Phenotypic and Genotypic Characterization of Extended-Spectrum β-Lactamase Producing *Escherichia coli* Isolated from Pig Feces in Farms, Muang Phayao

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Abstract

Extended-Spectrum β-Lactamase (ESBL)-producing *Escherichia coli* has been reported increasingly worldwide. This study aimed to investigate the prevalence, phenotypic and genotypic resistance of ESBL-producing *E. coli* isolated from pig feces in Muang District, Phayao Province. Pig feces were screened on MacConkey agar supplemented with (1 μg/mL) cefotaxime or ceftazidime. Suspected ESBL isolates on screening media were identified with biochemical tests. Then, suspected-ESBL-producing *E. coli* isolates were confirmed ESBL phenotype with combination disk method. Antimicrobial susceptibility and detection of ESBL genes were performed in a total 47 confirmed-ESBL-producing *E. coli* with 21 antimicrobial agents by disk diffusion and PCR methods, respectively. The results showed the prevalence of ESBL-producing *E. coli*, 58.8% (47/80). All 47 isolates were multidrug resistance (MDR), with resistant to AMP, CTX, CRO and CPD. However, they were still susceptible to AMC, FOX, ETP, IMP, MEM, FOS and TGC. Moreover, the phenotypic β-lactam resistance can be grouped into 6 patterns, with the predominance of AMP CTX CRO CPD ATM FEP in 48.9% (23/47). Genotypic characterization showed that all isolates possessed $bla_{CTX-M-9}$ and 7 genotypic-resistant patterns have been divided. The most frequently genotypic-resistant pattern was $bla_{CTX-M-9}$ (31.9%), followed by $bla_{CTX-M-1+CTX-M-9}$ (21.3%) and $bla_{CTX-M-1+CTX-M-9+TEM}$ (19.1%). However, there was no bla_{SHV} gene found. High prevalence of ESBL-producing *E. coli* in pig farms indicated that pigs were an important reservoir of MDR. Therefore, increasing of ESBL isolates in pig farms might increase the risk of transmission ESBL from feces to the human.

Keywords: *Escherichia coli*, ESBL, Antimicrobial Resistance, Pig Feces

Introduction

Multidrug resistance (MDR) is one of the most important current threats to public health (Van Duin & Paterson, 2016). Using of antimicrobial agents in agriculture and livestock has been linked to the development of resistance in animals (Liebana et al., 2013; Nuangmek et al., 2018). Transferring of resistance from animals to humans is possible and might cause harm to the public health. ESBL-producing *E. coli* can survive in the gastrointestinal tract of food-producing animals and can also cause infections (Smet et al., 2010; Marshall & Levy, 2011)

Infections with extended-spectrum β-lactamases (ESBL)-producing *E. coli* are a major global public health concern because it related to the increasing of morbidity, mortality, and healthcare costs (Dallenne, Da Costa, Decre, Favier, & Arlet, 2010; Smet et al., 2010; Li et al., 2015). Several studies showed food-producing animals have been regarded as a reservoir for ESBL-producing *E. coli* (Smet et al., 2010; Marshall & Levy, 2011; Liebana et al., 2013). ESBL are capable of inducing resistance to penicillin and to first-, second- and third-generation cephalosporin and monobactams (susceptible to cephamycins and carbapenems) (Ur Rahman et al., 2018). Extended-spectrum cephalosporins (ESCs) are effective drugs against such infections in veterinary clinical use. The causes of resistance may occur from using of ESCs (drug abuse and excessive usage) in food

animal production. These causes are considered to be associated with the mergence and high prevalence of ESBL-producing *E. coli* in animals (Liebana et al., 2013; Nuangmek et al., 2018). Many studies reported ESBL-producing *E. coli* in farm worker, food animals, river and environment (Hanson, Kaneene, Padungtod, Hirokawa, & Zeno, 2002; Dohmen et al., 2015; Gao et al., 2015; Li et al., 2015; Zhang et al., 2016; Nuangmek et al., 2018). The ESBL-producing *E. coli* in animal farms could influence public health through environment pollution and contaminated animal products (Gao et al., 2015). The ESBL producer carries resistant genes located on plasmid and can transfer to same or other strains and make to be resistant property as well (Smet et al., 2010). Pigs, especially feces, have been regarded as a potential source for ESBL-producing *E. coli* and have attracted considerable attention (Smet et al., 2010; Gao et al., 2015; Zhang et al., 2016; Nuangmek et al., 2018).

