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Cefalosporin resistance genes and antibacterial activity of *Pereskia aculeata* against ESBL- producing *Escherichia coli* isolated from broiler chickens

Genes de resistência a cefalosporinas e atividade antibacteriana de *Pereskia aculeata* em *Escherichia coli* ESBL de frangos de corte

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Abstract: Antimicrobial resistance in *Escherichia coli* strains has contributed to the search for alternative antimicrobial products, such as plant extracts. The objective of this study was to evaluate the profile of cephalosporin-resistance genes in *E. coli* isolates with an extended-spectrum ß-lactamase (ESBL) production phenotype, and the phenolic composition and antibacterial activity of the ethanolic extract of *Pereskia aculeata* against strains isolated from 1-day-old and growing chickens. To determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the ethanolic extract of *P. aculeata*, *E. coli* isolates that exhibited an ESBL profile when isolated from either 1-day-old chicks or growing chickens were selected. The resistance genes $bla_{CTX\text{-}M\text{-}1}$, $bla_{CTX\text{-}M\text{-}2}$, $bla_{CTX\text{-}M\text{-}8}$, and bla_{SHV} were detected in a higher percentage of isolates from growing chickens. The *P. aculeata* extract was found to contain several phenolic compounds, with malic acid and rutin being predominant. Determination of MIC was only possible for two *E. coli* isolates (one from Broiler house A (growing chickens) and the other from Broiler house B (1-day-old chicks) - and for the standard strain *E. coli* ATCC 25922; in all cases, the MIC was 20 mg/mL. These findings indicate that *E. coli* isolates from both age groups carried cephalosporin-resistance genes and exhibit similar susceptibility to the *P. aculeata* extract, regardless of ESBL phenotype. These results highlight the need for further research into plant-based antimicrobials.

Key-words: $bla_{CTX\text{-}M\text{-}1}$; minimum inhibitory concentration; minimum bactericidal concentration; *Ora-pro-nóbis*.

Resumo: A resistência antimicrobiana em cepas de *Escherichia coli* tem contribuído para a busca por produtos alternativos ao seu uso, como os extratos vegetais. O objetivo deste estudo foi avaliar o perfil dos genes de resistência a cefalosporinas em isolados de *E. coli* com fenótipo de produção de beta-lactamase de espectro estendido (ESBL), a composição fenólica e a atividade antibacteriana do extrato etanólico de *Pereskia aculeata* contra cepas isoladas de frangos de um dia de vida e em crescimento. Para determinar

a Concentração Inibitória Mínima (CIM) e a Concentração Bactericida Mínima (CBM) do extrato etanólico de *P. aculeata*, foram selecionados isolados de *E. coli* que apresentaram perfil ESBL em uma das origens (pintos de um dia ou frangos em crescimento). Os genes de resistência $bla_{CTX\text{-}M\text{-}1}$, $bla_{CTX\text{-}M\text{-}2}$, $bla_{CTX\text{-}M\text{-}8}$ e bla_{SHV} foram detectados com maior percentual em isolados de frangos em crescimento. O extrato de *P. aculeata* apresentou diversos compostos fenólicos, sendo o ácido málico e a rutina os predominantes. A determinação da CIM foi possível em apenas dois isolados de *E. coli*, um do Aviário A (frangos em crescimento) e outro do Aviário B (pintos de um dia). Em ambos os casos, a CIM foi de 20 mg/mL. Na cepa ATCC 25922 de *E. coli*, utilizada como cepa padrão para a avaliação do teste, a CIM foi de 20 mg/mL. Conclui-se que isolados de *E. coli* provenientes de pintos de um dia e frangos em crescimento carregam genes de resistência a cefalosporinas, e que não há diferença na CIM do extrato etanólico de *P. aculeata* Mill. quando comparadas cepas com perfil fenotípico ESBL àquelas que não apresentam esse perfil, demonstrando a necessidade de estudos adicionais com plantas com potencial antimicrobiano.

Palavras-chave: $bla_{CTX\text{-}M\text{-}1}$; concentração inibitória mínima; concentração bactericida mínima; *Ora-pro-nóbis*.

