Ciência Animal Brasileira
Brazilian Animal Science

DOI: 10.1590/1809-6891v26e-79298E

e-ISSN 1809-6891
Animal science | Research article

Antibiotic resistance gene occurrence in poultry farms in northeast Brazil

Ocorrência de genes de resistência a antibióticos em granjas avícolas localizadas no nordeste do Brasil

Henrique Francisco de Almeida[1], Paulo Ricardo Conceição Marques Trindade[1], César Roberto Viana Teixeira[1], Claudson Oliveira Brito[1], Silvio Santana Dolabella[1], Sona Jain[1], Maíra Pompeu Martins[1], Ana Andréa Teixeira Barbosa*[1]

1 Universidade Federal de Sergipe, São Cristóvão, Sergipe, Brazil.
*Corresponding author: ana.barbosaufs@yahoo.com.br

Received: May 07, 2024. Accepted: October 11, 2024. Published: February 06, 2025. Editor: Rondineli P. Barbero

Abstract: The misuse of antibiotics in food-producing animal farming practices exerts selective pressure on bacterial strains, intensifying the spread of pathogenic and commensal bacteria carrying antibiotic resistance genes (ARGs). We conducted a study aiming to investigate ARGs in chicken litter from farms in the State of Sergipe, Northeast Brazil. A total of 14 chicken litter samples were collected from twelve farms and subjected to total DNA extraction. The presence of ARGs in the obtained material was tested by Polymerase Chain Reaction (PCR) using primers for selected ARGs. ARGs were confirmed in all samples, and the highest resistance positivity was obtained for tetracyclines (*tet*A, *tet*M, and *tet*G), quinolones (*gyr*A and *qnr*S), beta-lactams (*bla*TEM), macrolides (*erm*B) and sulfonamides (*sul*-1). Sequencing and comparison with the GenBank database confirmed the identity of the ARGs. Some of the sequences that were amplified by PCR were similar to resistance factors found in Gram-positive and Gram-negative bacteria of different species, mostly enterobacteria. Furthermore, similarity was observed for resistance determinants located both on the chromosome and on plasmids, transposons, and integrons. Our results indicate the potential of poultry farming for the environmental dissemination of ARGs in the State of Sergipe.

Keywords: Antibiotic-resistant bacteria; poultry manure; environmental dissemination; antimicrobial; avian

Resumo: O uso indevido de antibióticos na produção animal pode exercer pressão seletiva sobre cepas bacterianas, intensificando a disseminação de bactérias patogênicas e comensais portadoras de genes de resistência a antibióticos (GRAs). O objetivo deste estudo foi investigar a presença de GRAs em camas de frango provenientes de granjas avícolas localizadas no Estado de Sergipe, no Nordeste do Brasil. Um total de 14 amostras de cama de frango foram coletadas de doze fazendas e submetidas à extração de DNA total. A presença de GRAs foi testada por Reação em Cadeia da Polimerase (PCR) usando primers para as principais classes de antibióticos. GRAs foram confirmados em todas as amostras, e a maior positividade para resistência foi obtida para tetraciclinas (*tet*A, *tet*M,

and *tet*G), quinolonas (*gyr*A and *qnr*S), beta-lactâmicos (*bla*TEM), macrolídeos (*erm*B) e sulfonamidas (*sul*-1). O sequenciamento e a comparação com o banco de dados GenBank confirmaram a identidade dos GRAs. Algumas das sequências amplificadas por PCR eram semelhantes a fatores de resistência encontrados em bactérias Gram-positivo e Gram-negativo de diferentes espécies, principalmente enterobactérias. Além disso, foi observada semelhança para determinantes de resistência localizados tanto no cromossomo quanto em plasmídeos, transposons e integrons. Nossos resultados indicam o potencial da criação de aves para a disseminação ambiental de GRAs no Estado de Sergipe.

Palavras-chave: Bactérias resistentes a antibióticos; esterco de aves; disseminação ambiental; antimicrobiano; aves

1. Introduction

The use of antibiotics in food-producing animals, particularly in chicken production, for prophylactic and therapeutic purposes as well as growth promoters has been identified as one of the activities that lead to the spread of antibiotic resistance in the environment [1,2]. Antibiotic resistance has become a serious and widespread public health problem, and farming activities can intensify the spread of pathogenic and commensal bacteria carrying antibiotic resistance genes (ARGs) [3–5]. Antibiotic resistance determinants include antibiotics, antibiotic-resistant bacteria (ARB) and ARGs. When bacteria are in the environment, antibiotics can kill ARBs and allow commensal strains to get ARGs through horizontal gene transfer (HGT) [6–8]. In the environment, these resistance determinants can reach human and animal pathogenic bacterial strains, representing a serious problem [9,10].

Poultry production eliminates antibiotic resistance determinants in poultry excreta, which forms the widely used organic fertilizer [11–13]. Some studies have shown that the use of poultry manure as fertilizer is responsible for the introduction of ARB and ARGs into the soil [14–16], resulting in the accumulation and absorption of these micropollutants by plants, thus reaching humans and animals through the food chain [9,10,15].

Since the late 1990s, Brazil has observed a progressive reduction in the use of antibiotic agents as growth promoters in animals [17]. The Ministry of Agriculture, Livestock and Food Supply (MAPA) implemented the Antimicrobial Resistance Surveillance and Monitoring Program, through Normative Instructions, prohibiting the use of tetracyclines, beta-lactams (benzylpenicillin and cephalosporins), quinolones, sulfonamides, colistin, tylosin, lincomycin, and tiamulin as growth promoters, aiming to contain the advance of antimicrobial resistance [17–20]. Despite restrictive surveillance measures, the available Brazilian data on this topic reveal a wide variety of resistance profiles [21].

Considering the growing consumption of antibiotics in animal production, despite efforts to reduce their use and the relevance of Brazil as a food producer and exporter of poultry meat, the aim of the present study was to verify the presence of ARGs in poultry manure from different farms located in Sergipe state, Northeast Brazil.

2. Material and methods

2.1. Study area and sample collection

A total of 14 samples were collected, with ten (10) originating from poultry litter (poultry broiler) and four (4) from the poultry manure layer (designated as G1 to G14 in Table 1). These samples were gathered from twelve farms situated across seven municipalities in the State of Sergipe, Brazil, spanning the period from September 2021 to February 2022. Specifically, the sampling points were distributed as follows: Estância (n=5), Areia Branca (n=4), Umbaúba (n=1), Nossa Senhora da Glória (n=1), Carira (n=1), Frei Paulo (n=1), and Campo do Brito (n=1). Figure 1 displays the locations of the municipalities included in the study.