Therefore, the aim of this study was to investigate the prevalence, phenotypic and genotypic resistance of ESBL-producing *E. coli* isolated from pig feces in Muang District, Phayao Province. Antimicrobial resistant data in this area are still limited. The data helps to know more about the resistant situation in farm in this area. It is useful in management of antimicrobial agent usage in farms properly.

Materials and Methods

Sample size determination and sample collection

The sample size of this study was determined according to W.G. Cochran formula (1997) at confidence level at 90% (Z = 1.65, e = 0.10, P = 0.30). Eighty pig feces were obtained from 4 pig farms (20 samples per farm) in Muang District, Phayao Province during September to October, 2016. All feces were collected using sterile swabs, preserved in Cary-Blair transport medium, kept in icebox and transferred to the Clinical Microbiology Laboratory, School of Allied Health Sciences, University of Phayao. The samples were performed in the same day of sample collection.

Screening of ESBL-producing *E. coli*

Pig feces were randomly collected from fresh samples on the floor in different pig pens, kept in Cary-Blair medium and placed on two selective media plates, MacConkey agar supplemented with (1 μg/mL) cefotaxime or ceftazidime. All plates were incubated plate at 37°C for 18-24 hours. Suspected ESBL-producing *E. coli* colonies were identified with biochemical tests (Oxoid), triple sugar iron medium, motile-indole-lysine medium, urea medium, Simmons'citrate medium, malonate medium, methyl-red medium and Voges-Proskauer medium. All biochemical tests were incubated at 37°C for 18-24 hours. Both colony appearance and biochemical testing results were used for interpretation of suspected ESBL-producing *E. coli*. All suspected isolates were subjected to ESBL confirmation test.

Confirmation of ESBL-producing *E. coli*

Confirmation of ESBL was performed by using combination disk method comparing between cefotaxime (30 μg) and cefotaxime/clavulanic acid (30/10 μg) or between ceftazidime (30 μg) and ceftazidime/clavulanic acid (30/10 μg). The difference of inhibition zone between cefotaxime alone and cefotaxime + clavulanic acid or between ceftazidime alone and ceftazidime + clavulanic acid with the size of inhibition zone more than 5 mm confirmed as ESBL producer. *K. pneumoniae* ATCC® 700603 (positive control) and *E. coli* ATCC® 25922

(negative control) were used as quality control of ESBL confirmation test. Positive samples for both screening and confirmatory test were calculated for the prevalence.

Antimicrobial susceptibility testing

Forty seven ESBL-producing *E. coli* isolates were tested for antimicrobial susceptibility using disk diffusion method according to the guidelines the Clinical and Laboratory Standards Institute (CLSI), 2016 with 21 antimicrobial agents (Oxoids); ampicillin (10 μg), amoxicillin-clavulanate (20/10 μg), cefotaxime (30 μg), ceftazidime (30 μg), ceftriaxone (30 μg), cefepime (30 μg), cefoxitin (30 μg), cefpodoxime (10 μg), aztreonam (30 μg), ertapenem (10 μg), imipenem (10 μg), meropenem (10 μg), gentamicin (10 μg), trimethoprim- sulfamethoxazole (1.25/23.75 μg), fosfomycin (200 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), levofloxacin (5 μg), norfloxacin (10 μg), tetracycline (30 μg) and tigecycline (15 μg). *E. coli* ATCC 25922 was used as quality control for antimicrobial susceptibility testing according to CLSI, 2016 recommendations. The percentage of antimicrobial resistance was calculated.

Genotypic analysis for ESBL

All 47 ESBL positive isolates were brought to DNA extraction process, pure colonies of each sample were picked up, suspended in 500 μL sterile deionized water in 1.5 mL microcentrifuge tube and boiled at 95°C for 10 minutes. After that the tube was centrifuged at 6,000 rpm for 10 minutes (Dallenne et al., 2010). The supernatant was used as template for PCR analysis.