1. Introduction

Poultry farming is a prominent economic activity at both national and international levels. Productivity in poultry farming can be enhanced via improvements in several aspects, including genetics, nutrition, production management, environment (facilities), and health [1]. The production of high-density broiler chickens is a strategy that aims to enhance productivity without incurring additional expenses for producers [2]. However, intensive high-density breeding may increase the risk of developing diseases [3], particularly when errors occur in animal management. This necessitates the use of additives as performance enhancers to increase growth rates and feed efficiency and reduce mortality [4].

The emergence of antimicrobial resistance has prompted changes in poultry farming approaches. Since 2010, the Tripartite Alliance formed by the Food and Agriculture Organization of the United Nations (FAO), World Organization for Animal Health (WOAH), and Pan American Health Organization (PAHO) has made a firm commitment to combat antimicrobial resistance to mitigate risks from the One Health perspective [5]. Among various pathogens of importance in poultry farming, *Escherichia coli* stands out for its role in the development of several infections and can act as a primary or secondary agent [6]. Risk factors associated with the emergence of antimicrobial resistance in pathogenic *E. coli* strains include the use of antimicrobials along with inadequate hygiene practices, which leads to selection pressure for avian pathogenic *E. coli* strains (APEC) [7].

Hu *et al.* [8] reported that APEC strains cause severe respiratory and systemic diseases in several bird species, posing significant problems for the poultry industry, along with challenges associated with food safety and animal welfare. Furthermore, APEC strains have been associated with ß-lactam-resistance genes [9], which makes it a One Health problem, with the genes most commonly detected in strains isolated from infected animals belonging to the bla_{TEM}, bla_{SHV}, and $bla_{CTX\text{-}M}$ families [10].

These challenges have motivated the search for additives, such as plant extracts, that can serve as alternatives to antimicrobials. In Brazil, the species *Pereskia aculeata* and Pereskia grandifolia are commonly known as Ora-pro-nóbis, and their leaves are consumed as food in some regions because of their high nutritional value, which includes minerals and proteins [11]. Many studies have evaluated the antioxidant properties of Ora-pro-nóbis leaves [12-13]; however, further studies are needed to assess their antimicrobial effects.

Nevertheless, evidence indicates that the *Pereskia aculeata* extract exhibits significant antimicrobial potential, as demonstrated by Pimenta *et al.* [14], showing positive results against both Gram-positive and Gram-negative bacteria as well as specific fungal genera. Garcia *et al.* [15] also observed the antimicrobial activity of Ora-pro-nóbis extracts against Gram-positive bacteria, including *Enterococcus faecalis, Listeria monocytogenes*, and methicillin-resistant *Staphylococcus aureus*, as well as Gram-negative bacteria, such as *E. coli*, *Klebsiella pneumoniae*, *Morganella morganii, Proteus mirabilis*, and *Pseudomonas aeruginosa*, highlighting the broad-spectrum action of the extract.

According to Moraes *et al.* [16], the presence of phenolic compounds in the leaves of *P. aculeata* explains its antioxidant activity. Differences in the extraction method were found to affect the activity, with the greatest activity noted in the acetone extract. Further, upon conducting a bibliometric analysis of articles discussing antioxidant activity, Agostini-Costa *et al.* identified that not only phenolics, but also other substances, such as carotenoids, are associated with antioxidant activity [12, 17].

Therefore, the objective of the present work was to detect genes encoding extended-spectrum β-lactamase (ESBL) enzymes (bla_{CTX}, bla_{TEM}, bla_{SHV}) in *E. coli* strains with an ESBL phenotypic profile isolated from 1-day-old chicks and growing broilers, and to determine the antibacterial activity of the ethanolic extract of *P. aculeata* Mill. against the isolated strains.

2. Material and methods

This study was approved by the Animal Use Ethics Committee (CEUA) of the Universidade Paranaense (UNIPAR), under protocol number 40065/2023. This study was conducted using *E. coli* isolates collected from 1-day-old chicks via cloacal swab samples and subsequently from the same batch when the chicks were in the growth phase (28 days of age). The samples were collected by veterinarians using a moistened urethral swab in Brain Heart Infusion (BHI) medium, compressing it with a rotary movement in the birds' cloaca.