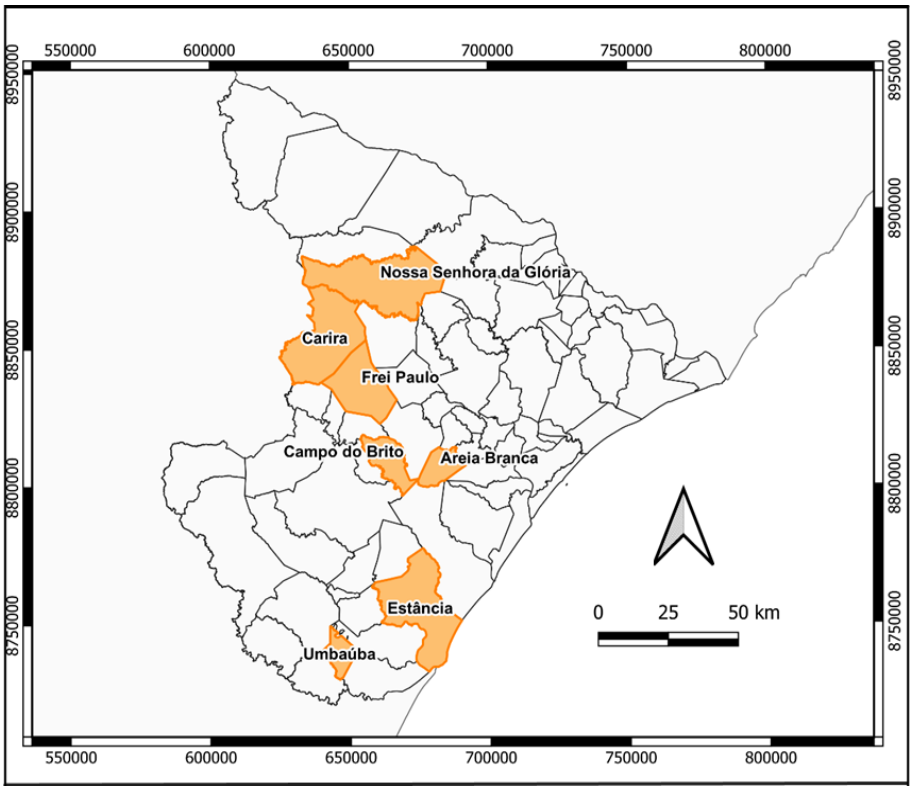

Figure 1. The state of Sergipe, Brazil, and the locations of the municipalities in the study area (highlighted in orange).

Samples from G11 to G14 were collected from the same farm, representing distinct phases of chicken development: G11 during the brooding phase, encompassing the first ten weeks of the hens' lives; G12 during the rearing phase, spanning from the 10th and 17th week of development; and G13 and G14 at the beginning and end, respectively, of the laying phase, covering the 18th to the 72nd week of the chicken growth cycle [22]. To create a composite sample, a minimum of 20 subsamples were collected using the zigzag method and subsequently homogenized to produce a fraction of approximately 300 g (total sample). These samples were then placed in plastic bags, labeled for identification, and transported to the Animal Science Department of the Federal University of Sergipe. The samples were stored at -20°C until further processing and analysis. Detailed information regarding the samples collected in this study is provided in Table 1.

Table 1. Identification of the samples with their respective municipalities, type of poultry production and growth promoter used.

Sample	Municipality	Geographical location	Type of production	Duration of housing (days)	Growth promoter
G1	Estância	S 12°49'55" W 38°32'33"	broiler	110	Enramycin 8%
G2	Estância	S 12°47'27" W 38°40'55"	broiler	130	Enramycin 8%
G3	Estância	S 12°47'2" W 38°37'45"	broiler	165	Enramycin 8%
G4	Estância	S 12°47'15" W 38°40'55"	broiler	210	Enramycin 8%
G5	Umbaúba	S 12°38'49" W 38°20'9"	broiler	240	Enramycin 8%
G6	Estância	S 12°46'54" W 38°38'16"	broiler	260	Enramycin 8%
G7	Nossa Senhora da Glória	S 11°41'59" W 38°34'42"	broiler	42	Uninformed
G8	Carira	S 11°34'7" W 38°10'43"	broiler	45	Halquinol + Monensin[3]
G9	Campo do Brito	S 11°12'38" W 38°20'29"	broiler	52	Salinomycin[4]
G10	Frei Paulo	S 11°28'9" W 38°29'53"	broiler	120	Halquinol + Salinomycin
G11[1]	Areia Branca	S 11°14'6" W 38°40'50"	laying	21	Halquinol + Salinomycin
G12[1]	Areia Branca	S 11°14'6" W 38°40'50"	laying	70	Halquinol + Salinomycin
G13[2]	Areia Branca	S 11°13'39" W 38°37'48"	laying	140	Zinc Bacitracin
G14[2]	Areia Branca	S 11°13'39" W 38°37'48"	laying	546	Zinc Bacitracin

[1]G11 and G12; [2]G13 and G14: same farm but different aviaries. [3,4]Monensin and Salinomycin: used against avian coccidiosis.

2.2. Sample processing and DNA extraction

Sample processing was performed according to Subirats et al. [(23)], with adaptations. From the total sample of chicken litter, 20 g was diluted in 200 mL of saline solution (0.85% NaCl), and the suspensions were manually stirred for approximately five minutes. Then, the samples were filtered, distributed in 50 mL Falcon tubes, and centrifuged (5000 rpm for 12 minutes at 4°C). The supernatant was discarded, and the pellet was washed twice with a saline solution (5000 rpm for 12 minutes at 4°C). The pellet was resuspended in saline solution and stored at -20°C until DNA extraction. For DNA extraction, the QIAamp Fast DNA Stool Kit (QIAGEN, Valencia, CA, United States) was used according to the manufacturer's instructions. Quantification of the extracted genetic material was performed using a spectrophotometer (Epoch, Microplate Spectrophotometer, Biotek, Agilent®).

2.3. Detection of antibiotic resistance genes

The extracted DNA was subjected to polymerase chain reaction (PCR) using primers to first detect the 16S rRNA (control) and later to identify the key resistance genes that have been linked to poultry farming (Table 2) using the following conditions: initial denaturation at 95°C for 5 minutes; followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing from 55°C to 60°C (30 seconds), and extension at 72°C for 30 seconds. The final extension was performed at 72°C for 10 minutes. The positive controls for the *tet*A, *tet*B and *mcr*-1 genes were isolated from a strain of *Klebsiella pneumoniae*[56] and provided by the Laboratory of Molecular Genetics of Bacteria of the Federal University of Viçosa- Minas Gerais. For the other evaluated genes, positive controls were provided by the Laboratory of Molecular Biology at Federal University of Sergipe [24].