The PCR technique was used for determination of ESBL genes, $bla_{CTX-M-1}$, $bla_{CTX-M-2}$, $bla_{CTX-M-8/25}$, and $bla_{CTX-M-9}$, bla_{TEM} and bla_{SHV}. PCR was performed in 20 μL mixture containing 2 μL template DNA, 1 μL of each primer (10 pmol/μL), 10 μl 2 × PCR Master mix solution (i-Taq™, iNtRON biotechnology), 6 μL ddH2O. All PCRs were done as follows: initiation denaturation (94°C, 5 min); followed by 30 cycles of denaturation (94°C, 20 sec), annealing (55-58°C, 10 sec) and extension (72°C, 45 sec); and final extension (72°C, 5 min) using a PCR thermocycler (Bio-Rad)(Xu, An, Wang and Zhang, 2015). DNA from positive strains *K. pneumoniae* ATCC® 700603 was used as a positive control of detection. Presence of ESBL-resistant genes was used for calculation of the percentage of occurrence.

Results

ESBL-producing *E. coli*

A total of 80 samples of pig feces were collected in this study. Fecal samples were screened for ESBL-producing *E. coli* on MacConkey agar supplemented with cefotaxime (or ceftazidime) and identified with biochemical tests. The result showed the number of suspected ESBL-producing *E. coli* were 67.5% (54/80). Fifty-four screening positive isolates were confirmed with combination disk method, the results showed the percentage of positive for ESBL were 58.8% (47/80). The range of ESBL positive were 30-80% (Table 1).

Table 1 The percentage of ESBL-producing *E. coli* from screening test and confirmatory test

Farm	Prevalence (%)	
	Screening test	Confirmatory test
1	18 (90)	16 (80)
2	14 (70)	11 (55)
3	16 (80)	14 (70)
4	6 (30)	6 (30)
Total	**54 (67.5)**	**47 (58.8)**

Note: Positive for screening test: any isolates grew on screening media (MacConkey agar supplemented with (1 μg/mL) cefotaxime or ceftazidime)

Positive for confirmatory test: any isolates had the difference of inhibition zone between cefotaxime alone and cefotaxime + clavulanic acid or between ceftazidime alone and ceftazidime + clavulanic acid with inhibition zone size more than 5 mm.

Antimicrobial resistance of ESBL-producing *E. coli*

A total of 47 ESBL positive isolates were determined for antimicrobial susceptibility with 21 agents using disk diffusion method according to CLSI guidelines. The results revealed percentage of antimicrobial resistance with 100% were AMP, CTX, CRO and CPD followed by CAZ (61.7%), FEP (68.1%), ATM (87.2%), CN (78.7%), SXT (70.2%), C (91.5%) and TE (83%). In addition, this study found resistance to CIP (6.4%), LEV (2.1%) and NOR (2.1%). However, all isolates were no resistance (100% susceptible) to AMC, FOX, ETP, IMP, MEM, FOS, and TGC. (Table 2).

Moreover, there were 7 phenotypic β-lactam resistant patterns, AMP CTX CRO CPD (10.6%), AMP CTX CRO CPD ATM (10.6%), AMP CTX CRO CPD ATM FEP (17%), AMP CTX CRO CPD ATM CAZ (10.6%), AMP CTX CRO CPD FEP CAZ (2.1%) and AMP CTX CRO CPD ATM FEP CAZ (48.9%)-the most common phenotypic pattern. A total of 47 isolates as ESBL positive was MDR strain. (Table 3).

Genotypic analysis for ESBL

Analysis of genotypic-resistant genes in 47 ESBL positive isolates with PCR method, the results found predominant ESBL encoding *bla* genes were $bla_{\text{CTX-M-1}}$(53.2%), $bla_{\text{CTX-M-9}}$ (100%) and bla_{TEM} (44.7%). This study found only 2 isolates carrying $bla_{\text{CTX-M-2}}$ (14.9%), and did not found $bla_{\text{CTX-M-8/25}}$ and bla_{SHV}. The results revealed the genotypic resistance in 7 patterns that were $bla_{\text{CTX-M-9}}$ (31.9%), $bla_{\text{CTX-M-1 + CTX-M-9}}$ (21.3%), $bla_{\text{CTX-M-9 + TEM}}$ (12.8%), $bla_{\text{CTX-M-1 + CTX-M-2 + CTX-M-9}}$ (2.1%), $bla_{\text{CTX-M-1 + CTX-M-9 + TEM}}$ (19.1%), $bla_{\text{CTX-M-2+ CTX-M-9 + TEM}}$ (2.1%) and $bla_{\text{CTX-M-1 + CTX-M-2 + CTX-M-9 + TEM}}$ (10.6%). More importantly, all ESBL isolates carried at least $bla_{\text{CTX-M-9}}$ and $bla_{\text{CTX-M-9}}$, $bla_{\text{CTX-M-1+ CTX-M-9}}$ and $bla_{\text{CTX-M-1+ CTX-M-9+ TEM}}$ were the most 3 predominant patterns (Table 3).

