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Prevalence, Antibiogram, and Resistance Profile of Extended-Spectrum β-Lactamase-Producing *Escherichia coli* Isolates from Pig Farms in Luzon, Philippines

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This cross-sectional study was conducted to determine the prevalence, antibiogram, and resistance profile of extended-spectrum β-lactamase-producing *Escherichia coli* (ESBL-EC) isolates from healthy pigs and pig farms in Luzon, Philippines. A total of 162 rectal samples from healthy finisher and breeder pigs and boot swab samples from pig houses were collected from 54 randomly selected pig farms. Bacteria were isolated and screened using MacConkey agar plate supplemented with 1 mg/L cefotaxime. Identification of bacteria and antimicrobial susceptibility test were carried out through Vitek® 2 and combined disk test. PCR amplifications were carried out in all isolates targeting $bla_{\text{CTX-M}}$ and its five major groupings, bla_{TEM}, and bla_{SHV}. The farm prevalence of ESBL-EC was 57.41% (95% confidence interval [CI] = 43.21–70.77). A total of 48 (29.63%) ESBL-EC isolates were isolated from samples that showed 14 different phenotypic multidrug resistance patterns. The prevalence of $bla_{\text{CTX-M}}$ gene was 91.67% (95% CI = 80.02–97.68). All major $bla_{\text{CTX-M-groups}}$ except $bla_{\text{CTX-M-25group}}$ were detected. The $bla_{\text{CTX-M-1}}$ was the most prevalent $bla_{\text{CTX-M}}$ gene, 75.0% (95% CI = 60.40–86.36). The prevalence of bla_{TEM} and bla_{SHV} genes was 91.67% (95% CI = 80.02–97.68) and 60.42% (95% CI = 45.27–74.23), respectively. Coexistence of different $bla_{\text{CTX-M}}$, bla_{TEM}, and bla_{SHV} genes was observed in 44 isolates with 20 different genotypic patterns. High prevalence, diverse antibiogram profile, and genotypic resistance pattern of ESBL-EC isolates from healthy pigs and pig farms were observed in this study that could result in possible transmission to farm workers, susceptible bacteria, and the environment.

Keywords: CTX-M, *E. coli*, ESBL, pig, Philippines

Introduction

Antimicrobial resistance is a natural phenomenon that has been documented as early as a few years from the discovery of penicillin. Today, antimicrobial-resistant bacteria are continually emerging and contributing to mortality and morbidity of not only humans but animals as well. Recently, the World Health Organization listed the extended-spectrum β-lactamase-producing *E. coli* (ESBL-EC) as a priority pathogen for the research and development of newer drugs.[1]

Isolations and characterization of this bacterium have been reported in human, livestock, companion animals, and the environment. The acquisition and expression of the ESBL resistance genes lead to the ability of *E. coli* to hydrolyze and resist several types of β-lactam antibiotics, including the third-generation cephalosporins, monobactams except cephamycins and carbapenems.[2–4] Apart from ESBL resistance genes, it has been established that other plasmid-mediated antimicrobial resistance genes can be co-transferred to susceptible bacteria resulting in multidrug-resistant isolates.[5]

Swine farms use antimicrobials for growth promotion, prophylaxis, and treatment of nonbacterial infection that creates an ideal situation for development and selection of antimicrobial-resistant bacteria. These antimicrobial resistance genes can be transferred to other enteric bacteria that may serve as reservoirs and may cause persistence of bacterial infection.[6] Previous studies have demonstrated that

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isolates from pigs and farm houses were genetically related to human isolates that suggests the public health importance of isolation and characterization of ESBL *E. coli* in farm animals such as pigs.[7–9]

At present, there are no data on the occurrence of ESBL-EC in pigs and pig farms in the Philippines. The detection of the ESBL-encoding genes in bacterial isolates from pigs and pig farms will be beneficial in drafting policies for surveillance studies and antimicrobial resistance mitigation strategies.