Table 2. *Primers* used for the detection of 16S rRNA and antibiotic resistance genes in poultry litter samples.

Gene	Primer	Sequence (5′ → 3′)	Annealing Temperature (° C)	Amplification Length (bp)	Source
16S rRNA	FW RV	AGAGTTTGATCCTGGCTCAG GGTTACCTTGTTACGACTT	55	1500	[25]
*tet*A	FW RV	GCTACATCCTGCTTGCCTTC CATAGATCGCCGTGAAGAGG	58	210	[26]
*tet*B	FW RV	TTGGTTAGGGGCAAGTTTTG GTAATGGGCCAATAACACCG	55	659	[26]
*tet*G	FW RV	GCTCGGTGGTATCTCTGCTC AGCAACAGAATCGGGAACAC	58	468	[26]
*tet*M	FW RV	TTTATCTGTATCACCGCTTCCG ACAATCCGTCACATTCCAACC	60	154	[27]
*gyr*A	FW RV	AGCGACCTTGCGAGAGAAAT GGAACCGAAGTTACCCTGACC	60	330	[27]
*qnr*S	FW RV	TTGCCCATCAAGTGAGTAATCG AGGATAAACAACAATACCCAGTGC	60	341	[27]
*bla*TEM	FW RV	CATTTCCGTGTCGCCCTTATTC CGTTCATCCATAGTTGCCTGAC	60	800	[28]
*erm*B	FW RV	TAACGACGAAACTGGCTAAAATAAG AACATCTGTGGTATGGCGGG	60	419	[27]
sul-1	FW RV	CGCACCGGAAACATCGCTGCAC TGAAGTTCCGCCGCAAGGCTCG	56	163	[27]
mcr-1	FW RV	CGGTCAGTCCGTTTGTTC CTTGGTCGGTCTGTAGGG	55	309	[29]

2.4. Sequencing

The PCR-amplified bands were purified using the Promega Purification Kit, quantified using a spectrophotometer (Epoch, Microplate Spectrophotometer, Biotek, Agilent®) and sequenced at the Federal University of Pernambuco, Brazil. The sequences obtained were compared using the BLAST Basic Local Alignment Search Tool (GenBank, NCBI – National Center for Biotechnology Information). Samples for sequencing were chosen based on the strongest amplification performance of a single gene from each farm.

3. Results

3.1 Detection of ARGs

All tested samples were positive for at least one of the investigated ARGs. Five of the tested genes, *tet*M, *gyr*A, *bla*TEM, *erm*B, and *sul*-1, were positive in all analyzed samples (Table 3). The *qnr*S and *mcr*-1 were not detected in samples G13 and G14, respectively. All tetracycline resistance genes were detected in samples G1 (Estância), G3 (Estância), G5 (Umbaúba), G7 (Nossa Senhora da Glória) and G9 (Campo do Brito), obtained from poultry litter, highlighting the spread of antimicrobial resistance genes in Sergipe State poultry farms. Samples GI, G3 and G5 exhibited positive results for all the primers tested (Table 3).

Table 3. Detection frequency of antibiotic resistance genes in poultry farms samples (G1 to G14) collected from september 2021 to february 2022, Sergipe State, Brazil.

Antibiotic Class	Gene	Samples															
		G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	Total	%
Tetracyclines	*tet*A	+	-	+	+	+	-	+	-	+	+	+	+	+	+	11	78,6
	*tet*B	+	-	+	-	+	-	+	-	-	+	+	+	+	+	9	64,3
	*tet*G	+	-	+	+	+	-	+	-	+	+	+	+	+	+	11	78,6
	*tet*M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14	100
Quinolones	*gyr*A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14	100
	*qnr*S	+	+	+	+	+	-	+	+	+	+	+	+	-	+	12	85,7
Beta-lactams	*bla*TEM	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14	100
Macrolides	*erm*B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14	100
Sulfonamides	*sul*-1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14	100
Polymyxins	*mcr*-1	+	+	+	+	+	+	-	-	+	-	+	+	+	-	10	71,4
	Total	10	7	10	9	10	6	9	6	9	9	10	10	9	10	123	87,86

3.2 Sequencing

Sequencing and comparison with the GenBank database confirmed the identity of the genes (Table 4). They showed similarity with resistance determinants present in bacteria of different species, demonstrating their ubiquitous character. The results were the same for both Gram-positive and Gram-negative strains, with enterobacteria being the most similar. This was to be expected since the samples came from birds' digestive systems [2, 7, 12] (Table 4).

For *tet*A gene, the analyzed sequence showed a 100% identity with genes present in the genome of strains of *Escherichia coli*, *Shigella flexneri*, *Salmonella enterica*, and *Klebsiella pneumoniae*. A 100% identity was also observed with *Acinetobacter baumannii* for *tet*B, while other strains, such as *Vibrio cholerae*, showed a 99.85% identity (Table 4). Among the tetracycline genes, *tet*G showed lower identity with reference strains: the highest identity recorded was 92.27% with an uncultured bacterial clone. Other strains like *Proteus mirabilis* and *Pseudomonas aeruginosa* showed lower identity percentages around 90.34%. For *tet*M, all strains analyzed had a 95.79% identity, including those from *Streptococcus agalactiae* and *Enterococcus faecalis* (Table 4).

High identity percentages of 100% with *gyr*A gene were noted for *E. coli* and *Salmonella* sp., while *S. flexneri* and *Shigella dysenteriae* had identities of around 99.35% and 99.68%, respectively. Complete sequences from various *K. pneumoniae* and *P. aeruginosa* strains showed 100% identity with gene *qnr*S (Table 4). For *bla*TEM, identity percentages ranged from 99.71% for multiple strains, including *E. coli* and *A. baumannii.* The *erm*B gene showed 99.05% identity with genetic determinants from *Streptococcus suis*, *Clostridium perfringen*s, and *E. faecalis*. The sequence of *sul*1 gene exhibited a 100% identity with genetic determinants from *Enterobacter cloacae, E. coli* and *K. pneumoniae*. For *mcr*-1 gene, 100% identity was found with genes located in the genome of *E. coli and K. pneumoniae, Salmonella* Typhimurium and *Raoultella ornithinolytica* (Table 4).

