JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Colistin and β-lactam resistance in *Escherichia coli* isolates from bovines, swine, and humans

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Abstract

Introduction: Colistin and β-lactams are widely investigated because of their effectiveness in the treatment of human diseases. This study investigated the phenotypic and genotypic profiles of colistin- and β-lactam-resistant *Escherichia coli* (n = 235) obtained from bovines, swine, and workers from a mixed slaughterhouse in Brazil.

Methodology: The disk diffusion method was used to test the resistance against β-lactams (amoxicillin, ampicillin, cefaclor, cefazolin, cefepime, cefotaxime, ceftazidime, ceftriaxone, imipenem, meropenem, and aztreonam). In order to test colistin resistance, the isolates were subjected to the minimum inhibitory concentration (MIC) technique using the broth microdilution method (BMD; 0.5 to 16 µg/mL) and polymerase chain reaction (PCR) assays targeting colistin- (*mcr-1* to *mcr-5*) and β-lactam- (*blaTEM*, *blaCTX-M*, *blaSHV*, *ampC*) genes. The *pmrAB* mutation was further investigated.

Results: The isolates presented resistance, especially to ampicillin (cattle: 14/106, swine: 62/100, humans: 10/29) and amoxicillin (cattle: 7/106, swine: 61/100, humans: 8/29). One swine isolate was characterized as extended spectrum β-lactamase (ESBL) producer. The isolates obtained from swine presented higher frequencies of colistin resistance (13/100) when compared to isolates from bovines (5/106) and humans (0/29). Molecular assays concluded that the isolates presented *blaTEM* (swine: 67/100, humans: 7/29), *ampC* (swine: 1/100), and *blaCTXM* (swine: 1/100). The *pmrAB* complex presented mutations (T31S, P42A, I128N, G144S, H2R, N358Y, D283G, K15I).

Conclusions: This study highlights the presence of antimicrobial resistance and presents a method to verify these factors in the animal production chain.

Key words: *bla*; *mcr*; *pmrAB*; resistance.

J Infect Dev Ctries 2025; 19(1):49-57. doi:10.3855/jidc.20143

(Received 22 March 2024 – Accepted 24 June 2024)

Introduction

The emergence of antimicrobial resistance amongst bacteria is a worldwide concern since the ineffectiveness of antibiotics jeopardizes medical treatment and represents a threat to public health. This issue has intensified over time, and one of the reasons is the usage of antibiotics in the food production chain [1,2].

Among the bacteria associated with antimicrobial resistance, *Escherichia coli* plays an important role in the food production chain, contributing significantly to the dissemination of resistance genes among animals, humans, and the environment [3]. *E. coli* frequently has resistance to several antibiotics, such as beta-lactams — the broadest class of antibiotics and most commonly used for the treatment of infections caused by Gram-negative bacteria. However, due to alterations in porins and penicillin-binding proteins, or due to the production of enzymes called beta-lactamase (usually associated with *blaTEM*, *blaCTX-M*, *blaSHV*, *ampC*), the treatment of these infections can be seriously compromised [4,5].

Colistin (COL) is another antibiotic of concern worldwide. COL was not used for a certain period of time since its discovery due to its toxicity and was replaced by less toxic antibiotics. However, with the increase in antimicrobial resistance, its use has resumed in recent decades, making it a last-choice drug [3,6,7]. Unfortunately, resistance to COL has also been reported in *E. coli* due to factors such as changes in the structure and charge of the lipopolysaccharide (LPS) structure of COL, mutations in the two-component system

(*PmrA*/*PmrB* and *PhoP*/*PhoQ*), and the presence of genes in mobile elements (*mcr*) [1,8].

These antibiotics are widely used in the food production chain, and resistance to them has been frequently reported in *E. coli* isolated from livestock. Considering that Brazil is a major producer and exporter of beef and pork, it is of great importance to analyze the panorama of bacterial resistance associated with farmed animals and workers involved in these production chains. Therefore, the aim of this work was to investigate the resistance profiles of β-lactams and colistin in *E. coli* isolates from cattle, swine, and workers from a slaughterhouse that processes swine and cattle.

Methodology

A total of 235 *E. coli* isolates from bovine (n = 106) and swine (n = 100) feces collected at the rectus occlusion step, and slaughterhouse workers' (n = 29) feces were selected for this study. Briefly, the samples were collected in 2019 from a meat processing plant of bovines and swine located at Uberlândia, Minas Gerais, Brazil; by the official federal inspection services. This slaughterhouse processed 170 bovine and 200 swine per week.

Informed consent was obtained from all humans for feces collection. The protocol was approved by the Ethics Committee for Research with Human Beings — Comitê de Ética em Pesquisa (CEP) com Seres Humanos (protocol number 3.366.306), and the Commission for Ethics in the Use of Animals of the Universidade Federal de Viçosa (UFV) Comissão de Ética no Uso de Animais (CEUA) da UFV (protocol number 49/2019).

All isolates were reevaluated by microbiological analyses, cultured on MacConkey agar, and subjected to biochemical tests — motility, gas production, urease, H_2S production, indole production, lysine decarboxylation, and citrate production. The same methodology was used to investigate *E. coli* in all the samples.

Phenotypical antimicrobial resistance for β-lactams and colistin

The *E. coli* isolates were characterized by their β-lactam resistance profiles against 11 antibiotics: amoxicillin (AMO, 10 μg), ampicillin (AMP, 10 μg), cefaclor (CFC, 30 μg), cefazolin (CFZ, 30 μg), cefepime (CPM, 30 μg), cefotaxime (CTX, 30 μg), ceftazidime (CAZ, 30 μg), ceftriaxone (CRO, 30 μg), imipenem (IPM, 10 μg), meropenem (MPM, 10 μg), and aztreonam (ATM, 30 μg), using the disk diffusion assay as described by Bauer *et al*. [9]. Briefly, the isolates were cultivated in brain heart infusion (BHI; OXOID, Basingstoke, UK) at 37 °C overnight, adjusted to 0.5 McFarland standard, and plated onto Müller–Hinton agar (OXOID, Basingstoke, UK). The 11 antibiotic disks were added to each isolate plate and incubated at 37 °C for 24 hours. After incubation, the inhibitory zone was read, following the protocol of CLSI [10], and the isolates characterized as resistant or susceptible.