This study established the prevalence, antibiogram, phenotypic and genotypic resistance profile of commensal ESBL-EC isolates from healthy pigs and commercial pig farms in Luzon, Philippines.

Materials and Methods

Farm selection

From the top four pig-producing provinces in Central Luzon (Fig. 1), a sampling frame of commercial swine farms was made and 406 farms were listed. Using the following assumptions, 80% prevalence, 10% accepted error, and 95% level of confidence, the sample size was calculated. Random selection of 54 farms was carried out in province 1 ($n=26$), province 2 ($n=15$), province 3 ($n=8$), and province 4 ($n=5$) using probability proportional to size sampling. Before the start of the study, owners and managers of all selected pig farms were contacted to ask for their consent to participate.

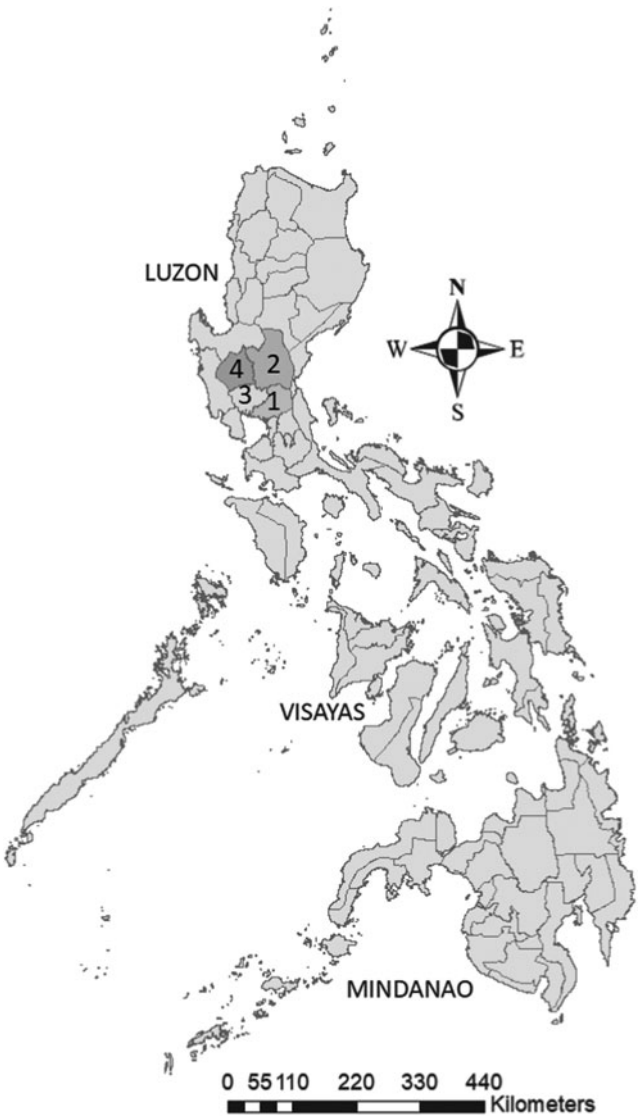

FIG. 1. Map of the Philippines showing the study provinces.

Sampling and bacterial isolation

Fresh fecal samples were collected directly from the rectum of 10 randomly selected pigs from each farm. The pooled fecal samples were placed in 500 beaker containing Luria–Bertani (LB) broth (Merck, Darmstadt, Germany). A pair of boot swab was worn to obtain samples from the environment by walking in the aisle, inside the pig house. Samples from boot swabs were put in a 500 mL beaker containing LB broth for enrichment. Microbiological analysis was performed in 162 fecal and boot swab samples (54 fecal samples each from fattener and breeder pigs and 54 boot swabs) from 54 pig farms following a previously reported protocol.[10] Pure cultures were preserved using 20% glycerol in Mueller–Hinton broth until further evaluation.