Table 4. Result of sequencing analysis of antibiotic resistance genes amplified from chicken litter samples using GenBank database.

Gene	Reference strain: species and source	Identity (%)	GenBank Access Number	Aligned Region	Gene Location
*tet*A	*Escherichia coli PBM64, tet*Atetracycline efflux transporter MFS gene, partial cds.	100	OQ625508.1	2 a 210	1..210
	Shigella flexneri 2nd strain Sflex 21-42, unnamed plasmid 4, complete sequence.	100	CP121221.1	21440 a 21648	21318..22517
	Salmonella enterica subsp. enterica serovar Uganda strain RM018, plasmid pRM018_1, complete sequence.	100	CP117383.1	26328 a 26536	26206..27405
	Klebsiella pneumoniae strain IM007 plasmid pIM007_ESBL, complete sequence.	100	CP095430.1	8091 a 8299	7222..8421

*tet*B	*Acinetobacter baumannii strain* S402, *tet*B gene, partial cds.	100	MK506781.1	1 a 656	1..656
	Vibrio cholerae strain BY369, plasmid pBY369-1, complete sequence.	99,85	CP090380.1	35342 a 35999	35190..36395
	Avibacterium paragallinarum strain AG21-0333, chromosome, complete genome.	99,85	CP104914.1	81099 a 81756	80703..81908
	Escherichia coli strain CMCY6 tetracycline efflux MFS transporter gene, *tet*(B) allele, complete cds.	99,85	OM977025.1	397 a 1054	1..1206
*tet*G	Uncultured bacteria clone G0-10 class G tetracycline resistance protein gene (*tet*G), partial cds.	92,27	KJ603177.1	2 a 415	1..468
	Proteus mirabilis HN2p strain, HN2p chromosome, complete sequence.	90,34	CP046048.1	5799 a 6212	5346..6521
	Pseudomonas aeruginosa strain AR_0111, chromosome, complete genome.	90,34	CP032257.1	2703701 a 2704114	2703248..2704423
	Klebsiella pneumoniae strain 309074, plasmid p309074-1, complete sequence.	90,34	CP030297.1	99949 a 100362	99496..100671
*tet*M	*Streptococcus agalactiae* strain PHEGBS0463, transposon Tn7539, complete sequence.	95,79	OP715847.1	19907 a 20001	18569..20488
	Enterococcus faecalis strain W5, plasmid pW5-2, complete sequence.	95,79	CP118757.1	8140 a 8234	7653..9572
	Gallibacterium anatis strain IMT49310, chromosome, complete genome.	95,79	CP110225.1	1611696 a 1611790	1611209..1613128
	Staphylococcus aureus, chromosome N09CSA16, complete genome.	95,79	CP091525.1	1671366 a 1671460	1670028..1671947
*gyr*A	*Escherichia coli strain 128,* DNA gyrase A *(gyrA)* gene, partial cds.	100	KC493126.1	1 a 328	1..626
	Salmonella sp. Chromosome S13, complete genome.	100	CP047094.1	2928257 a 2928585	2925962..2928589
	Shigella flexneri strain B36 DNA gyrase subunit A *(gyr*A) gene, partial cds.	99,35	KU586842.1	1 a 309	1..645
	Shigella dysenteriae strain NK3898, DNA gyrase subunit A *(gyr*A) gene, partial cds.	99,68	KU586846.1	1 a 309	1..645
*qnr*S	*Klebsiella* pneumoniae isolate KSH203, plasmid pKSH203-*qnr*S, complete sequence.	100	CP034326.1	146224 a 146562	146216..146872
	Pseudomonas aeruginosa, plasmid pP6qnrS1, complete sequence.	100	MH061383.1	68516 a 68854	68206..68862
	Enterobacter cloacae strain 3849, plasmid p3846_IncN_VIM-1, complete sequence.	100	CP052872.1	16839 a 17177	16529..17185
	Escherichia coli MN067 *qnr*S gene for quinolone resistance pentapeptide repeat protein *qnr*S12, complete CDS	100	NG_059276.1	411 a 749	101..757
*bla*TEM	*Escherichia coli* strain BLG15, broad-spectrum plasmid class A beta-lactamase gene TEM-1(*bla*TEM), *bla*TEM-1 allele, complete cds.	99,71	OQ625507.1	80 a 763	1..861
	Klebsiella pneumoniae strain KPN6328, plasmid pK6328_1, complete sequence.	99,71	CP124838.1	98097 a 98780	98018..98878
	Acinetobacter baumannii strain Aba_C-34HGM2020 HAS family class A beta-lactamasegene(*bla*TEM), partial cds. lactamase (*bla*TEM), cds parciais.	99,71	OP745943.1	28 a 711	1..754
	Pseudomonas aeruginosa strain Emad-H6 family TEM beta-lactamase gene (*bla*TEM), partial cds.	99,71	OQ784849.1	80 a 763	1..857

ermB	*Streptococcus suis* strain STC78 ICensui78-*tet*O-*erm*Bmobile element, complete sequence.	99,05	ON944185.1	26620 a 27038	1..55758
	Clostridium perfringens strain QHY-2, plasmid pQHY-2, complete sequence.	99,05	CP118266.1	20960 a 21378	20833..21570
	Enterococcus faecalis strain W5, plasmid pW5-2, complete sequence.	99,05	CP118757.1	62670 a 63088	62478..63215
	Gallibacterium anatis strain IMT49310, chromosome, complete genome	99,05	CP110225.1	1613935 a 1614353	1613808..1614545
sul-1	*Enterobacter cloacae* 2017-266 intI, blaIMP-1, *aac(6')-Ic,* qacEdelta1, *sul*-1 genes, complete cds.	100	LC508022.1	3763 a 3924	3218..4057
	Escherichia coli strain E dihydropteroate synthase(*sul*-1) gene, partial cds.	100	MN527466.1	502 a 663	1..775
	Proteus mirabilis HN2p strain, HN2p chromosome, complete sequence.	100	CP046048.1	143413 a 143574	143280..144118
	Klebsiella pneumoniae strain THC-2, sulfonamide resistance protein *sul*-1 gene, partial cds.	100	MK620997.1	216 a 377	1..388
mcr-1	*Klebsiella* pneumoniae strain NH54, phosphoethanolamine-lipid A transferase (*mcr*-1) gene, complete cds.	100	MF149969.1	143 a 451	110..1735
	Escherichia coli strain HKSH_MCR_161114268_EC, phosphoethanolamine lipid A transferase gene (*mcr*-1), mcr1.9 allele, complete cds.	100	KY685071.1	34 a 342	1..1626
	Salmonella enterica subsp. enterica serovar Typhimurium strain P22, phosphoethanolamine transferase (*mcr*-1) gene, partial cds.	100	MH654791.1	69 a 377	36..693
	Raoultella ornithinolytica strain TS48CTX, plasmid pHNTS48-1, complete sequence.	100	MF135534.1	178989 a 179297	177705..179330