Table 2 The percentage of antimicrobial agent resistance of ESBL-producing *E. coli*

Farm	Resistance (%)																		
	β-lactam													Non-β-lactam					
	Penicillins	β-lactam combination agents	Cephems						Monobactams	Penems	Aminoglycosides	Folate pathway antagonists	Fosfomycins	Phenicols	Fluoroquinolones			Tetracyclines	
	AMP	AMC	CTX	CAZ	CRO	FEP	CPD	FOX	ATM	ETP, IMP, MEM	CN	SXT	FOS	C	CIP	LEV	NOR	TE	TGC
1	16 (100)	0	16 (100)	4 (25)	16 (100)	8 (50)	16 (100)	0	13 (81.3)	0	15 (93.8)	12 (75)	0	16 (100)	0	0	0	15 (93.8)	0
2	11 (100)	0	11 (100)	8 (72.7)	11 (100)	9 (81.8)	11 (100)	0	10 (90.9)	0	3 (27.3)	10 (90.9)	0	8 (72.7)	2 (18.2)	0	0	11 (100)	0
3	14 (100)	0	14 (100)	13 (92.9)	14 (100)	14 (100)	14 (100)	0	13 (92.9)	0	14 (100)	6 (42.9)	0	13 (92.9)	1 (7.1)	1 (7.1)	1 (7.1)	7 (50)	0
4	6 (100)	0	6 (100)	4 (66.7)	6 (100)	1 (16.7)	6 (100)	0	5 (83.3)	0	5 (83.3)	5 (83.3)	0	6 (100)	0	0	0	6 (100)	0
Total	**47 (100)**	**0**	**47 (100)**	**29 (61.7)**	**47 (100)**	**32 (68.1)**	**47 (100)**	**0**	**41 (87.2)**	**0**	**37 (78.7)**	**33 (70.2)**	**0**	**43 (91.5)**	**3 (6.4)**	**1 (2.1)**	**1 (2.1)**	**39 (83)**	**0**

Note: AMP; ampicillin, AMC; amoxicillin-clavulanate, CTX; cefotaxime, CAZ; ceftazidime, CRO; ceftriaxone, FEP; cefepime, CPD; cefpodoxime, FOX; cefoxitin, ATM; aztreonam, ETP; ertapenem, IMP; imipenem, MEM; meropenem, CN; gentamicin, SXT; trimethoprim-sulfamethoxazole, FOS; fosfomycin, C; chloramphenicol, CIP; ciprofloxacin, LEV; levofloxacin, NOR; norfloxacin, TE; tetracycline and TGC; tigecycline.

Table 3 The percentage of phenotypic and genotypic characterization of ESBL-producing *E. coli*

Phenotypic β-lactam resistant pattern	No. of isolate (%)	No. of each genotypic resistant pattern (%)						
		bla						
		CTX-M-9	CTX-M-1+ CTX-M-9	CTX-M-9+ TEM	CTX-M-1+ CTX-M-2+ CTX-M-9	CTX-M-1+ CTX-M-9+ TEM	CTX-M-2+ CTX-M-9+ TEM	CTX-M-1+ CTX-M-2+ CTX-M-9+ TEM
AMP CTX CRO CPD	5 (10.6)	2 (4.3)	1 (2.1)	1 (2.1)	0	1 (2.1)	0	0
AMP CTX CRO CPD ATM	5 (10.6)	3 (6.4)	0	0	0	2 (4.3)	0	0
AMP CTX CRO CPD ATM FEP	8 (17)	5 (10.6)	0	1 (2.1)	0	1 (2.1)	0	1 (2.1)
AMP CTX CRO CPD ATM CAZ	5 (10.6)	0	1 (2.1)	1 (2.1)	0	2 (4.3)	0	1 (2.1)
AMP CTX CRO CPD FEP CAZ	1 (2.1)	0	0	0	0	1 (2.1)	0	0
AMP CTX CRO CPD ATM FEP CAZ	23 (48.9)	5 (10.6)	8 (17)	3 (6.4)	1 (2.1)	2 (4.3)	1 (2.1)	3 (6.4)
Total	**47 (100)**	**15 (31.9)**	**10 (21.3)**	**6 (12.8)**	**1 (2.1)**	**9 (19.1)**	**1 (2.1)**	**5 (10.6)**

Note: The percentage of presence of each ESBL gene from 47 ESBL positive isolates were $bla_{\text{CTX-M-1}}$ (53.2%), $bla_{\text{CTXM-2}}$ (14.9%), $bla_{\text{CTX-M-9}}$, (100%) and bla_{TEM} (44.7%).