Bacterial identification and antimicrobial susceptibility testing

Identification of bacterial isolates and antimicrobial susceptibility testing was performed using GN and AST-N261 cards of Vitek® 2 Compact (bioMérieux, Craponne, France). Seventeen different antimicrobials were tested as given in Fig. 2. The minimum inhibitory concentration data were evaluated using the Clinical and Laboratory Standards Institute (CLSI) M100-S24 clinical breakpoints.[11] CLSI standards were also used in programming Vitek 2 Compact. *E. coli* ATCC 25922 was used as quality control in the susceptibility testing.

Based on results of bacterial culture, another phenotypic confirmatory testing using the combined disk test (CDT) was performed on all suspected ESBL-EC isolates. Cefotaxime (30 μg) and ceftazidime (30 μg) alone and in combination with 10 μg clavulanic acid were tested. The presence of ESBL was confirmed if there is ≥5 mm increase in the zone diameter for either antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone.[11]

DNA extraction and PCR amplification

DNA of bacterial isolates was extracted using NucleoSpin Microbial DNA Purification kit (Macherey-Nagel, Germany) following manufacturer's protocol. Optimization of primers from published studies (Table 1) was carried out before PCR amplification of target genes of all isolates. The PCR assay was performed individually for each primer set using BioRad T100 thermal cycler (BioRad, Herts, United Kingdom) following a previously described protocol.[10] Electrophoresis was carried out by loading 3 μL of amplified products and 2 μL of loading buffer in 1.5% agarose gel with 0.5× Tris-Borate-EDTA buffer. The negative and positive control used were ATCC 25922 and ATCC 35218