4. Discussion

The use of antimicrobial substances in food-producing animals leads to extensive human exposure to bacteria carrying ARGs, including commensal bacteria present in poultry droppings[12]. In poultry farms, the extensive distribution of resistant bacteria and their related genes poses a recognized threat to human and animal health [2,3]. In our results, the *tet*M, *gyr*A, *bla*TEM, *erm*B and *sul*-1 genes were detected in all analyzed samples (Table 3). These ARGs confer resistance to tetracyclines, quinolones, beta-lactams, macrolides, and sulfonamides. These genes are among the most commonly detected genes in samples from poultry farming[30,31]. Eleven studied samples amplified *tet*A and *tet*G, while nine samples amplified *tet*B, the other genes conferring resistance to tetracyclines. Only two samples, G6 and G13 (Table 3), did not contain the *qnr*S gene, which encodes resistance to quinolones. Ten samples (Table 3) detected the *mcr*-1 gene, which encodes resistance to polymyxins, confirming its prevalence and persistence in poultry environments[32–34].

The resistance determinants of the amplified ARGs, selected from the GenBank database, were located both on the chromosome and on plasmids, transposons and integrons, showing similarities. The presence of these genes in mobile genetic elements such as plasmids, transposons and integrons facilitates their propagation between species, which may significantly contribute to their dissemination in the environment[35–37]. Most bacterial

strains selected from the database for similarity analysis had genes associated with both plasmids and chromosomes. Only one strain of *Streptococcus agalactiae* carried the *tet*M gene associated with transposon 7539, and one strain of *Enterobacter cloacae* had the *sul*-1 gene associated with class I integrons (Table 4). The macrolide resistance determinant *erm*B was found to be associated with the *tet*O gene in *Streptococcus suis*. During HGT events, both genes can be transferred simultaneously to another bacterial strain, leading to the spread of multidrug-resistant strains[38].

These resistance genes are related to antibiotics, whose use as growth promoters is prohibited by Brazilian legislation[17]. However, their use for disease prevention and treatment in animals is permitted under specific conditions and supervision. [39]. Therefore, the presence of these ARGs in most of the samples may be linked to the frequent use of these antibiotics for therapeutic or prophylactic purposes in poultry[30,31]. Data regarding antibiotic use for these two purposes during the poultry production cycle were not available from the farms under study, only information on growth promoters were available. The growth promoters used in the evaluated farms include enramycin (8%), Halquinol and the zinc bacitracin. Enramycin, a polypeptide antibiotic, primarily inhibits the synthesis of the cell wall in Gram-positive bacteria [40,41]. It ranks among the top three growth promoters, with high import rates of approximately 62.58 tons between 2017 and 2019, and is frequently added to chicken diets[42]. Enramycin was used by most of the farms included in this study (Table 1).

Halquinol is classified as a quinolone, but its mechanism of action differs from that of representatives of this class. It affects fungi and protozoa and is used as a growth promoter in swine and poultry farms[43,44]. In this study, only four establishments used this additive (Table 1). To date, no cases of microorganisms resistant to Halquinol have been reported in the literature[43,44]. Zinc bacitracin exhibits activity against Gram-positive bacteria and serves as a growth promoter in poultry. It is used in poultry production on two farms in the study area. It is used to treat *C. perfringens* infections in chickens and is also used topically in humans[45,46]. However, its inappropriate and widespread use has led to an increase in the prevalence of bacitracin-resistant strains of *C. perfringens*[47] and the detection of ARGs in foods such as meat, vegetables, and fruits[48]. The detection of the *tet*A, *tet*B, *bla*TEM and *sul*-1 genes in the present study may be related to the use of bacitracin in the studied farms. A study by Diarra et al.[49] linked the use of bacitracin as a growth promoter to the presence of multiresistant *E. coli* harboring the *tet*A, *tet*B, *bla*TEM and *sul*-1 genes. The use of this growth promoter was also associated with the presence of the ARGs *tetA* and *sul*-1 in *E. coli* strains isolated from chickens[50].

Monensin and salinomycin, authorized in Brazil for use as growth promoters in cattle, sheep and pigs, are used for prophylactic purposes in poultry to combat coccidiosis[51]. Due to their frequent use in poultry farming, cases of *Eimeria* spp. resistant to these anticoccidials have been reported[51]. Furthermore, the use of salinomycin as a growth promoter in chickens was associated with the isolation of *E. coli* carrying the following ARGs: *tet*A, *tet*B, *bla*TEM and *sul*-1[49]. All farms in the present study that used salinomycin as a growth promoter tested positive for these genes, except for G9, where *tet*B was not detected.

Therefore, the ARGs detected are not directly related to the drugs used as growth promoters on the farms under study. This is concerning, as it may indicate a lack of control over the use of antibiotics on farms or cross-resistance with growth promoters. It is important to note that despite global trends to reduce or prohibit the use of antibiotics in animal production, these measures do not effectively address the issue of bacterial resistance. The colistin resistance gene *mcr*-1, for example, has been shown to confer cross-resistance to bacitracin. Furthermore, mobile genetic elements such as plasmids, transposons, and integrons can carry various resistance determinants, facilitating the dissemination of HGT gene transfer[52,53]. Thus, the detection of ARGs in this study can be attributed to direct antibiotic consumption during the poultry production cycle, as well as other factors not directly related to these drugs in chickens. In addition, there are no reports in the literature that correlate the use of enramycin and halquinol with the detection of ARGs on the study farms, highlighting the need for further research to address this gap. These ARGs confer resistance to critical antibiotics used in the treatment of infectious diseases in humans [54].

The presence of these ARGs in chicken litter possesses a potential risk for their dissemination in the environment, particularly since using litter as fertilizer in plantations is a common practice [11]. An exacerbating factor is the repeated reuse of poultry litter across multiple growth cycles, which increases the diversity and concentration of ARGs and ARBs in poultry waste [7,11]. Most of the studied poultry farms reused chicken litter in multiple production cycles with only samples from G7, G8, and G9 having fresh litter (Table 1). Farm operators confirmed that they use the resulting manure in surrounding plantations. This practice promotes the spread of bacterial resistance by facilitating contact and genetic material exchange between enteric bacteria in manure and soil bacteria [41,55].