2.1 Sample collection and processing

Samples were collected from two Broiler house (A and B) located in the northwestern region of the state of Paraná, with 10 samples collected from each aviary. Each sample consisted of a pool of 10 birds. Immediately after collection, the samples were stored in BHI medium, refrigerated, and processed at the Laboratory of Preventive Veterinary Medicine and Public Health of the Postgraduate Program in Animal Science with Emphasis on Bioactive Products at Universidade Paranaense, Brazil. *E. coli* isolates were identified using biochemical tests, including lysine iron agar, Simmons citrate, motility, indole, ornithine (MIO) medium, triple sugar iron (TSI), and urea, according to the methodology described by Quinn *et al.* [18].

The isolates were subjected to the synergistic double-disk test to identify ESBL-producing strains according to the methodology described by the Clinical and Laboratory Standards Institute (CLSI) [19]. Briefly, separate discs containing cefotaxime (CTX), ceftazidime (CAZ), ceftriaxone (CRO), and aztreonam (ATM) were positioned 20 mm from a disc containing amoxicillin/clavulanic acid (AMC). The isolates were considered ESBL producers based on the formation of an irregular zone of inhibition (ghost zone) between the composite disc and the disc of one of the beta-lactam antimicrobials.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the ethanolic extract of Ora-pro-nóbis (*P. aculeata* Mill.), only *E. coli* isolates that presented ESBL profiles or profile variations (positive-negative/negative-positive) between the origins (1-day-old chick or growing chicken) were selected, totaling eight isolates from Broiler house A and six isolates from Broiler house B. The standard strain of *E. coli* ATCC 25922 was used as a control.

2.2 Molecular analysis of the presence of ESBL or cephalosporin-resistance genes

From the isolated samples, DNA was extracted by boiling at 100 °C for 10 min, followed by centrifugation and removal of the supernatant to search for bla_{CTX}, bla_{TEM}, and bla_{SHV} genes (20-21) (Table 1). PCR was performed using the Promega PCR Master Mix (Promega, USA), and the amplified material was subject to electrophoresis on a 1.5% agarose gel and visualized via staining with Gel Red (Biotium). The presence of an amplified band at a specific region indicated a positive result.

Table 1. Primers used for DNA amplification and sequencing to examine the presence of ESBL genes.

Target	Gene	Pb	Oligonucleotide sequence (5' to 3')	Reference
bla_{ESBL}	$bla_{CTX\text{-}M\text{-}1}$	415	F- AAA AAT CAC TGC GCC AGT TC R- AGC TTA TTC ATC GCC ACG TT	19
	$bla_{CTX\text{-}M\text{-}2}$	552	F- CGA CGC TAC CCC TGC TAT T R- CCA GCG TCA GAT TTT TCA GG	
	$bla_{CTX\text{-}M\text{-}8}$	666	F- TCG CGT TAA GCG GAT GAT GC R- AAC CCA CGA TGT GGG TAG C	
	$bla_{CTX\text{-}M\text{-}9}$	205	F- CAA AGA GAG TGC AAC GGA TG R- ATT GGA AAG CGT TCA TCA CC	
	$bla_{CTX\text{-}M25}$	327	F- GCA CGA TGA CAT TCG GG R- AAC CCA CGA TGT GGG TAG C	
	bla_{TEM}	800	F- CAT TTC CGT GTC GCC CTT ATT C R- CGT TCA TCC ATA GTT GCC TGA C	20
	bla_{SHV}	713	F- CAC TCA AGG ATG TAT TGT G R- TTA GCG TTG CCA GTG CTC G	21

Pb: Pair of bases

2.3 Ethanol extract of *P. aculeata*

The leaves of Ora-pro-nóbis (*P. aculeata* Mill.) were collected from the Medicinal Garden of the Universidade Paranaense, located at latitude 23°46'10.9" S and longitude 53°16'39.6" W, in the city of Umuarama, state of Paraná, early in the morning, during the summer. Botanical material from Ora-pro-nóbis was deposited in the Horto Medicinal Herbarium under registration number 88 and was registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge, SisGen, under registration number AA63755.