The isolates that were resistant to CPM, CTX, CAZ, or CRO were subjected to the double-disk diffusion assay test for extended spectrum β-lactamase (ESBL) detection [10,11]. For ESBL detection, the isolates were grown in BHI (OXOID), normalized in 0.5 of McFarland standard, and plated on Müller–Hinton agar plates (OXOID, Basingstoke, UK) with five antibiotic disks (CRO 30 μg; CTX, 30 μg; CPM, 30 μg; CAZ, 30 μg; and amoxicillin–clavulanic acid, 30 μg). The plates were incubated at 37 °C for 20 hours, and then the isolates were considered positive for ESBLs based on the presence of a "ghost zone" — an enlargement of the inhibition zone between the disks [11].

The isolates were subjected to the minimum inhibitory concentration (MIC) following the protocol to CLSI for investigating colistin resistance [10]. The *E. coli* isolates were reactivated in BHI broth (OXOID, Basingstoke, UK) and incubated overnight at 37 °C. Subsequently, they were spread on MacConkey agar and incubated under the same conditions. Colonies from all isolates were added to tubes with Müller–Hinton II cation-adjusted broth (BD, OXOID, Basingstoke, UK) up to the 0.5 McFarland standard concentration. After this, 20 μL of the bacterial suspension was added to each well of a sterilized 96-well plate containing 180 μL of Müller–Hinton II cation-adjusted broth with colistin sulfate concentrations of 0.5, 1, 2, 4, 8, and 16 μg/mL. The plate was incubated at 33 °C for 20 hours, the inhibitory concentration was annotated, and the isolate was characterized according to the standards established by the European Committee on Antimicrobial Susceptibility Testing (EUCAST). A concentration > 2 μg/mL were defined as resistant, and ≤ 2 μg/mL as sensitive [12]. All tests were performed in duplicate. *E. coli* ATCC 25922 was used as a quality control strain for all tests.

Investigation of resistance genes for COL and β-lactam

All isolates used in this experiment were investigated for resistance genes. The DNA of each *E.*

coli isolate was obtained by the boiling technique. The isolates were screened by polymerase chain reaction (PCR) [13–18] for the presence of *mcr-1*, *mcr-2*, *mcr-3*, *mcr-4*, and *mcr-5* (COL); and *blaTEM*, *blaCTX-M*, *blaSHV*, *ampC* (β-lactam) genes (Supplementary Table 1). The PCR mixtures for the amplification were composed of GoTaq Green Master Mix (Promega, Madison, USA), 200 nMol of each primer, 5 µL DNA samples, and nuclease-free water up to a final volume of 25 µL. The PCR products were electrophoresed in 1.5% agarose gel, stained with Unisafe® intercalator (UniScience, Osasco, Brazil), and visualized using L-PIX-HE (Loccus Biotecnologia, Cotia, Brazil). All positive controls used in this study were wild type strains that were whole genome sequenced (WGS) and from our collection.

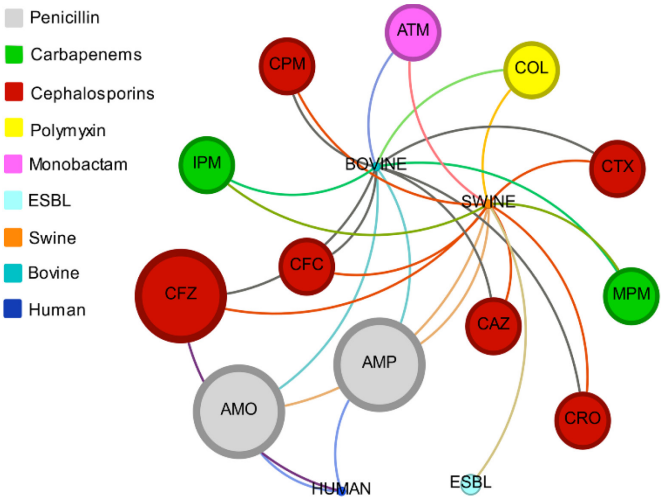
Investigation of colistin mutation at the pmrAB complex

The *pmrAB* gene complex of the isolates that showed COL-resistance were sequenced. Briefly, *pmrA* and *pmrB* were amplified by the PCR technique [19] (Supplementary Table 1). The amplified products were purified using the kit Wizard® SV Gel and the PCR Clean-Up System (Promega, Madison, USA), and the product was sequenced by an external service provider (ACTGene Análises Moleculares, Brazil). The sequences and mutations were analyzed using the software MEGA 11 [20]. The *pmrA* and *pmrB* genes of the isolates were compared with *E. coli* ATCC 25922 (TaxID: 1322345; Locus: CP009072.1), and the mutation regions were annotated.

Statistical analysis

The antimicrobial resistance among *E. coli* isolates was compared by Chi-square or Fisher's exact test (0 = sensitive and 1 = resistant) using RStudio software [21], and $p < 0.05$ was considered as the significance level. The GEPHI figure was built using the modularity algorithm [22].

Figure 1. Networking analysis comparing the groups (bovine, swine, and human) to the phenotypical antimicrobial resistance profile. Higher diameters indicate strong presence.



Results

In our analysis of phenotypical antimicrobial resistance, β-lactam resistance was detected in 59.6% (140/235) of all isolates tested, highlighting resistance to AMP and AMO in all production chains (Table 1 and Figure 1). We also detected resistance to other antibiotics, albeit at lower frequencies and mainly in swine. *E. coli* of human origin showed resistance only to the penicillin group and to CFZ. In addition, carbapenem resistance was identified only in 1 isolate from bovines and 3 from swine. There was no significant difference ($p > 0.05$) in antibiotic resistance found among the isolates from the 3 groups (bovine, swine, and human).

Among all samples, only 1 isolate from swine was characterized as an ESBL producer. Among the β-lactam genes, *blaTEM* was detected in samples from swine (67%, 67/100), and humans (24.14%, 7/29); *ampC* was detected at low frequencies in samples from

Table 1. Frequency of antibiotic resistance in *Escherichia coli* isolates from bovine, swine and human feces.