5. Conclusion

This study evaluates poultry residues harboring ARGs, together with the potential risk they represent for the spread of bacterial resistance in the environment. These resistance determinants can reach humans through contact with contaminated soil, food and water, thereby increasing the risk of treatment failures of the infections caused by resistant microorganisms. The current study, a pioneer in Sergipe, Brazil, will thus help raising awareness among producers about the judicious use of antibiotics across all sectors of society, including animal husbandry. Proper treatment of litter and chicken manure before disposal into the environment is crucial. This approach can help poultry farming play a role in reducing the dissemination of bacterial resistance. Developing management strategies to mitigate the spread of antibiotics, ARBs, and ARGs is a priority for the animal production sector.

Declaration of conflict of interest

The authors declare no conflicts of interest.

Data availability statement

The data will be provided upon request.

Author contributions

Conceptualization: A.A.T.Barbosa. Formal analysis: A.A.T.Barbosa. Methodology: A.A.T. Barbosa, S. Jain, S.S. Dolabella and C.O. Brito. Supervision: A.A.T.Barbosa. Investigation: H.F. Almeida, P.R.C.M. Trindade and C.R.V. Teixeira. Visualization: A.A.T. Barbosa, S. Jain, S.S. Dolabella, C.O. Brito, M.P. Martins and C.R.V. Teixeira. Writing (original draft): H.F. Almeida. Writing (review & editing): A.A.T.Barbosa, S. Jain, C.O.Brito, S.S. Dolabella and M.P. Martins.

References

1. van Cuong N, Kiet BT, Hien VB, Truong BD, Phu DH, Thwaites G, et al. Antimicrobial use through consumption of medicated feeds in chicken flocks in the Mekong Delta of Vietnam: A three-year study before a ban on antimicrobial growth promoters. PLoS One. 2021;16(4): e0250082. http://doi.org/10.1371/journal.pone.0250082

2. Langata LM, Maingi JM, Musonye HA, Kiiru J, Nyamache AK. Antimicrobial resistance genes in *Salmonella* and *Escherichia coli* isolates from chicken droppings in Nairobi, Kenya. BMC Research Notes. 2019;12(1):22. https://doi.org/10.1186/s13104-019-4068-8

3. Farooq M, Smoglica C, Ruffini F, Soldati L, Marsilio F, Di Francesco CE. Antibiotic resistance genes occurrence in conventional and antibiotic-free poultry farming, Italy. Animals (Basel). 2022;12(18):2310. https://doi.org/10.3390/ani12182310

4. Huemer M, Mairpady Shambat S, Brugger SD, Zinkernagel AS. Antibiotic resistance and persistence - Implications for human health and treatment perspectives. EMBO Reports. 2020;21(12): e51034. https://doi.org/10.15252/embr.202051034

5. Larsson DGJ, Flach CF. Antibiotic resistance in the environment. Nature Reviews Microbiology. 2022;20(5):257–269. https://doi.org/10.1038/s41579-021-00649-x

6. Alalam H, Graf FE, Palm M, Abadikhah M, Zackrisson M, Boström J, et al. A high-throughput method for screening for genes controlling bacterial conjugation of antibiotic resistance. mSystems. 2020;5(6):e01226-20. https://doi.org/10.1128/mSystems.01226-20

7. Oladeinde A, Abdo Z, Zwirzitz B, Woyda R, Lakin SM, Press MO, et al. Litter commensal bacteria can limit the horizontal gene transfer of antimicrobial resistance to *Salmonella* in chickens. Applied and Environmental Microbiology. 2022;88(9):e0251721. https://doi.org/10.1128/aem.02517-21

8. Virolle C, Goldlust K, Djermoun S, Bigot S, Lesterlin C. Plasmid transfer by conjugation in Gram-negative bacteria: From the cellular to the community level. Genes (Basel). 2020;11(11):1239. https://doi.org/10.3390/genes11111239

9. Lima T, Domingues S, Silva GJ Da. Manure as a potential hotspot for antibiotic resistance dissemination by horizontal gene transfer events. Veterinary Sciences. 2020;7(3):110. https://doi.org/10.3390/vetsci7030110

10. Vumazonke S, Khamanga SM, Ngqwala NP. Detection of pharmaceutical residues in surface waters of the Eastern Cape Province. International Journal of Environmental Research and Public Health. 2020;17(11)4067. https://doi.org/10.3390/ijerph17114067

11. Ngogang MP, Ernest T, Kariuki J, Mouiche MMM, Ngogang J, Wade A, et al. Microbial contamination of chicken litter manure and antimicrobial resistance threat in an urban area setting in Cameroon. Antibiotics (Basel). 2021;10(1):20. https://doi.org/10.3390/antibiotics10010020

12. Wang L, Chai B. Fate of antibiotic resistance genes and changes in bacterial community with increasing breeding scale of layer manure. Frontiers in Microbiology. 2022;13:857046. https://doi.org/10.3389/fmicb.2022.857046

13. Oxendine A, Walsh AA, Young T, Dixon B, Hoke A, Rogers EE, et al. Conditions necessary for the transfer of antimicrobial resistance in poultry litter. Antibiotics (Basel). 2023;12(6):1006. https://doi.org/10.3390/antibiotics12061006

14. Chen Q, An X, Li H, Su J, Ma Y, Zhu YG. Long-term field application of sewage sludge increases the abundance of antibiotic resistance genes in soil. Environment International. 2016;92–93:1–10. https://doi.org/10.1016/j.envint.2016.03.026

15. Duan M, Gu J, Wang X, Li Y, Zhang R, Hu T, et al. Factors that affect the occurrence and distribution of antibiotic resistance genes in soils from livestock and poultry farms. Ecotoxicology and Environmental Safety. 2019;180:114–122. https://doi.org/10.1016/j.ecoenv.2019.05.005

16. Fatoba DO, Abia ALK, Amoako DG, Essack SY. Rethinking manure application: Increase in multidrug-resistant enterococcus spp. in agricultural soil following chicken litter application. Microorganisms. 2021;9(5):885. https://doi.org/10.3390/microorganisms9050885