After collection, the leaves were selected, washed, and dried in a forced-air oven at 35°C for 15 days. Subsequently, the material was crushed to a particle size of 850 µm. The powder was subjected to dynamic maceration with solvent renewal (cereal ethyl alcohol 70º GL) every 2 days for approximately 20 days in a proportion of 50 g to 1 L of solvent. At each solvent renewal step, the material was filtered using gauze and a funnel. The filtrate was stored and subsequently concentrated under reduced pressure in a rotary evaporator (Tecnal TE-211) at 35 °C until the crude extract was obtained. Subsequently, the extract was stored frozen at –20 °C until further use to determine the phenolic composition, MIC, and MBC.

2.4 Analysis of phenolic composition using high-performance liquid chromatography (HPLC) coupled to mass spectrometry (MS)

The extract was resuspended in methanol (MS grade) and purified using a solution of 1 M barium hydroxide and 5% zinc sulfate. It was then filtered through a PVDF hydrophobic membrane (pore size 0.45 µm and 25 mm diameter) and analyzed using HPLC. Phenolic compounds were detected using HPLC (Shimadzu, model NEXERA X2) coupled to a mass detector (MS/MS, Shimadzu, model 8050) and a C18 Shimadzu column (5 µm, 150 × 4.6 mm). For elution, a linear gradient composed of Milli-Q water (A) and methanol (B) was used as follows: 1–9 min (20% B), 10–15 min (40% B), and 16–30 min (10% B). The temperature was set at 35 °C, and the injection volume was 1 µL. The MS/MS detector was operated in scan mode for 15 min. Analysis for the presence of phenolic compounds was conducted using Insight Software (Shimadzu), based on the analytical curves (10–250 µg/L) of the following compounds: catechol, morin, isovanillin, gallic acid, quercetin, hydroxybenzaldehyde, naringenin, syringaldehyde, chlorogenic acid, syringic acid, protocatechuic acid, vanillic acid, salicylic acid, vanillin, ferulic acid, p-hydroxybenzoic acid, naringin, p-coumaric acid, caffeic acid, coniferyl aldehyde, sinapic acid, syringaldazine, catechin, sinapaldehyde, luteolin, rutin, theobromine, epicatechin, baicalin, chrysin, quinic acid, malic acid, kaempferol, coumarin, caffeine, resorcylic acid, nicotinic acid, and fumaric acid [22].

2.5 Determination of the MIC of *P. aculeata* ethanolic extract

MIC was determined using the microdilution method in 96-well plates with Mueller–Hinton broth according to the CLSI [23], with modifications for natural compounds. The extract was dissolved in Tween 80 and evaluated at concentrations ranging from 20 to 0.039 mg/mL. The bacterial suspension was standardized in saline solution (0.85%) according to the 0.5 McFarland scale (approximately 1.5 × 108 CFU/mL) and subsequently diluted 1:10 (1.5 × 107 CFU/mL). After serial microdilution (1:2) of the extract, 50 µL (1.5 × 105 CFU/mL) of each inoculum was added. Subsequently, the microplates were incubated for 24 h at 37 ºC, and the plates were read to determine the MIC using 2,3,5-triphenyltetrazolium chloride as a growth indicator. MIC was defined as the lowest concentration of the extract (mg/mL) that inhibited visible bacterial growth, as indicated by the addition of the growth indicator [24]. These assays were performed in triplicate.

2.6 Determination of the MBC of the ethanolic extract of *P. aculeata*

The MBC was determined by transferring 5 µL of the culture from each well of the plate used for MIC determination to a Mueller–Hinton agar plate using a replicator, and then incubating the plate at 36 °C for 24 h. MBC was determined based on the absence of visible bacterial growth after the incubation period (25).