Group	Antibiotics	Phenotypical resistance (%)			
		Bovine (n = 106)	Swine (n = 100)	Human (n = 29)	Total (n = 235)
Polymyxin	Colistin	4.7	13	0	7.7
Penicillin	Ampicillin	13.2	62	34.5	36.6
	Amoxicillin	6.6	61	27.6	32.3
Cephalosporins	Cefazolin	2.8	39	27.6	21.3
	Cephaclor	0.9	11	0	5.1
	Cefotaxime	1.9	12	0	6.0
	Ceftriaxone	1.9	10	0	5.1
	Ceftazidime	1.9	10	0	5.1
	Cefepime	0.9	5	0	2.6
Carbapenems	Imipenem	0.9	2	0	1.3
	Meropenem	0.9	2	0	1.3
Monobactams	Aztreonam	2.8	12	0	6.4

Table 2. All characteristics (phenotypical resistance profile, β-lactams genes, colistin genes, MIC breakpoint, and pmrAB complex mutations) of the isolates resistant to colistin.

Isolate	Species	Phenotypical resistance profile	β-lactams				Colistin							
			blaTEM	*blaSHV*	*blaCTXM*	*AmpC*	MIC	*pmrA*	*pmrB*	*mcr-1*	*mcr-2*	*mcr-3*	*mcr-4*	*mcr-5*
152	Bovine	COL, AMP	-	-	-	-	4	T31S, I128N, G144S	H2R	-	-	-	-	-
173	Bovine	COL	-	-	-	-	4	T31S, I128N, G144S	H2R, N358Y	-	-	-	-	-
196	Bovine	COL	-	-	-	-	> 16	T31S, I128N, G144S	H2R, N358Y	-	-	-	-	-
208	Bovine	COL	-	-	-	-	> 16	T31S, I128N	H2R, N358Y	-	-	-	-	-
209	Bovine	COL	-	-	-	-	> 16	T31S, I128N	H2R, N358Y	-	-	-	-	-
301	Swine	COL, CAZ, ATM	-	-	-	-	16	T31S, I128N, G144S	H2R, N358Y	-	-	-	-	-
303	Swine	COL	+	-	-	+	> 16	T31S, I128N, G144S	H2R, D283G	-	-	-	-	-
309	Swine	COL, AMP, AMO	+	-	-	-	> 16	T31S, I128N, G144S	H2R, D283G	-	-	-	-	-
332	Swine	COL, AMP, AMO	+	-	-	-	> 16	T31S, P42A, I128N, G144S	H2R, D283G	-	-	-	-	-
348	Swine	COL,AMP,AMO,CFZ,CFC,CTX,CRO,CAZ,CPM,ATM	+	-	-	-	> 16	T31S, I128N, G144S	H2R, N358Y	-	-	-	-	-
369	Swine	COL,AMP,AMO,CTX,CRO,CAZ,ATM	+	-	-	-	> 16	T31S, I128N, G144S	H2R, D283G	-	-	-	-	-
377	Swine	COL,CFZ,CFC,CTX,CRO,CAZ,CPM, ATM	+	-	-	-	> 16	None	K15I	-	-	-	-	-
394	Swine	COL, AMP, AMO	+	-	-	-	16	T31S, I128N, G144S	H2R, D283G	-	-	-	-	-
399	Swine	COL, AMP, AMO	+	-	-	-	4	T31S, I128N, G144S	H2R, D283G	-	-	-	-	-
415	Swine	COL,AMP,AMO,CFZ,CFC,CTX,CRO,CAZ,CPM,IPM,ATM	+	-	-	-	> 16	None	none	-	-	-	-	-
422	Swine	COL, CFC	+	-	-	-	> 16	T31S, I128N, G144S	H2R, D283G	-	-	-	-	-
427	Swine	COL, AMP, AMO	+	-	-	-	4	none	H2R	-	-	-	-	-
436	Swine	COL, AMP, AMO, CFZ	+	-	-	-	4	T31S, I128N, G144S	H2R	-	-	-	-	-

Resistance to colistin was tested by the minimum inhibitory concentration (MIC) method, and resistance to β-lactams was tested by the disc diffusion assay. AMO: amoxicillin; AMP: ampicillin; ATM: aztreonam; CAZ: ceftazidime; CFC: cefaclor; CFZ: cefazolin; COL: colistin; CPM: cefepime; CRO: ceftriaxone; CTX: cefotaxime; IPM: imipenem; MPM: meropenem.

swine (1%, 1/100); and only 1 isolate from swine was positive for *blaCTXM* (1%, 1/100).

A total of 7.7% (18/235) *E. coli* isolates showed phenotypic resistance to COL. The isolates from swine showed the highest resistance to this antibiotic (13.0%), followed by the isolates from bovines (4.7%). None of the tested isolates presented *mcr* (*mcr-1* to *mcr-5*). However, sequence analysis showed various mutations in the *pmrAB* complex, such as T31S, I128N, G144S, P42A, in *pmrA*; and H2R, N358Y, D283G, K15I, H2R, in *pmrB* (Table 2).

The antibiotic resistance profiles varied, and there were a total of 25 different combinations (Table 3). It is important to highlight that among the species, swine presented the highest number of resistance profiles (67), whereas many bovine and human isolates did not show any resistance.

The swine isolates exhibited 21 antibiotic resistance profiles; the bovine isolates had 7 resistance profiles,

Table 3. Antibiotic resistance profiles of *Escherichia coli* isolates from bovine, swine, and human feces.

Antibiotic resistance profile	Species (isolates)			Total
	Bovine	Swine	Human	
COL	4	1	-	5
AMP	1	-	-	1
AMO	1	-	-	1
ATM	1	1	-	2
COL, AMP	1	-	-	1
COL, CFC	-	1	-	1
AMP, AMO	-	18	1	19
COL, AMP, AMO	-	5	-	5
COL, CAZ, ATM	-	1	-	1
AMP, AMO, CFZ	-	25	6	31
COL, AMP, AMO, CFZ	-	1	-	1
AMP, AMO, CFZ, CFC	-	2	-	2
AMP, AMO, CFZ, CTX	-	1	-	1
AMP, AMO, CFZ, CFC, CTX	-	1	-	1
CTX, CRO, CAZ, CPM, ATM	-	1	-	1
AMP, AMO, CTX, CRO, CAZ, ATM	1	-	-	1
COL, AMP, AMO, CTX, CRO, CAZ, ATM	-	1	-	1
AMP, AMO, CFZ, CTX, CRO, CAZ, ATM	-	1	-	1
COL, CFZ, CFC, CTX, CRO, CAZ, CPM, ATM	-	1	-	1
AMP, AMO, CFZ, CFC, CTX, CRO, CAZ, ATM	-	1	-	1
AMP, AMO, CFZ, CFC, CTX, CRO, MPM, ATM	-	1	-	1
AMP, AMO, CFZ, CFC, CTX, CRO, CAZ, ATM, ESBL	-	1	-	1
COL, AMP, AMO, CFZ, CFC, CTX, CRO, CAZ, CPM, ATM	-	1	-	1
COL, AMP, AMO, CFZ, CFC, CTX, CRO, CAZ, CPM, IPM, ATM	-	1	-	1
AMP, AMO, CFZ, CFC, CTX, CRO, CAZ, CPM, IPM, MPM, ATM	1	1	-	2
Total	**10**	**67**	**7**	**84**