17. Lentz SAM. Atualização sobre Uso Racional de Antimicrobianos e Boas Práticas de Produção. Opas/Mapa. 2022;1–50. Available from: https://www.gov.br/agricultura/pt-br/assuntos/insumos-agropecuarios/insumos-pecuarios/resistencia-aos-antimicrobianos/publicacoes/Apostila_AtualizaosobreUsoRacionaldeAntimicrobianoseBoasPrticasdeProduo.pdf

18. Brasil. Instrução Normativa Nº26, de 10 de julho de 2009. Ministério da Agricultura Pecuária e Abastecimento - MAPA. 2009;9. https://wikisda.agricultura.gov.br/dipoa_baselegal/in_9-2009_listeria.pdf

19. Brasil. Instrução Normativa Nº 45, De 22 De Novembro De 2016. Dou. 2016;4. https://www.gov.br/agricultura/pt-br/assuntos/insumos-agropecuarios/insumos-pecuarios/alimentacao-animal/arquivos-alimentacao-animal/legislacao/instrucao-normativa-no-45-de-22-de-novembro-de-2016.pdf

20. Brasil. Ministério da Saúde. Plano de Ação Nacional de Prevenção e Controle da Resistência aos Antimicrobianos no Âmbito da Saúde Única. 2019;1. https://bvsms.saude.gov.br/bvs/publicacoes/plano_prevencao_resistencia_antimicrobianos.pdfantimicrobianos/plano-nacional-antimicrobianos-pan-br-14fev19-isbn.pdf/view

21. Rabello RF, Bonelli RR, Penna BA, Albuquerque JP, Souza RM, Cerqueira AMF. Antimicrobial resistance in farm animals in Brazil: An update overview. Animals (Basel). 2020;10(4):552. https://doi.org/10.3390/ani10040552

22. SENAI. Manual de Segurança e Qualidade para a Avicultura de Postura Manual de Segurança e Qualidade para a Avicultura de Postura. Embrapa. 2004;1–100.

23. Subirats J, Murray R, Scott A, Lau CHF, Topp E. Composting of chicken litter from commercial broiler farms reduces the abundance of viable enteric bacteria, Firmicutes, and selected antibiotic resistance genes. Science of the Total Environment. 2020;746:141113. https://doi.org/10.1016/j.scitotenv.2020.141113

24. Trindade, P. R. C. M., Dolabella, S. S., Jain, S., Nascimento, L. F. . de J., Sacramento, A. . G., Barbosa, F. H. F., & Teixeira Barbosa, A. A. (2024). Ocorrência e persistência de genes de resistência a antibióticos em estações de tratamento de esgoto em Aracaju/SE/Brasil. Scientia Plena, 20(10). https://doi.org/10.14808/sci.plena.2024.106201

25. Limayem A, Micciche A, Nayak B, Mohapatra S. Prokaryotic community profiling of local algae wastewaters using advanced 16S rRNA gene sequencing. Environmental Science and Pollution Research. 2018;25(1):704–711. https://doi.org/10.1007/s11356-017-0078-z

26. Cornejo J, Yevenes K, Avello C, Pokrant E, Maddaleno A, Martin BS, et al. Determination of chlortetracycline residues, antimicrobial activity and presence of resistance genes in droppings of experimentally treated broiler chickens. Molecules. 2018;23(6):1264. https://doi.org/10.3390/molecules23061264

27. Jia J, Guan Y, Cheng M, Chen H, He J, Wang S, et al. Occurrence and distribution of antibiotics and antibiotic resistance genes in Ba River, China. Science of the Total Environment. 2018;642:1136–1144. https://doi.org/10.1016/j.scitotenv.2018.06.149

28. Furlan JPR, dos Santos LDR, Moretto JAS, Ramos MS, Gallo IFL, Alves G de AD, et al. Occurrence and abundance of clinically relevant antimicrobial resistance genes in environmental samples after the Brumadinho dam disaster, Brazil. Science of the Total Environment. 2020;726:138100. https://doi.org/10.1016/j.scitotenv.2020.138100

29. Ayandiran TO, Falgenhauer L, Schmiede J, Chakraborty T, Ayeni FA. High resistance to tetracycline and ciprofloxacin in bacteria isolated from poultry farms in Ibadan, Nigeria. Journal of Infection in Developing Countries. 2018;12(6):462–470. https://doi.org/10.3855/jidc.9862

30. Collignon PJ, McEwen SA. One health-its importance in helping to better control antimicrobial resistance. Tropical Medicine and Infectious Disease. 2019;4(1):22. https://doi.org/10.3390/tropicalmed4010022

31. Gibson JS, Wai H, Oo SSML, Hmwe EMM, Wai SS, Htun LL, et al. Antimicrobials use and resistance on integrated poultry-fish farming systems in the Ayeyarwady Delta of Myanmar. Scientific Reports. 2020;10(1):16149. https://doi.org/10.1038/s41598-020-73076-2

32. Anyanwu MU, Jaja IF, Okpala COR, Jaja CJI, Oguttu JW, Chah KF, et al. Potential sources and characteristic occurrence of mobile colistin resistance (mcr) gene-harbouring bacteria recovered from the poultry sector: A literature synthesis specific to high-income countries. PeerJ. 2021;9:e11606. https://doi.org/10.7717/peerj.11606

33. Wang Y, Lyu N, Liu F, Liu WJ, Bi Y, Zhang Z, et al. More diversified antibiotic resistance genes in chickens and workers of the live poultry markets. Environment International. 2021;153:106534. https://doi.org/10.1016/j.envint.2021.106534

34. Xu S, Lu W, Qasim MZ. High-throughput characterization of the expressed antibiotic resistance genes in sewage sludge with transcriptional analysis. Ecotoxicology and Environmental Safety. 2020;205:111377. https://doi.org/10.1016/j.ecoenv.2020.111377

35. Yang Q, Tian T, Niu T, Wang P. Molecular characterization of antibiotic resistance in cultivable multidrug-resistant bacteria from livestock manure. Environmental Pollution. 2017 Oct 1;229:188–198. https://doi.org/10.1016/j.envpol.2017.05.073

36. Leungtongkam U, Thummeepak R, Tasanapak K, Sitthisak S. Acquisition and transfer of antibiotic resistance genes in association with conjugative plasmid or class 1 integrons of *Acinetobacter baumannii*. PLoS One. 2018;13(12):e0208468. https://doi.org/10.1371/journal.pone.0208468

37. Dionisio F, Zilhão R, Gama JA. Interactions between plasmids and other mobile genetic elements affect their transmission and persistence. Plasmid. 2019;102:29–36. https://doi.org/10.1016/j.plasmid.2019.01.003