Discussion

ESBL are widely distributed in *Enterobacteriaceae*, particularly in *E. coli*, and the rapid mergence and spread of ESBL-producing *E. coli* have been reported in food animals globally (Smet et al., 2010). In Thailand, ESBL-producing *E. coli* has been frequently reported from pig farm (Luvsansharav et al., 2011; Nuangmek et al., 2018). However, there has no data from pig farm in Phayao province. Thus, this investigation aimed to explore the prevalence in both phenotypic and genotypic characteristics of antimicrobial resistance in ESBL-producing *E. coli* isolated from pig farm in Phayao province. A total number of 80 swabs of pig feces from

4 pig farms have been screened and 47 *E. coli* isolates have been confirmed as ESBL. The percentage of positive samples for ESBL-producing *E. coli* was 58.8% (47/80), with the ranging between 30-80% in 4 farms. Several studies reported the prevalence of ESBL-producing *E. coli* in fecal pig samples including at least 3 reports from China, which were Shandong 56.7% (Zhang et al., 2016), Shandong 43% (Gao et al., 2015), Taian 31% (Li et al., 2015) and 19.7% in Taiwan (Lee & Yeh, 2017). In this study, there were 7 isolates that confirmed with combination disk method were not ESBL producer. A total of 7 isolates showed the phenotypic characterization in two pattern, AMP CTX CRO CPD ATM (n=4) or AMP CTX CRO CPD ATM CAZ (n=3). These isolates might mask ESBL production in the phenotypic confirmation test, causing false-negative ESBL result. They may contain the inhibitor-resistant TEMs (IRTs), hyper-production of TEM and/or SHV β-lactamases or existence of AmpC-type β-lactamases and ESBL (Tzouvelekis, Vatopoulos, Katsanis, & Tzelepi, 1999; Srisangkaew & Vorachit, 2004). Previous report showed highly related data between ESBL-producing *E. coli* from feces and environmental samples within the same farm and suggested that the ESBL-producers in the environment might originate from the pig farm (Gao et al., 2015). It can infect human causing mobility and mortality and disseminate into environment that might return to cause problems in animal and human again (Hammerum et al., 2014; Chang, Wang, Regev-Yochay, Lipsitch, & Hanage, 2015; Dohmen et al., 2015; Li et al., 2015; Zhang et al., 2016; Nuangmek et al., 2018).

Phenotypic results revealed 100% resistance to AMP, CTX, CRO and CPD. Additionally, more than 60% of isolates resistant to CAZ, FEP, ATM, CN, SXT, C and TE. The low frequency of resistance to fluoroquinolones (CIP, LEV, NOR) was found in only 2 samples from 2 farms. However, there was no isolate resistant to β-lactam combination agents (AMC), Cephems (FOX), Penems (ETP, IMP, MEM), Fosfomycins (FOS) and Tetracyclines (TGC). Some results are concomitant with other study, but some of them show the differences of drug resistance prevalence. Consistency, a study by Hu et al showed that the 100% resistance of CTX and CRO, while other agents were CAZ (16.1%), FEP (9.7%), ATM (61.3%) and no resistance to FOX (Hu et al., 2013). Additionally, the study by Gao L, et al, 2015 showed that antimicrobial resistance of fecal isolates isolated from pig farm were AMP, ATM, CN, SXT, C and TE and no resistance to AMC (Gao et al., 2015). Interestingly, the most of ESBL-*E. coli* isolates in our study are still susceptible to fluoroquinolones, while the high prevalence of CIP (48.4%) and (LEV (76.9%) were found in a study by Hu et al. (2013). This may causes the antibiotic abuse in the farms in each areas, type of antibiotic usage should be pursued in further study. All 47 isolates can be grouped into 7 β-lactam resistant patterns. All 7 patterns contain the resistance to AMP, CTX, CRO, CPD, but they were different in the capability of breaking down of FEP, ATM and CAZ. Pattern of AMP CTX CRO CPD ATM FEP CAZ was the most predominant resistance. The high level of antimicrobial resistance in farms may occur from inappropriate use and/or overuse of antimicrobial agents (Xu et al., 2015; Nuangmek et al., 2018). Our limitation of this study is the relation of antimicrobial agent used in pig farms in Phayao and drug resistance patterns. However, several agents have been used in the pig farms in Northern of Thailand such as tiamulin (83.1%), amoxicillin (71.2%), penicillin/streptomycin (66.1%) and tylosin (50.8%) including cefotaxime (15.3%) (Nuangmek et al., 2018).