Resistance to colistin was tested by the minimum inhibitory concentration (MIC) method, and resistance to β-lactams was tested by the disc diffusion assay. AMO: amoxicillin; AMP: ampicillin; ATM: aztreonam; CAZ: ceftazidime; CFC: cefaclor; CFZ: cefazolin; COL: colistin; CPM: cefepime; CRO: ceftriaxone; CTX: cefotaxime; ESBL: extended-spectrum β-lactamase; IPM: imipenem; MPM: meropenem.

and the human isolates had 2 resistance profiles. The profiles AMP-AMO-CFZ, COL, and AMP-AMO-CFZ, respectively, were the most identified in each species from this study.

Discussion

Overall, 7.7% (18/235) of the isolates showed resistance to COL, mostly isolates from swine (13%) followed by those from bovines (4.7%). Studies conducted on bovine feces in different regions of China have reported varying frequencies of COL resistance. Zhang *et al.* [23] reported a frequency of 26.92%, whereas Hassen *et al.* [24] reported lower frequencies, indicating that the prevalence of COL resistance can vary according to the region. However, the use of COL in the beef production chain is uncommon.

The pork production chain showed a higher range of COL-resistance frequencies. In this study, the frequency was 13%, which is low compared to the findings of Huang *et al.* [25] and Zhang *et al.* [23], who reported frequencies of 24.3% and 54.25%, respectively. COL-resistant isolates can be attributed to the intensive rearing system employed for these animals to prevent diseases during this period, which justifies the administration of this antibiotic [2,23,25].

The use of COL in the pork production chain started in the 1960s, mainly to treat diseases or due to its metaphylactic or prophylactic actions. After its discovery, many countries introduced this drug in the pork production chain to improve the results [26]. Because of the high prevalence and new genes identified in COL-resistant bacteria, new regulations have been introduced [27], and in 2016 Brazil banned the use of COL as a growth promoter in the pork production industry [28].

Such action occurred after the reintroduction of polymyxins in human treatment, in the 2000s, due to the ineffectiveness in the treatment of infections caused by multidrug resistant Gram-negative bacteria [3,7,27]. However, traces of this antibiotic in Brazil have been reported, caused by the continuous use of β-lactams and COL in preventive or therapeutic applications in the pork production chain [3,29] or because resistance genes remained in the bacterial genetic system over several years, which resulted in continuous resistance [30].

COL resistance in human isolates was reported by other studies [31,32]. In this study, COL-resistant isolates were not identified. However, two isolates from slaughterhouse workers presented MIC results of 2 μg/mL, raising concerns about initial resistance to this medication. COL serves as a last-resort treatment option for certain infections, emphasizing the significance of this finding.

The prediction of *mcr* (*1 to 5*) genes for COL resistance was negative in all isolates. It is important to mention that other variants of this gene were not screened [27], which is a limitation of this study. These genes are important resistance factors [23,25,33], and because they are inserted in the plasmid, presence of resistance in bacteria is somehow unpredictable [26]. Zurfluh *et al.* [32] also reported the phenotypic presence of COL resistance but not the *mcr* gene, similar to one of the isolates of this study, which showed resistance to COL but lacked both *mcr* genes and mutations in the *pmrAB* complex.

Mutations in specific genes, such as those in the two-component systems (TCS) *pmrAB* are other causes of COL resistance. In this study, mutations found in the *pmrA* (T31S, P42A, I128N, and G144S) and *pmrB* genes (H2R, N358Y, D283G, and K15I) were related to COL-resistant and susceptible *E. coli* isolates [8,34,35].

Huang *et al.* highlighted the mutation at position 358 (N358Y) in *pmrB* contributing to COL resistance, which seems to affect the protein function [34]. The functionality of *pmrAB* genes is associated with the maintenance of the microbial membrane, mainly in the electrical charge support, and any change or mutation in this structure can provide such resistance [36]. Thus in the absence of *mcr*, the main hypothesis is that resistance is provided by some mutation in the *pmrAB* complex or another mutation in the *etk e mgrR* genes and in the *PhoP-PhoQ* system, as reported by previous studies [14,31,37,38].

Regarding resistance to β-lactams, *E. coli* isolates from pigs and cattle showed a higher frequency for AMP and AMO resistance, which is in agreement with previous findings [33,39,40]. This might have been caused by the frequent use of these antibiotics in veterinary medicine. Even though there is no official record about the antibiotics sold in Brazil, data from the United States show that penicillin is the second most sold class of antibiotics for veterinary purposes, second to those of the tetracycline group. Furthermore, cephalosporins rank seventh, polymyxins are not independently reported, and there are no records for carbapenems by the Food and Drug Administration Center for Veterinary Medicine [41].

Comparing the production chains, swine isolates showed a higher frequency of antibiotic resistance than bovine isolates. In the swine production chain, a high level of antibiotics is administered on the farms, mostly due to management practices [2,42,43]. Bovines are

mostly kept in extensive systems at lower densities compared to swine, thus minimizing antibiotic use [2].

Human isolates showed more resistance to penicillin. The high level of resistance to AMP and AMO is in agreement with other reports [44,45]. This is worrying because penicillin is widely used in human treatment and highly effective against infections. In this context, resistance caused by ESBL, for example, could compromise its effectiveness [46].

The ESBL-producing *E. coli* are frequently reported [4,47,48]. Despite the low frequency identified in the present study (1 ESBL-producing isolate from swine), the swine production system may be a facilitator due to the intense use of antibiotics of different classes. The way through which these animals acquire ESBL-producing bacteria is complex, including several ports of entry. Factors beyond cross-contamination during slaughtering should be considered, such as transport vehicles, trading places, the introduction of new animals on the farm, and environmental factors such as drinking water, surface water, and wastewater [49].