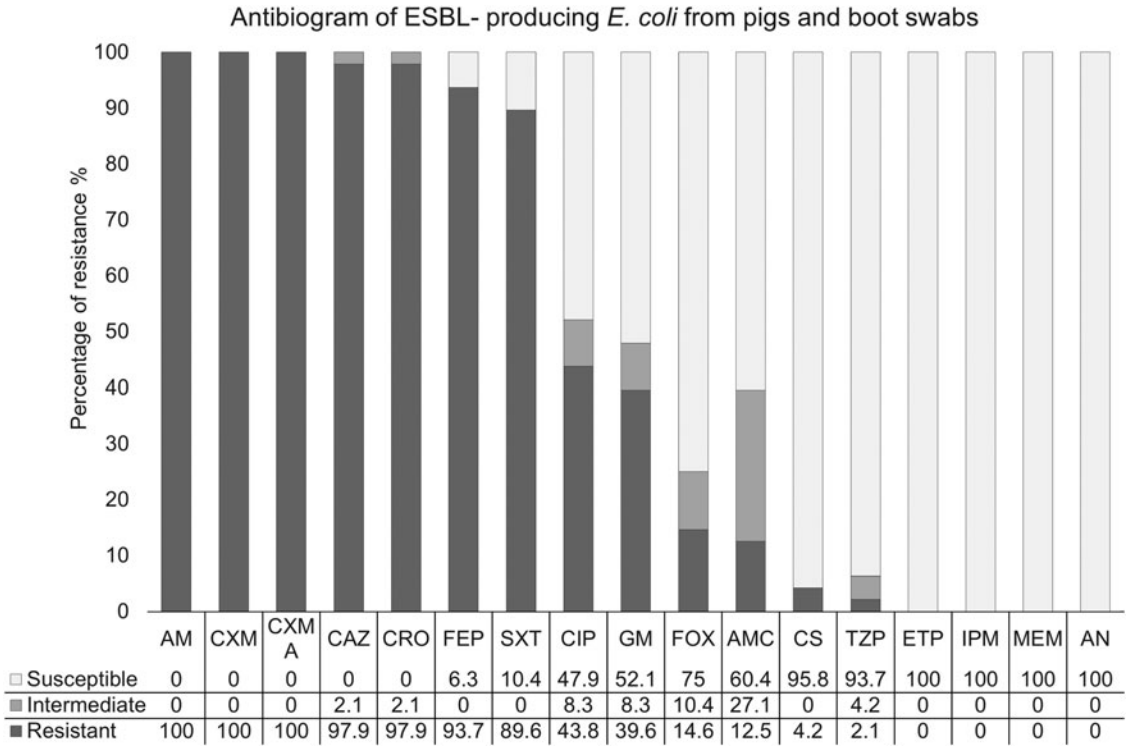

FIG. 2. Antimicrobial resistance pattern of extended-spectrum β-lactamase-producing *Escherichia coli* isolates from pig farms. AM, ampicillin; AMC, amoxicillin/clavulanic acid; TZP, piperacillin/tazobactam; CXM, cefuroxime; CXMA, cefuroxime axetil; FOX, cefoxitin; CAZ, ceftazidime; CRO, ceftriaxone; FEP, cefepime; ETP, ertapenem; IPM, imipenem; MEM, meropenem; AN, amikacin; GM, gentamicin; CIP, ciprofloxacin; CS, colistin; SXT, trimethoprim/sulfamethoxazole.

TABLE 1. PRIMERS USED TO DETECT RESISTANCE GENES AND GENOTYPES IN *ESCHERICHIA COLI* ISOLATES

Target gene	*Primer*	*Sequence (5′ → 3′)*	*Annealing temp (°C)*	*Size (bp)*	*References*
$bla_{\text{CTX-M}}$	CTX-M—F CTX-M—R	ATGTGCAGYACCAGTAARGTKATGGC TGGGTRAARTARGTSACCAGAAYSAGCGG	55	592	[12]
$bla_{\text{CTX-M-1group}}$	CTX-M—1-F CTX-M—1-R	GGTTAAAAAATCACTGCGTC TTACAAACCGTYGGTGACGA	50	873	[12]
$bla_{\text{CTX-M-15group}}$	CTX-M—15-F CTX-M—15-R	CACACGTGGAATTTAGGGACT GCCGTCTAAGGCGATAAACA	50	995	[13]
$bla_{\text{CTX-M-2group}}$	CTX-M—2-F CTX-M—2-R	ATGATGACTCAGAGCATTCGCCGC TCAGAAACCGTGGGTTACGATTTT	56	876	[14]
$bla_{\text{CTX-M-8group}}$	CTX-M—8-F CTX-M—8-R	TGATGAGACATCGCGTTAAG TAACCGTCGGTGACGATTTT	52	875	[15]
$bla_{\text{CTX-M-9group}}$	CTX-M—9-F CTX-M—9-R	GTGACAAAGAGAGTGCAACGG ATGATTCTCGCCGCTGAAGCC	55	856	[16]
$bla_{\text{CTX-M-25group}}$	CTX-M—25-F CTX-M—25-R	GCACGATGACATTCGGG AACCCACGATGTGGGTAGC	52	327	[17]
bla_{TEM}	TEM-F TEM-R	TTGGGTGCACGAGTGGGTTA TAATTGTTGCCGGGAAGCTA	55	506	[18]
bla_{SHV}	SHV-F SHV-R	TCGGGCCGCGTAGGCATGAT AGCAGGGCGACAATCCCGCG	52	628	[18]
mcr-1[a]	CLR5-F CLR5-R	CGGTCAGTCCGTTTGTTC CTTGGTCGGTCTGTA GGG	58	305	[19]

[a]Target gene for colistin resistance.