Genotypic results, the percentage of ESBL-encoding genes (*bla* genes) were $bla_{CTX-M-1}$ (53.2%), $bla_{CTX-M-9}$ (100%) and bla_{TEM} (44.7%). Interestingly, all 47 ESBL producing *E. coli* isolates carried $bla_{CTX-M-9}$. The most common genotypic-resistant patterns were only $bla_{CTX-M-9}$ (31.9%), $bla_{CTX-M-1+CTX-M-9}$ (21.3%) and

$bla_{CTX-M-9+TEM}$ (12.8%). However, $bla_{CTX-M-8/25}$ and bla_{SHV} were not found in this study. Our results are consistent with other reports that $bla_{CTX-M-1}$, $bla_{CTX-M-9}$ and bla_{TEM} were the most common of ESBL genes (Xu et al., 2015; Zhang et al., 2016). Additionally, the previous common ESBL subtypes included $bla_{CTX-M-1}$ [$bla_{CTX-M-3, -15, -55, -64, -123}$], $bla_{CTX-M-9}$ [$bla_{CTX-M-14, -24, -27, -65, -104, -125}$], bla_{TEM} [$bla_{TEM-1, -52, -116}$] (Hu et al., 2013; Gao et al., 2015; Xu et al., 2015; Lee & Yeh, 2017). The presence of bla_{SHV} genes was not found similar to other study (Xu et al., 2015; Zhang et al., 2016). Monitoring the prevalence of ESBL-encoding genes should be done due to these genes are located on mobile genetic elements and can disseminate through horizontal gene transfer between bacteria, and even between different species (Li et al., 2015; Nuangmek et al., 2018; Ur Rahman et al., 2018). The presence of each subtypes varied depending on the geographic region and different farms (Ewers, Bethe, Semmler, Guenther, & Wieler, 2012; Dahms et al., 2015; Gao et al., 2015). All isolates were confirmed as ESBL negative following the combination disk method. However, their genotypic characteristics may need further investigation.

This study's results showed a high prevalence of ESBL-producing *E. coli* in the samples from the 4 farms in the Phayao region, and can be used to increase awareness of antimicrobial agent use and control on pig farms. Using veterinary medicine improperly might increase the development of ESBL-producing *E. coli* resistance in human medicine. Moreover, molecular typing of ESBL-producing *E. coli* is useful for hospital epidemiologists, microbiologists, and clinicians in guiding surveillance studies, monitoring outbreak situations, and tracking the spread of emerging pathogens. However, the spreading of drug resistant bacteria could be possible from the farm to the human via the product or soil and water contamination. To complete the chain of gene transfer, further study should be genotypic comparative analysis of ESBL-producing *E. coli* from farm worker, food animals and farm environment in order to determine the horizontal gene transfer.

Conclusion

The prevalence of ESBL-producing *E. coli* was high in the pig feces in 4 farms in Phayao province, with the percentage of 58.8% (47/80). A total of 47 ESBL positive isolates was 100% resistant to AMP, CTX, CRO and CPD and 100% susceptible to AMC, FOX, ETP, IMP, MEM, FOS and TGC. All ESBL positive isolates were MDR. Phenotypic characterization showed that the most common β-lactam resistant pattern was AMP CTX CRO CPD ATM FEP CAZ (48.9%). Genotyping for ESBL genes demonstrated the predominant of $bla_{CTX-M-9}$ (31.9%), followed by $bla_{CTX-M-1+CTX-M-9}$ (21.3%) and $bla_{CTX-M-9+TEM}$ (12.8%). Awareness regarding antimicrobial usage on farms should be addressed. Comprehensive surveillance systems to monitor the antimicrobial resistance should be established in order to reduce the selective pressure downstream on humans.

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