Furthermore, even though the humans in this research did not present ESBL-producing isolates, the identification of an ESBL-producing isolate is a worrying factor as ESBL-producing *E. coli* isolates are associated with co-resistance to other classes of antibiotics and have resistance genes that can be transferred between animals and humans [47].

The *blaTEM* was the main gene distributed among the tested isolates (67% of swine and 24.14% of humans), as also observed in other studies [47,50]. In addition, depending of the variant, up to 90% of AMP resistance in *E. coli* can be caused by this gene, as described by Hussain *et al.* [5]. In this study, 86.49% (64/74) of the isolates resistant to AMP were reservoirs of this gene.

Another important gene found is the *ampC* gene. It can hydrolyze different drugs such as penicillin, cephalosporins, and monobactams [51], increasing its range to extended-spectrum cephalosporins [52,53]. Despite the low detection of *ampC* in this work, and although it is weakly expressed in *E. coli*, the increased use of cephalosporins in livestock has increased its detection rate in the food production chain [54].

In this study, 42.55% (100/235) of all isolates presented at least one phenotypical resistance, but the main concern was multidrug resistance. A total of 25 different resistance profiles are identified in this study, and there is concern regarding a specific profile of a swine isolate considered an ESBL producer and resistant to AMP, AMO, CFZ, CFC, CTX, CRO, CAZ, and ATM. This seems to be common in Brazil as other studies described similar profiles, and their isolates were considered multidrug-resistant, meaning they exhibited resistance to at least one agent in three or more antimicrobial categories [29,55,56].

Carbapenem resistance was found in some profiles. The identification of these strains in food production animals is of high interest for the scientific community. Carbapenems are among the few antibiotics that can effectively combat ESBL-producing bacteria. The Centers for Disease Control and Prevention (CDC) reported that in 2017 these bacteria were responsible for approximately 13,100 hospitalizations and 1,100 deaths [57]. Additionally, some studies reported resistance to carbapenems in swine isolates, further exacerbating the situation [47,58].

Antimicrobial resistance is a threat to public health, making it important to implement surveillance, control, and awareness programs regarding the use of antibiotics. Initiatives such as the National Action Plan for the Prevention and Control of Antimicrobial Resistance are crucial for strengthening efforts to prevent and control antimicrobial resistance [59].

Conclusions

The results of this study highlight the importance of the food production chain in the spread of antimicrobial resistance; and provide evidence that microorganisms, such as *E. coli*, are involved in this process. The pork production chain is a major concern, contributing as a disseminator of resistant bacteria. The results of this study highlight the need for mandatory surveillance programs to control the potential spread of antimicrobial resistance through the final product.

Acknowledgements

This work was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brasília, DF, Brazil, Financial Code 001), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brasília, DF, Brazil), and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG, Belo Horizonte, MG, Brazil).

Authors' contributions

Research activity planning, including mentorship to the team: MVCC, LAN, RSY; molecular and phenotypical data analysis: NKOV, LRF, FAS; manuscript draft: NKOV

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Conflict of interests

No conflict of interests is declared.