Table 2. Prevalence of Extended-Spectrum β-Lactamase-Producing *Escherichia coli* in Pig Farms ($N=54$) in Selected Provinces in Luzon

Farm/samples	*No. of positives*	*Prevalence %*	*95% Confidence interval*	
			Lower	*Upper*
Farm	31	57.41	43.21	70.77
Pooled fecal samples from breeders	19	35.19	22.68	49.38
Pooled fecal samples from finishers	15	27.78	16.46	41.64
Boot swabs	14	25.93	14.96	39.65

E. coli strains (Microbiologics, MN), respectively. The latter is suitable as a positive control for detection of bla_{TEM} gene, but its use in other genes was also tested. For confirmation of target genes, DNA sequencing analysis was carried out on PCR products of representative isolates through the services of First Base Laboratories (Axil Scientific Pte Ltd., Singapore), and then analyzed using Basic Local Alignment Search Tool.

Statistical analysis

Descriptive statistics was used to analyze the data. The farm prevalence was calculated and the difference in proportions was tested by determining the 95% confidence intervals (CIs) using the exact binomial confidence limits with a significance level of ≤0.05.

Results

Prevalence

Thirty-one farms were found to have at least one sample being positive for ESBL-EC, either from pooled fecal sample or boot swab sample, with a farm prevalence of 57.41% (95% CI = 43.21–70.77). Positive isolates from fecal and boot swab samples were observed in seven farms. From 19 pooled fecal samples (35.19%) from breeders, 15 pooled fecal samples (27.78%) from finishers and 14 boot swab samples from 54 pig farms in Luzon, Philippines, a total of 48 (29.63%) ESBL-EC were isolated (Table 2). There is no significant difference in the prevalence rate between pooled fecal samples from both breeders and finishers ($p=0.407$). Likewise, the prevalence of ESBL-EC in fecal and boot swabs has no significant difference ($p=0.466$).

Antibiogram of ESBL isolates

The antibiogram of ESBL-EC isolates is given in Fig. 2. All isolates were sensitive to carbapenems and amikacin. A high sensitivity to colistin and piperacillin/tazobactam was also observed. A moderately high resistance rate was observed in gentamicin, ciprofloxacin, and amoxicillin with clavulanic acid. Absolute resistance was observed in ampicillin and cefotaxime in comparison with varying resistance to different cephalosporin drugs.

Table 3 provides the antimicrobial drug resistance patterns of ESBL-EC isolates from swine. A total of 14 multidrug resistance patterns were observed. The most common combinations were penicillin–cephems–fluoroquinolone–folate pathway inhibitor (18.75%) followed by penicillin–cephem–aminoglycoside–folate pathway inhibitor (16.67%). It was observed that the common denominator of these patterns was penicillin–cephems–folic acid inhibitor (45.83%).

Genotypic resistance profile of ESBL isolates

The prevalence of ESBL genotype among ESBL-EC isolates is given in Table 4. Forty-four isolates (91.67%, 95% CI = 80.02–97.68) have $bla_{CTX\text{-}M}$ ESBL gene type. Among the $bla_{CTX\text{-}M}$ groups, the most prevalent gene was $bla_{CTX\text{-}M\text{-}1group}$, observed at 75.0% (95% CI = 60.40–86.36), followed by $bla_{CTX\text{-}M\text{-}8group}$, 45.83% (95% CI = 31.37–60.83), $bla_{CTX\text{-}M\text{-}9group}$, 18.75% (95% CI = 8.95–32.63), and $bla_{CTX\text{-}M\text{-}2group}$, 6.25% (95% CI = 1.31–17.20). No $bla_{CTX\text{-}M\text{-}25group}$ gene was detected in any of the 48 isolates. The $bla_{CTX\text{-}M\text{-}15}$, a gene for subtype of $bla_{CTX\text{-}M\text{-}1group}$, has a prevalence of 35.42% (95% CI = 22.16–50.54). Both bla_{TEM} and bla_{SHV} have prevalence of 91.67% (95% CI = 80.02–97.68) and 60.42% (95% CI = 45.27–74.23), respectively.