2.7 Data analysis

Descriptive analysis of the data was performed by calculating absolute (n) and relative (%) frequencies.

3. Results and discussion

In the present study, 9 out of 10 (90%) *E. coli* isolates were identified from 1-day-old chicks on Broiler house A, and 6 out of 10 (60%) from Broiler house B. Among these isolates, the highest percentage of strains with an ESBL profile was found in Broiler house B (Figure 1). When the chicks reached 28 days of

age, fresh cloacal swabs were collected from Broiler houses A and B. In this case, 100% of the isolates were identified as *E. coli* (Figure 1). Of these isolates, 50% from both broiler houses presented ESBL profiles. Only isolates with an ESBL profile were evaluated for the presence of ESBL genes (bla_{CTX-M}, bla_{TEM}, and bla_{SHV}).

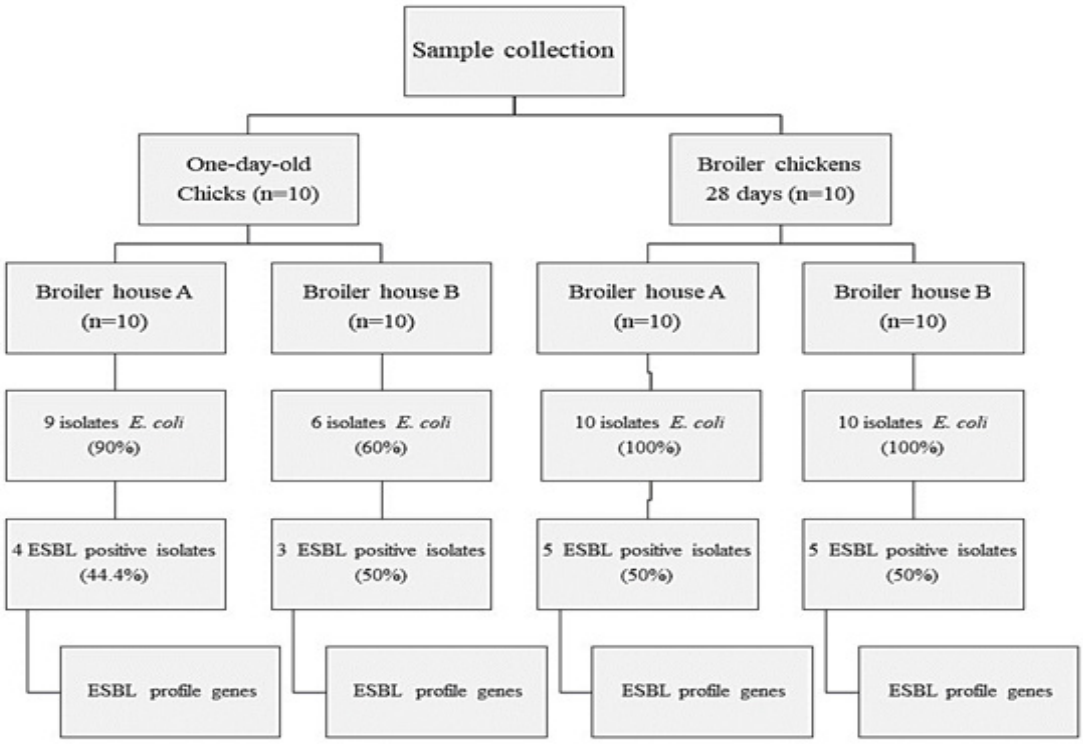

Figure 1. Flowchart of *E. coli* strain isolation results, ESBL phenotypic profile, and isolates selected for ESBL gene profile analysis (bla_{CTX-M}, bla_{TEM}, and bla_{SHV}).

Isolates from Broiler house A presented a lower number of isolates with an ESBL phenotypic profile (44.4%) in 1-day-old chicks (Figure 1), and this percentage increased numerically in isolates from growing chickens (50%). In contrast, the isolates from poultry farm B exhibited a 50% ESBL phenotypic profile even at the chick stage.