References

1. Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, Doi Y, Tian G, Dong B, Huang X, Yu LF, Gu D, Ren H, Chen X, Lv L, He D, Zhou H, Liang Z, Liu JH, Shen J (2016) Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis 16: 161–168. doi: 10.1016/S1473-3099(15)00424-7.
2. Rabello RF, Bonelli RR, Penna BA, Albuquerque JP, Souza RM, Cerqueira AMF (2020) Antimicrobial resistance in farm animals in Brazil: an update overview. Animals 10: 1–43. doi: 10.3390/ani10040552.
3. Barlaam A, Parisi A, Spinelli E, Caruso M, Di Taranto P, Normanno G (2019) Global emergence of colistin-resistant *Escherichia coli* in food chains and associated food safety implications: a review. J Food Prot 82: 1440–1448. doi: 10.4315/0362-028X.JFP-19-116.
4. Brisola MC, Crecencio RB, Bitner DS, Frigo A, Rampazzo L, Stefani LM, Faria GA (2019) *Escherichia coli* used as a biomarker of antimicrobial resistance in pig farms of Southern Brazil. Sci Total Environ 647: 362–368. doi: 10.1016/j.scitotenv.2018.07.438.
5. Hussain HI, Aqib AI, Seleem MN, Shabbir MA, Hao H, Iqbal Z, Kulyar MF, Zaheer T, Kun L (2021) Genetic basis of molecular mechanisms in β-lactam resistant Gram-negative bacteria. Microb Pathog 158: 105040. doi: 10.1016/j.micpath.2021.105040.
6. Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M, Monnet DL, Pulcini C, Kahlmeter G, Kluytmans J, Carmeli Y, Ouellette M, Outterson K, Patel J, Cavaleri M, Cox EM, Houchens CR, Grayson ML, Hansen P, Singh N, Theuretzbacher U, Magrini N, Aboderin AO, Al-Abri SS, Jalil NA, Benzonana N, Bhattacharya S, Brink AJ, Burkert FR, Cars O, Cornaglia G, Dyar OJ, Friedrich AW, Gales AC, Gandra S, Giske CG, Goff DA, Goossens H, Gottlieb T, Blanco MG, Hryniewicz W, Kattula D, Jinks T, Kanj SS, Kerr L, Kieny MP, Kim YS, Kozlov RS, Labarca J, Laxminarayan R, Leder K, Leibovici L, Levy-Hara G, Littman J, Malhotra-Kumar S, Manchanda V, Moja L, Ndoye B, Pan A, Paterson DL, Paul M, Qiu H, Ramon-Pardo P, Rodríguez-Baño J, Sanguinetti M, Sengupta S, Sharland M, Si-Mehand M, Silver LL, Song W, Steinbakk M, Thomsen J, Thwaites GE, Meer JWM, Kinh NV, Vega S, Villegas MV, Wechsler-Fördös A, Wertheim HFL, Wesangula E, Woodford N, Yilmaz FO, Zorzet A (2017) Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Infect Dis 18: 318–327. doi: 10.1016/S1473-3099(17)30753-3.
7. Trimble MJ, Mlynárčik P, Kolář M, Hancock REW (2016) Polymyxin: alternative mechanisms of action and resistance. Cold Spring Harb Perspect Med 6: 1–22. doi: 10.1101/cshperspect.a025288.
8. Wang CH, Kristopher Siu L, Chang FY, Tsai YK, Lin YT, Chiu SK, Huang L-Y, Lin J-C (2020) A novel deletion mutation in *pmrB* contributes to concurrent colistin resistance in carbapenem-resistant *Escherichia coli* sequence type 405 of clinical origin. Antimicrob Agents Chemother 64: 1–6. doi: 10.1128/AAC.00220-20.
9. Bauer AW, Kirby WM, Sherris JC, Turck M (1966) Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 45: 493–496. doi: 10.1093/ajcp/45.4_ts.493.
10. Clinical and Laboratory Standards Institute (2020) M100: performance standards for antimicrobial susceptibility testing. 30th informational supplement. Performance Standards for Antimicrobial Susceptibility Testing. 282 p.
11. Jarlier V, Nicolas MH, Fournier G, Philippon A (1988) Extended broad-spectrum β-lactamases conferring transferable resistance to newer β-lactam agents in Enterobacteriaceae: hospital prevalence and susceptibility patterns. Clin Infect Dis 10: 867–878. doi: 10.1093/clinids/10.4.867.
12. The European Committee on Antimicrobial Susceptibility Testing (2023) Breakpoint tables for interpretation of MICs and zone diameters. Version 13.0. European Committee on Antimicrobial Susceptibility Testing. Available: http://www.eucast.org. Accessed: 21 June 2024.
13. Lima Barbieri N, Nielsen DW, Wannemuehler Y, Cavender T, Hussein A, Yan SG, Nolan LK, Logue CM (2017) *mcr-1* identified in avian pathogenic *Escherichia coli* (APEC). PLoS One 12: e0172997. doi: 10.1371/journal.pone.0172997.
14. Rebelo AR, Bortolaia V, Kjeldgaard JS, Pedersen SK, Leekitcharoenphon P, Hansen IM, Guerra B, Malorny B, Borowiak M, Hammerl JA, Battisti A, Franco A, Alba P, Perrin-Guyomard A, Granier SA, De Frutos Escobar C, Malhotra-Kumar S, Villa L, Carattoli A, Hendriksen RS (2018) Multiplex PCR for detection of plasmid-mediated colistin resistance determinants, *mcr-1*, *mcr-2*, *mcr-3*, *mcr-4*, and *mcr-5* for surveillance purposes. Eurosurveillance 23: 1–11. doi: 10.2807/1560-7917.ES.2018.23.6.17-00672.
15. Lu H, Wang X, Lang X, Wang Y, Dang Y, Zhang F, Tang J, Li X, Feng X (2010) Preparation and application of microarrays for the detection of antibiotic resistance genes in samples isolated from Changchun, China. Mol Biol Rep 37: 1857–1865. doi: 10.1007/s11033-009-9621-4.
16. Edelstein M, Pimkin M, Palagin I, Edelstein I, Stratchounski L (2003) Prevalence and molecular epidemiology of CTX-M extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in Russian hospitals. Antimicrob Agents Chemother 47: 3724–3732. doi: 10.1128/AAC.47.12.3724-3732.2003.
17. Ibrahim DR, Dodd CE, Stekel DJ, Ramsden SJ, Hobman JL (2016) Multidrug resistant, extended spectrum β-lactamase (ESBL)-producing *Escherichia coli* isolated from a dairy farm. FEMS Microbiol Ecol 92: fiw013. doi: 10.1093/femsec/fiw013.
18. Pérez-Pérez FJ, Hanson ND (2002) Detection of plasmid-mediated *AmpC* beta-lactamase genes in clinical isolates by using multiplex PCR. J Clin Microbiol 40: 2153–2162. doi: 10.1128/JCM.40.6.2153-2162.2002.
19. Quesada A, Porrero MC, Téllez S, Palomo G, García M, Domínguez L (2015) Polymorphism of genes encoding PmrAB in colistin-resistant strains of *Escherichia coli* and *Salmonella enterica* isolated from poultry and swine (2015) J Antimicrob Chemother 70:71–74. doi: 10.1093/jac/dku320.