38. Karim MR, Zakaria Z, Hassan L, Mohd Faiz N, Ahmad NI. Antimicrobial resistance profiles and co-existence of multiple antimicrobial resistance genes in mcr-harbouring colistin-resistant enterobacteriaceae isolates recovered from poultry and poultry meats in Malaysia. Antibiotics (Basel). 2023;12(6):1060. https://doi.org/10.3390/antibiotics12061060

39. OIE. World Organisation for Animal Health. List of Antimicrobial Agents of Veterinary Importance. 2021, (Resolution No. XXVIII) Available from: https://www.woah.org/app/uploads/2021/06/a-oie-list-antimicrobials-june2021.pdf

40. Belote BL, Tujimoto-Silva A, Hümmelgen PH, Sanches AWD, Wammes JCS, Hayashi RM, et al. Histological parameters to evaluate intestinal health on broilers challenged with *Eimeria* and *Clostridium perfringens* with or without enramycin as growth promoter. Poultry Science. 2018;97(7):2287–2294. https://doi.org/10.3382/ps/pey064

41. Yang Y, Ashworth AJ, Willett C, Cook K, Upadhyay A, Owens PR, et al. Review of antibiotic resistance, ecology, dissemination, and mitigation in U.S. broiler poultry systems. Frontiers in Microbiology. 2019;10:2639. https://doi.org/10.3389/fmicb.2019.02639

42. Umair M, Orubu S, Zaman MH, Wirtz VJ, Mohsin M. Veterinary consumption of highest priority critically important antimicrobials and various growth promoters based on import data in Pakistan. PLoS One. 2022;17(9):e0273821. http://doi.org/10.1371/journal.pone.0273821

43. Basit MA, Kadir AA, Loh TC, Aziz SA, Salleh A, Zakaria ZA, et al. Comparative efficacy of selected phytobiotics with halquinol and tetracycline on gut morphology, ileal digestibility, cecal microbiota composition and growth performance in broiler chickens. Animals (Basel). 2020;10(11):2150. https://doi.org/10.3390/ani10112150

44. Habib MA, Haque MA, Islam MS, Liton MR. Effect of dietary Halquinol supplementation on the productive performances, carcass traits and blood profile of Sonali chicken. Asian Journal of Medical and Biological Research. 2020;5(4):316–323. https://doi.org/10.3329/ajmbr.v5i4.45270

45. Díaz Carrasco JM, Redondo EA, Pin Viso ND, Redondo LM, Farber MD, Fernández Miyakawa ME. Tannins and bacitracin differentially modulate gut microbiota of broiler chickens. BioMed Research International. 2018;2018:1879168. https://doi.org/10.1155/2018/1879168

46. Yu Y, Zhao H, Lin J, Li Z, Tian G, Yang YY, et al. Repurposing non-antibiotic drugs auranofin and pentamidine in combination to combat multidrug-resistant gram-negative bacteria. International Journal of Antimicrobial Agents. 2022;59(5)106582. https://doi.org/10.1016/j.ijantimicag.2022.106582

47. Aranda MIR, Gómez GAT, De Barros M, Dos Santos MH, De Oliveira LL, Pena JL, et al. Antimicrobial and synergistic activity of 2,2',4-trihydroxybenzophenone against bacterial pathogens of poultry. Frontiers in Microbiology. 2019;10:490. https://doi.org/10.3389/fmicb.2019.00490

48. Li BB, Zhi LL, Peng ZY, Ma XX, Li J. Contrasting distribution of antibiotic resistance genes and microbial communities in suspended activated sludge versus attached biofilms in an integrated fixed film activated sludge (IFAS) system. Science of the Total Environment. 2020;742:140481. https://doi.org/10.1016/j.scitotenv.2020.140481

49. Diarra MS, Silversides FG, Diarrassouba F, Pritchard J, Masson L, Brousseau R, et al. Impact of feed supplementation with antimicrobial agents on growth performance of broiler chickens, *Clostridium perfringens* and enterococcus counts, and antibiotic resistance phenotypes and distribution of antimicrobial resistance determinants in *Escherichia coli* isolates. Applied and Environmental Microbiology. 2007;73(20):6566–6576. https://doi.org/10.1128/AEM.01086-07

50. Yang C, Rehman MA, Yin X, Carrillo CD, Wang Q, Yang C, et al. Antimicrobial resistance phenotypes and genotypes of *Escherichia coli* isolates from broiler chickens fed encapsulated cinnamaldehyde and citral. Journal of Food Protection. 2021;84(8):1385–1399. https://doi.org/10.4315/JFP-21-033

51. Shen W, Chen Y, Wang N, Wan P, Peng Z, Zhao H, et al. Seasonal variability of the correlation network of antibiotics, antibiotic resistance determinants, and bacteria in a wastewater treatment plant and receiving water. Journal of Environmental Management. 2022;317:115362. https://doi.org/10.1016/j.jenvman.2022.115362

52. Bengtsson-Palme J, Kristiansson E, Larsson DGJ. Environmental factors influencing the development and spread of antibiotic resistance. FEMS Microbiology Reviews. 2018;42(1):68–80. https://doi.org/0.1093/femsre/fux053

53. Xu F, Zeng X, Hinenoya A, Lin J. MCR-1 confers cross-resistance to bacitracin, a widely used in-feed antibiotic. mSphere. 2018;3(5):e00411-18. https://doi.org/10.1128/msphere.00411-18

54. Frost I, Laxminarayan R, McKenna N, Chai S, Joshi J. World Health Organization. Technical serie on primary health care: Antimicrobial resistance and primary health care. [Internet]. 2018;62p. Available from: https://iris.who.int/bitstream/handle/10665/326454/WHO-HIS-SDS-2018.56-eng.pdf

55. Agga GE, Kasumba J, Loughrin JH, Conte ED. Anaerobic digestion of tetracycline spiked livestock manure and poultry litter increased the abundances of antibiotic and heavy metal resistance genes. Frontiers in Microbiology. 2020;11:614424. https://doi.org/10.3389/fmicb.2020.614424

56. Ramos PI, Picão RC, Vespero EC, Pelisson M, Zuleta LF, Almeida LG, Gerber AL, Vasconcelos AT, Gales AC, Nicolás MF. Pyrosequencing-based analysis reveals a novel capsular gene cluster in a KPC-producing *Klebsiella pneumoniae* clinical isolate identified in Brazil. BMC Microbiology. 2012, 12:173. https://doi.org/10.1186/1471-2180-12-173