According to Dame-Korevaar *et al.* [26], chickens are important reservoirs of ESBL-producing bacterial strains. In their review aimed at describing possible transmission routes for ESBL-producing strains, the authors identified vertical transmission routes (via contamination of the ovary or vagina, or contamination of the eggshell) in the hatchery, horizontal transmission between aviaries, and indirect horizontal transmission between poultry houses through contact with humans, other animals, equipment, and the environment.

Considering that the samples obtained from 1-day-old chicks were taken at the time of unloading the birds on the farm, it is assumed that contamination of these birds with *E. coli* strains with an ESBL profile may have occurred vertically, during the eggs' stay in the hatchery, or even during hatching. It should also be noted that the increase in the percentage of ESBL-producing strains when the birds were 28-days-old (growth phase) can be explained by environmental contamination, as studies have reported the presence of ESBL-producing strains in the litter or creation environment [27,28], flies [29], and drinking water [30], among others.

On the other hand, Dierikx *et al.* [31] evaluated the presence of ESBL-producing *E. coli* strains using the phenotypic method. They confirmed their findings using a genotypic method in broiler chicken farms in the United Kingdom and Ireland. The authors found that the prevalence of ESBL-positive birds increased in the first week, from 0 to 24% to 96 to 100%, regardless of the use of antimicrobials, and remained at 100% until slaughter, corroborating the increase in ESBL-positive strains in growing birds compared with day-old chicks observed in aviary A.

Among the isolates with an ESBL phenotypic profile submitted for the analysis of resistance gene profiles, 100% and 66.6% of isolates from 1-day-old chicks from Broiler house A and B, respectively, harbored the $bla_{\text{CTX-M-1}}$ gene, which was higher than the prevalence of the $bla_{\text{CTX-M-2}}$ and $bla_{\text{CTX-M-8}}$ genes. However, in *E. coli* strains isolated from growing chickens, a higher percentage was found for the $bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-2}}$ genes were present in an equal percentage of isolates from Broiler house A and B (80% and 60%, respectively). None of the *E. coli* strains isolated from broiler chickens in poultry Broiler house A harbored the $bla_{\text{CTX-M-8}}$ gene (Table 2). Notably, none of the *E. coli* strains from either farm harbored the $bla_{\text{CTX-M-9}}$, $bla_{\text{CTX-M-25}}$, and bla_{TEM} genes.

Kim *et al.* [9], evaluating the presence of ß-lactam-resistance genes in APEC isolates from liver lesions of chickens with colibacillosis in South Korea, detected the presence of $bla_{\text{TEM-1}}$, $bla_{\text{CTX-M-1}}$, and $bla\text{CTX}_{\text{-M-15}}$ in 29.1%, 5.1%, and 3.8% of the isolates, respectively. Notably, the prevalence of the $bla_{\text{CTX-M-1}}$ gene in the study by Kim et al. was lower than that observed in the present study, despite the isolates evaluated in this study being obtained from batches of healthy broiler chickens.

Ilyas *et al.* [30] detected $bla_{\text{CTX-M}}$, $bla_{\text{CTX-M-1}}$, and bla_{TEM} in 71.2%, 67.5%, and 62.2% of *E. coli* strains, respectively, isolated from the ceca of birds originating from live poultry markets in various areas of Islamabad and Rawalpindi, Pakistan, which partially corroborates the percentages found in the present study. Tseng, Liu, and Liu [10] reported that the most commonly detected genes in animals are those from the bla_{TEM}, bla_{SHV}, and $bla_{\text{CTX-M}}$ families; however, in the present work, no bla_{TEM} gene was detected.

According to Kim *et al.* [9], the variety of beta-lactam-resistance genes identified, along with their association with plasmids, facilitates transmission between bacteria, highlighting the need for continuous monitoring to track strains of *E. coli* pathogenic to birds on poultry farms. Koga *et al.* [32] further emphasized that the production of ESBL confers multi-resistance and poses a health risk, as they are often associated with plasmids, facilitating transmission between bacteria from different hosts and suggesting zoonotic risks.