20. Tamura K, Stecher G, Kumar S (2021) MEGA11: molecular evolutionary genetics analysis. Version 11. Mol Biol Evol 38: 3022–3027. doi: 10.1093/molbev/msab120.
21. Studio R (2012) RStudio: integrated development environment for R. RStudio Inc, Boston, Massachusetts: 74.
22. Bastian M, Heymann S, Jacomy M (2009) Gephi: an open source software for exploring and manipulating networks. BT - International AAAI Conference on Weblogs and Social, Int. AAAI Conf. Weblogs Soc. Media 361–362. doi: 10.1609/icwsm.v3i1.13937.
23. Zhang X, Zhang B, Guo Y, Wang J, Zhao P, Liu J, He K (2019) Colistin resistance prevalence in *Escherichia coli* from domestic animals in intensive breeding farms of Jiangsu Province. Int J Food Microbiol 291: 87–90. doi: 10.1016/j.ijfoodmicro.2018.11.013.
24. Hassen B, Saloua B, Abbassi MS, Ruiz-Ripa L, Mama OM, Hassen A, Hammami S, Torres C (2019) *mcr-1* encoding colistin resistance in CTX-M-1/CTX-M-15- producing *Escherichia coli* isolates of bovine and caprine origins in Tunisia. First report of CTX-M-15-ST394/D *E. coli* from goats. Comp Immunol Microbiol Infect Dis 67: 101366. doi: 10.1016/j.cimid.2019.101366.
25. Huang X, Yu L, Chen X, Zhi C, Yao X, Liu Y, Wu S, Guo Z, Yi L, Zeng Z, Liu JH (2017) High prevalence of colistin resistance and *mcr-1* gene in *Escherichia coli* isolated from food animals in China. Front Microbiol. 8: 1–5. doi: 10.3389/fmicb.2017.00562.
26. Rhouma M, Beaudry F, Letellier A (2016) Resistance to colistin: what is the fate for this antibiotic in pig production? Int J Antimicrob Agents 48: 119–126. doi: 10.1016/j.ijantimicag.2016.04.008.
27. Rhouma M, Madec JY, Laxminarayan R (2023) Colistin: from the shadows to a one health approach for addressing antimicrobial resistance. Int J Antimicrob Agents 61: 106713. doi: 10.1016/j.ijantimicag.2023.106713.
28. Ministry of Agriculture, Livestock, and Supply (2016) Normative instruction no. 45, of November 22, 2016. 4 p. Available: http://www2.aneel.gov.br/aplicacoes/audiencia/arquivo/2016/038/resultado/ren2016745.pdf. Accessed: 21 June 2024.
29. De Azevedo EC, Martins BTF, Tiba Casas MR, Possebon FS, Araújo Junior JP, Nero LA, Yamatogi RS (2021) Multidrug resistance and virulence profiles of *Salmonella* isolated from swine lymph nodes. Microb Drug Resist 27: 562–70. doi: 10.1089/mdr.2020.0120.
30. Poirel L, Madec J-Y, Lupo A, Schink A-K, Kieffer N, Nordmann P, Schwarz S (2018) Antimicrobial resistance in *Escherichia coli*. Microbiol Spectr 6: 1–27. doi: 10.1128/microbiolspec.ARBA-0026-2017.
31. Sharahi JY, Hashemi A, Ardebili A, Davoudabadi S (2021) Molecular characteristics of antibiotic-resistant *Escherichia coli* and *Klebsiella pneumoniae* strains isolated from hospitalized patients in Tehran, Iran. Ann Clin Microbiol Antimicrob 20: 32. doi: 10.1186/s12941-021-00437-8.
32. Zurfluh K, Stephan R, Widmer A, Poirel L, Nordmann P, Nüesch HJ, Hächler H, Nüesch-Inderbinen M (2017) Screening for fecal carriage of MCR-producing Enterobacteriaceae in healthy humans and primary care patients. Antimicrob Resist Infect Control 6: 7–10. doi: 10.1186/s13756-017-0186-z.
33. Tong H, Liu J, Yao X, Jia H, Wei J, Shao D, Liu K, Qiu Y, Ma Z, Li B (2018) High carriage rate of *mcr-1* and antimicrobial resistance profiles of *mcr-1*-positive *Escherichia coli* isolates in swine faecal samples collected from eighteen provinces in China. Vet Microbiol 225: 53–57. doi: 10.1016/j.vetmic.2018.09.018.
34. Huang C, Shi Q, Zhang S, Wu H, Xiao Y (2021) Acquisition of the *mcr-1* gene lowers the target mutation to impede the evolution of a high-level colistin-resistant mutant in *Escherichia coli*. Infect Drug Resist 14: 3041–3051. doi: 10.2147/IDR.S324303.
35. Luo Q, Yu W, Zhou K, Guo L, Shen P, Lu H, Huang C, Xu H, Xu S, Xiao Y, Li L (2017) Molecular epidemiology and colistin resistant mechanism of *mcr*-positive and *mcr*-negative clinical isolated *Escherichia coli.* Front Microbiol 8: 2262. doi: 10.3389/fmicb.2017.02262.
36. Poirel L, Jayol A, Nordmanna P (2017) Polymyxins: antibacterial activity, susceptibility testing, and resistance mechanisms encoded by plasmids or chromosomes. Clin Microbiol Rev 30: 557–596. doi: 10.1128/CMR.00064-16.
37. Delannoy S, Devendec L Le, Jouy E, Fach P, Drider D, Kempf I (2017) Characterization of colistin-resistant *Escherichia coli* isolated from diseased pigs in France. Front Microbiol 8: 2278. doi: 10.3389/fmicb.2017.02278.
38. Janssen AB, Bartholomew TL, Marciszewska NP, Bonten MJM, Willems RJL, Bengoechea JA, van Schaik W (2020) Nonclonal emergence of colistin resistance associated with mutations in the BasRS two-component system in *Escherichia coli* bloodstream isolates. mSphere 5: 1–13. doi: 10.1128/mSphere.00143-20.
39. Souto MSM, Coura FM, Dorneles EMS, Stynen APR, Alves TM, Santana JA, Pauletti RB, Guedes RM, Viott AM, Heinemann MB, Lage AP (2017) Antimicrobial susceptibility and phylotyping profile of pathogenic *Escherichia coli* and *Salmonela entérica* isolates and pis in Minas Gerais, Brazil. Trop Anim Health Prod 49: 13–23. doi: 10.1007/s11250-016-1152-0.
40. Jiang HX, Lü DH, Chen ZL, Wang XM, Chen JR, Liu YH, Liao XP, Liu JH, Zeng ZL (2011) High prevalence and widespread distribution of multi-resistant *Escherichia coli* isolates in pigs and poultry in China. Vet J 187: 99–103. doi: 10.1016/j.tvjl.2009.10.017.
41. FDA (2021) Summary report on antimicrobials sold or distributed for use in food-producing animals. Available: https://www.fda.gov/media/163739/download. Accessed: 21 June 2024.
42. Cho I, Yamanishi S, Cox L, Methé BA, Zavadil J, Li K, Gao Z, Mahana D, Raju K, Teitler I, Li H, Alekseyenko AV, Blaser MJ (2012) Antibiotics in early life alter the murine colonic microbiome and adiposity. Nature 488: 621–626. doi: 10.1038/nature11400. doi: 10.1038/nature11400.
43. Salerno B, Furlan M, Sabatino R, Di Cesare A, Leati M, Volanti M, Barco L, Orsini M, Losasso C, Cibin V (2022) Antibiotic resistance genes load in an antibiotic free organic broiler farm. Poult Sci 101: 101675. doi: 10.1016/j.psj.2021.101675.
44. Aabed K, Moubayed N, Alzahrani S (2021) Antimicrobial resistance patterns among different *Escherichia coli* isolates in the Kingdom of Saudi Arabia. Saudi J Biol Sci 28: 3776–3782. doi: 10.1016/j.sjbs.2021.03.047.
45. Haghighatpanah M, Zeighami H, Mozaffari Nejad AS, Hajipour N (2022) Phylogenetic groups and antimicrobial resistance characteristics of *Escherichia coli* strains isolated from clinical samples in North Iran. Arab J Gastroenterol 23: 102–107. doi: 10.1016/j.ajg.2022.02.003.

