efflux alteration of antibiotic targets and antibiotic inactivation as shown in Figure 5.

The Acinetobacter genus was detected in a substantial proportion in our samples. Acinetobacter has been found to exhibit resistance to cephalosporins, a class of beta-lactam antibiotics, as demonstrated by various experiments (Pak and King, 2022). Enterococcus faecium and Enterococcus faecalis are the primary causative agents of the majority of health-related illnesses (Beukers *et al*., 2017). Scientists have determined that bovine feces contain *E. coli*. Faecium and E. are the subjects of discussion. According to Zalewska *et al*. (2021), faecalis may be a potential risk. Curiously, a single study discovered that *E. enterococcus* faecalis, a prevalent source of bovine mastitis, is frequently found in feces and natural bedding materials and acts as an opportunistic pathogen in the mammary glands. The citation is from Hoque *et al*. (2022). However, Enterococci and *Escherichia coli* serve as indicators of fecal contamination. The presence of these microorganisms is prevalent in the human gastrointestinal tract, and they are also linked to diseases in both animals and humans (Łuczkiewicz *et al*., 2010). Furthermore, *E. coli* strains that produce ESBL bacteria exhibit resistance to cefepime and fourth-generation cephalosporins, indicating a connection between wastewater treatment and resistance patterns. However, concerns persist over the finding of carbapenem-resistant Enterobacteriaceae in farm animals. CRE poses a significant health risk to humans due to its connection with the overuse of antibiotics in livestock farming. ESBL-producing enteric bacteria, particularly those resistant to third-generation cephalosporins, are currently mostly treated with carbapenems (Zhuang *et al*., 2021). Avoparcin, a glycopeptide antibiotic utilized in animal agriculture to enhance growth, is believed to significantly contribute to the emergence of vancomycin resistance. The rise of vancomycin resistance, namely in medically significant bacterial strains like Enterococcus and Staphylococcus species, presents a substantial obstacle in the field of human medicine (Zalewska *et al*., 2021). The prevalence of Escherichia coli ampC beta-lactamase enzymes was observed in all samples, including Patoki, Chakwal, salt range, and local. Plasmid-borne AmpC is a beta-lactamase enzyme found in Enterobacteriaceae

bacteria that breaks down cephalosporins and provides resistance to antibiotics. AmpC overexpression confers resistance to carbapenems, oxyimino-cephalosporins, and penicillins. The enzyme is commonly encoded by closely coordinated chromosomal genes and has been detected in multiple species of Enterobacteriaceae (Su *et al*., 2014). In a previous study, multi-drug resistance (bla-TEM) was identified in the wastewater environment of Pakistan (Saima *et al*., 2020) The blaCTX-M genes are commonly found on plasmids, but their occurrence on chromosomes is increasing. The isolates in the dataset under investigation had a uniform distribution of chromosomes and plasmids. Both the chromosomal and plasmid variants of the blaCTX–M–15 gene were discovered in pairs of isolates that are epidemiologically linked. This indicates that the plasmid-bound blaCTX–M–15 genes have been recently inserted into the chromosomes. This finding emphasizes the fast evolution of the site of resistance genes, irrespective of the patient. The migration of the blaCTX–M–15 gene has significant importance, since a recent study revealed varying rates of resistance when it was transferred onto a plasmid as opposed to the chromosome. The dissemination rate in a broad scenario may be modified by the spread of plasmids, as discussed by Walas *et al*. (2023). The identification of the blaCTXM-15 extended-spectrum beta-lactamase (ESBL) gene in the nearby vicinity, which is connected to resistance against Enterobacterales, specifically *Klebsiella* and *Escherichia coli* species, emphasizes the distinct resistance patterns in the local area, potentially influenced by the utilization of antibiotics in animal agriculture and hospital wastewater (Xie *et al*., 2023). Prior studies have established a correlation between these bacterial variants and urinary tract infections (UTIs) in the Nigerian populace, and they exhibit resistance to Enterobacterales (Ogbolu *et al*., 2018). In order to reduce the transmission of antibiotic-resistant bacteria (ARBs) in food production systems, it is crucial to recognize that uncontrolled and excessive antibiotic usage might result in environmental pollution (Zalewska et al., 2021).

CONCLUSIONS AND RECOMMENDARTIONS

The results of our study offer comprehensive knowledge on the abundance and spread of antibiotic resistance genes in dairy cow feces found in the environment. The presence of resistance genes linked to Enterobacteriaceae and Enterococcus species raises significant concerns regarding public health. These findings emphasize the essential requirement for proper antibiotic administration in agriculture and healthcare to reduce the occurrence of antibiotic resistance, preserve the efficacy of these life-saving drugs, and prevent significant public health emergencies. Additionally, further investigation is required to have a deeper comprehension of the mechanisms behind antibiotic resistance genes in this particular scenario and their possible consequences for public health.

Data availability

The data is uploaded to NCBI with the SRA identifier numbers SRR24770473, SRR24770470, SRR24770472 and SRR24770471 (Khan *et al*., 2023).

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NOVELTY STATEMENT

The studies on antibiotic gene resistance in Pakistan are very few specially identifying through next generation sequencing. This is among the pioneer studies using shotgun metagenomic approach to identify ARGs in cow feces from different ecological zones of Pakistan.

AUTHOR'S CONTRIBUTION

FAK, NPS, BWHEP, WN conceptualized the research, BA, AA collected the samples, FAK, NPS analyzed the samples, AA, FAK, WD, HC wrote the first draft and reviewed, FS RY BA FAK NPS BWHEP NPS, FAK reviewed and provided insightful input.

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Ethical statement

Ethics and animal welfare was strictly followed samples were obtained with the help of farmers who provided us access to their animals.

Conflict of interest

The authors have declared no conflict of interest.

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