Determination of MIC was only possible for two *E. coli* isolates, one from Broiler house A, isolated from growing chickens, and the other from Broiler house B, which were isolated from 1-day-old chicks. In both cases, the MIC of the extract was 20 mg/mL. Additionally, the MIC of the extract against the standard strain of E. coli ATCC 25922 was also 20 mg/mL.

Table 2. Frequency of resistance genes to cephalosporins in *E. coli* isolates from 1-day-old chicks and growing broilers, and the minimum inhibitory concentration (MIC) of the ethanol extract of *P. aculeata* Mill.

ID	Broiler house	ESBL Profile	Gene profile of 1-day-old chicks					ESBL Profile	Gene profile of growing chicken				
			bla$_{CTX}$			*bla*$_{SHV}$	MIC		*bla*$_{CTX}$			*bla*$_{SHV}$	MIC
			M-1	M-2	M-8				M-1	M-2	M-8		
1	A	+	+	+	-	-	>20	-	N/E	N/E	N/E	N/E	>20
2	A	+	+	-	+	-	>20	-	N/E	N/E	N/E	N/E	>20
3	A	-	N/E	N/E	N/E	N/E	>20	+	-	+	-	-	>20
5	A	-	N/E	N/E	N/E	N/E	>20	+	+	+	-	-	>20
6	A	-	N/E	N/E	N/E	N/E	>20	+	+	+	-	-	>20
7	A	+	+	+	-	+	>20	+	+	-	-	+	20
8	A	+	+	+	-	-	>20	-	N/E	N/E	N/E	N/E	>20
10	A	-	N/E	N/E	N/E	N/E	>20	+	+	+	-	-	>20
TOTAL			4/4 (100%)	3/4 (75%)	1/4 (25%)	1/4 (25%)			4/5 (80%)	4/5 (80%)	0 (0%)	1/5 (20%)	
1	B	+	+	-	-	-	>20	-	N/E	N/E	N/E	N/E	>20
2	B	+	-	+	-	+	>20	+	+	-	-	+	>20
3	B	-	N/E	N/E	N/E	N/E	20	+	+	+	-	-	>20
8	B	-	N/E	N/E	N/E	N/E	>20	+	+	-	+	+	>20
9	B	+	+	-	+	-	>20	+	-	+	+	-	>20
10	B	-	N/E	N/E	N/E	N/E	>20	+	-	+	-	-	>20
TOTAL			2/3 (66.6%)	1/3 (33.3%)	1/3 (33.3%)	1/3 (33.3%)			3/5 (60%)	3/5 (60%)	2/5 (40%)	2/5 (40%)	

ID: Identification; MIC: minimum inhibitory concentration; N/E: not evaluated; + = positive; - = negative.

Garcia *et al.* [15] confirmed the antimicrobial activity of *P. aculeata* leaf extract against Gram-positive bacteria (*Enterococcus faecalis, Listeria monocytogenes*, and methicillin-resistant *Staphylococcus aureus*) and Gram-negative (*E. coli, Klebsiella pneumoniae, Morganella morganii, Proteus mirabilis*, and *Pseudomonas aeruginosa*). Similarly, Colacite *et al.* [33] evaluated the methanolic and ethanolic extracts of the Ora-pro-nóbis leaf and found that the methanolic extract inhibited the growth of *K. pneumoniae* strains; however, they did not observe inhibition of *E. coli* strains.

These differences in antimicrobial activity may also be related to the bioactive compounds extracted from the plants. In this study, 14 phenolic compounds were detected, with malic acid exhibiting the highest concentration, followed by rutin and fumaric acid (Table 3).

Table 3. Mean ± standard deviation of the concentration (mg/100 g) of compounds present in the ethanolic extract of *P. aculeata* Mill. leaves detected using high-performance liquid chromatography.