46. Grumach AS, Ferraroni NR (2006) The role of penicillin in modern medicine. Brazilian Journal of Sexually Transmitted Diseases 18: 7–13. [Article in Portuguese].
47. Tamta S, Vinodh VK, Pruthvishree BS, Karthikeyan R, Rupner RN, Chethan GE, ZB Dubal, DK Sinha, Singh BR (2020) Faecal carriage of extended spectrum beta-lactamase (ESBL) and New Delhi metallo beta-lactamase (NDM) producing *Escherichia coli* between piglets and pig farmworkers. Comp Immunol Microbiol Infect Dis. 73: 101564. doi: 10.1016/j.cimid.2020.101564.
48. Anwar MA, Aziz S, Ashfaq K, Aqib AI, Shoaib M, Naseer MA, Alvi MA, Muzammil I, Bhutta ZA, Sattar H, Saleem A, Zaheer T, Khanum F, Mahmood A (2022) Trends in frequency, potential risks, and antibiogram of *E. coli* isolated from semi-intensive dairy systems. Pak Vet J 42: 167–172. doi: 10.29261/pakvetj/2022.018.
49. Bergšpica I, Kaprou G, Alexa EA, Prieto M, Alvarez-Ordóñez A (2020) Extended spectrum β-lactamase (ESBL) producing *Escherichia coli* in pigs and pork meat in the European Union. Antibiotics (Basel) 9: 1–23. doi: 10.3390/antibiotics9100678.
50. Dohmen W, Bonten MJM, Bos MEH, van Marm S, Scharringa J, Wagenaar JA, Heederik DJ (2015) Carriage of extended-spectrum β-lactamases in pig farmers is associated with occurrence in pigs. Clin Microbiol Infect. 21: 917–923. doi: 10.1016/j.cmi.2015.05.032.
51. MacDougall C (2011) Beyond susceptible and resistant, part I: treatment of infections due to Gram-negative organisms with inducible β-lactamases. J Pediatr Pharmacol Ther 16: 23–30. doi: 10.5863/1551-6776-16.1.23.
52. Rayamajhi N, Kang SG, Lee DY, Kang ML, Lee SI, Park KY, Lee HS, Yoo HS (2008) Characterization of TEM-, SHV- and AmpC-type β-lactamases from cephalosporin-resistant Enterobacteriaceae isolated from swine. Int J Food Microbiol 124: 183–187. doi: 10.1016/j.ijfoodmicro.2008.03.009.
53. Von Salviati C, Laube H, Guerra B, Roesler U, Friese A (2015) Emission of ESBL/AmpC-producing *Escherichia coli* from pig fattening farms to surrounding areas. Vet Microbiol. 175: 77–84. doi: 10.1016/j.vetmic.2014.10.010.
54. Hayer SS, Casanova-Higes A, Paladino E, Elnekave E, Nault A, Johnson T, Bender J, Perez A, Alvarez J (2022) Global distribution of extended spectrum cephalosporin and carbapenem resistance and associated resistance markers in *Escherichia coli* of swine origin — a systematic review and meta-analysis. Front Microbiol 13: 1–17. doi: 10.3389/fmicb.2022.853810.
55. Possebon FS, Tiba Casas MR, Nero LA, Yamatogi RS, Araújo JP, Pinto JPAN (2020) Prevalence, antibiotic resistance, PFGE and MLST characterization of *Salmonella* in swine mesenteric lymph nodes. Prev Vet Med 179: 105024. doi: 10.1016/j.prevetmed.2020.105024.
56. Viana C, Jagnow M, Pegoraro K, Seiti R, Ruben D, Bersot S, Nero LA (2019) Distribution, diversity, virulence genotypes and antibiotic resistance for Salmonella isolated from a Brazilian pork production chain. Int J Food Microbiol 310: 108310. doi: 10.1016/j.ijfoodmicro.2019.108310.
57. Centers for Disease Control and Prevention (2019) Antibiotic resistance threats in the United States. Available: https://www.cdc.gov/drugresistance/biggest-threats.html. Accessed: 21 June 2024.
58. Yuan J, Wang X, Shi D, Ge Q, Song X, Hu W, Wei D, Ge C, Li X, Hu C (2021) Extensive antimicrobial resistance and plasmid-carrying resistance genes in *mcr-1*-positive *E. coli* sampled in swine, in Guangxi, South China. BMC Vet Res 17: 1–10. doi: 10.21203/rs.2.13699/v4.
59. Brasil Ministério da Saúde (2019) National action plan for the prevention and control of antimicrobial resistance within the one health framework. Vol. 1. Available: https://www.gov.br/saude/pt-br/centrais-de-conteudo/publicacoes/svsa/antimicrobianos/plano-nacional-antimicrobianos-pan-br-14fev19-isbn.pdf/view. Accessed: 21 June 2024.

Annex – Supplementary items

Supplementary Table 1. Primers used for the detection of *mcr1-5*, *bla*TEM, *bla*CTX-M, *bla*SHV, *amp*C, *pmr*A, and *pmr*B genes.

Gene	Sequences 5'-3'	Fragment (bp)	Tm (°C)	Reference
mcr-1	CGGTCAGTCCGTTTGTTC CTTGGTCGGTCTGTAGGG	309	58	[13]
mcr-2	CAAGTGTGTTGGTCGCAGTT TCTAGCCCGACAAGCATACC	715	58	[14]
mcr-3	AAATAAAAATTGTTCCGCTTATG AATGGAGATCCCCGTTTTT	929	58	[14]
mcr-4	TCACTTTCATCACTGCGTTG TTGGTCCATGACTACCAATG	1116	58	[14]
mcr-5	ATGCGGTTGTCTGCATTTATC TCATTGTGGTTGTCCTTTTCTG	1644	58	[14]
bla_{TEM}	GAGTACTCACCATCACAGAAAAGC GACTTCCCGTCGTGTAGATAAC	489	51	[15]
bla_{CTX-M}	TTTGCGATGTGCAGTACCAGTAA CGATATCGTTGGTGGTGCCATA	544	51	[16]
bla_{SHV}	TTATCTCCCTGTTAGCCACC GATTTGCTGATTTCGCGCCG	796	55	[17]
ampC	AACACACTGATTGCGTCTGAC CTGGGCCTCATCGTCAGTTA	1226	60	[18]
*pmr*A	AGTTTTCCTCATTCGCGACCA TACCAGGCTGCGGATGATATTCT	714	68	[19]
*pmr*B	GGATGGCCTGATGTGACGCTGTC GCGCGGCTTTGGCTATATGCTG	1312	68	[19]

Bp: base pairs; Tm: melting temperature/annealing temperature.