Compounds	Mean ± standard deviation (mg/100g)
Caffeic acid	0.56±0.02
Chlorogenic acid	0.13±<0.01
Ferulic acid	1.17±0.05
Fumaric acid	4.32±0.10
Malic acid	46.12±0.99
Nicotinic acid	1.73±0.05
P-coumaric acid	1.65±0.07
P-hydroxybenzoic acid	1.33±0.08
Protocatechuic acid	1.98±0.01
Resorcylic acid	1.95±<0.01
Syringic acid	0.43±0.02
Vanillic acid	1.10±0.02
Isovaniline	1.68±0.07
Routine	20.66±0.98

Maciel *et al.* [34] reported the presence of coumarins, phenolic compounds, flavonoids, and tannins in an aqueous extract of *P. aculeata*. Garcia *et al.* [15] reported the presence of caffeic acid, caftaric acid, quercetin-3-O-rutinoside, kaempferol, and isorhamnetin-O-pentoside-O-rutinoside in the hydroethanolic extract of *P. aculeata*, demonstrating variations in the isolated bioactive compounds.

Macedo *et al.* [35] observed higher levels of chlorogenic acid (35-52 mg 100 g−1) and caffeic acid (~3 mg 100 g−1) in hydroethanolic extracts of *P. aculeata* leaves. In this study, the MIC for the Gram-positive bacterium *S. aureus* was determined to be 50 mg/mL, and for E. coli, it was not possible to determine the MIC [35], which corroborates the findings of the present study. Among the bioactive compounds evaluated, Wong *et al.* [36] detected the presence of quercetin in the aqueous extract of another species, P. bleo; however, as in the present study, they did not detect the presence of gallic acid and catechin. These studies suggest that there is a variation in the phenolic content in *P. aculeata* extracts, and this study contributes to elucidating this composition, as several phenolic compounds not previously reported in the literature for this species were detected.

The maximum concentration of the extract evaluated in this study was 20 mg/mL. Pandini *et al.* [37] classified extract concentrations of 12.5–25 mg/mL as moderately active, demonstrating that it is possible to evaluate higher concentrations of the extract. When comparing the strains for which MIC

determination was possible, only one of them presented an ESBL profile (Table 2), demonstrating that new studies are necessary to verify the possible relationship between bacteria with a resistance profile and different genes associated with resistance.

In veterinary medicine, *E. coli* infections are prevalent in the daily lives of those who work with farm animals [38]. Studies have demonstrated that strains of avian origin can cause infections in experimental mammalian models, thus strengthening the zoonotic potential thesis [39]. However, there is no consensus regarding the health risks associated with handling products of poultry origin or consumption, underscoring the need for further studies [38].

4. Conclusion

The results of this study indicated a presence of resistance genes in ESBL-producing *E. coli* isolates from broiler chickens, validating the growing concern over the spread of multidrug-resistant bacteria in poultry farms. Additionally, the ethanolic extract of *P. aculeata* showed limited antibacterial activity against these isolates, suggesting the need for improvements in extraction methods or their use in combination with other compounds. Future studies should investigate various extraction techniques, formulations, and concentrations, as well as conduct *in vivo* assays to further enhance the potential use of natural additives as viable alternatives to conventional antimicrobial agents.

Supplementary material

Graphical Abstract (only available in the electronic version).

Conflicts of interest statement

The authors declare that they have no conflict of interest.

Data availability statement

Data will be provided upon request.

Author contributions

Conceptualization: L. K. Otutumi and T. S. Mezalira. Data curation: L. K. Otutumi and Z. C. Gazim. Formal analysis: L. K. Otutumi. Funding acquisition: L. K. Otutumi. Project management: R. G. Ferreira, T. S. Mezalira, and D. A. Marchi. Methodology: L. K. Otutumi, Z. C. Gazim, R. K. K. Kobayashi, and B. C. B. Barros. Supervision: L. K. Otutumi, S. P. Ruiz, and Z. C. Gazim. Investigation: T. S. Mezalira, R. G. Ferreira, D. A. Marchi, G. C. C. Silva, J. S. Silva, D. L. G. Silva, L. A. Reati, and M. F. Menck-Costa. Visualization: R. G. Ferreira, L. K. Otutumi, and S. P. Ruiz. Writing (original draft): R. G. Ferreira. Writing (proofreading and editing): D. A. Marchi, L. K. Otutumi, and B. C. B. Barros.

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