The distribution of ESBL genotype among positive ESBL-EC isolates from rectal and boot swabs is given in Table 5. Coexistence of different $bla_{CTX\text{-}M}$, bla_{TEM}, and bla_{SHV} genes were observed in 44 isolates (91.67%) with 20 different genotypic patterns, 17 of which have a combination of three or more genes. Conversely, only one gene was

Table 3. Antimicrobial Drug Resistance Patterns of Extended-Spectrum β-Lactamase-Producing *Escherichia coli* Isolates from Pigs and Boot Swabs ($N=48$)

No. of isolates	*Multidrug resistance patterns*	*No. of antibiotic family*
2	PCN–CEPH	2/8
1	PCN–CEPH–AMGL	3/8
1	PCN–CEPH–FRQL	3/8
4	PCN–CEPH–FPI	3/8
3	PCN–BLIC–CEPH–FPI	4/8
8	PCN–CEPH–AMGL–FPI	4/8
9	PCN–CEPH–FRQL–FPI	4/8
1	PCN–BLIC–CEPH–AMGL–FRQL	5/8
1	PCN–BLIC–CEPH–LPP–FPI	5/8
4	PCN–BLIC–CEPH–AMGL–FPI	5/8
4	PCN–BLIC–CEPH–FRQL–FPI	5/8
4	PCN–CEPH–AMGL–FRQL–FPI	5/8
1	PCN–BLIC–CEPH–FRQL–LPP–FPI	6/8
5	PCN–BLIC–CEPH–AMGL–FRQL–FPI	6/8

PCN, penicillin; BLIC, β-lactam and β-lactam inhibitor combinations; CEPH, cephems; AMGL, aminoglycosides; FRQL, fluoroquinolones; LPP, lipopeptides; FPI, folate pathway inhibitor.

Table 4. Prevalence of Resistance Genes Among Extended-Spectrum β-Lactamase-Producing *Escherichia coli* Isolates ($N=48$) from Pig Farms

Genotype	*n*	*Prevalence %*	*95% CI*	
			LL	*UL*
$bla_{\text{CTX-M}}$	44	91.67	80.02	97.68
$bla_{\text{CTX-M-1group}}$	36	75.00	60.40	86.36
$bla_{\text{CTX-M-15}}$	17	35.42	22.16	50.54
$bla_{\text{CTX-M-2group}}$	3	6.25	1.31	17.20
$bla_{\text{CTX-M-8group}}$	22	45.83	31.37	60.83
$bla_{\text{CTX-M-9group}}$	9	18.75	8.95	32.63
$bla_{\text{CTX-M-25group}}$	0	—	—	—
bla_{TEM}	44	91.67	80.02	97.68
bla_{SHV}	29	60.42	45.27	74.23
mcr-1[a]	26	54.17	39.17	68.63

[a]Colistin resistance gene.
LL, lower limit; UL, upper limit; CI, confidence interval.

detected in four isolates (8.33%). One isolate yielded positive result for $bla_{\text{CTX-M}}$ gene but no $bla_{\text{CTX-M groups}}$ were detected. In addition to ESBL genes detected, Table 4 also gives the PCR results for *mcr-1* gene, the gene responsible for colistin resistance. Twenty-six isolates were found positive with a prevalence of 54.17% (95% CI = 39.17–68.63).

Discussion

This is the first report on high prevalence and diverse antibiogram and resistance profile of ESBL-EC in swine farms in the Philippines. The farm prevalence reported in this study is a cause for concern. Further risk assessment and appropriate risk management should be carried out to minimize the rate and proliferation of ESBL-EC. Because samples in this study are from healthy pigs, the isolates are commensal *E. coli*. This commensal, multidrug-resistant ESBL-EC has a potential to be zoonotic and can be a major opportunistic pathogen in pigs. ESBL-EC poses a huge risk both to pig production and public health, as this pathogen is more likely to reach the food chain and consequently facilitate the transmission of ESBL-EC to humans. Moreover, pigs and other animals infected with the bacteria can serve as a medium in spreading ESBL-EC through fecal contamination of their environment.[7,8]

The observed farm prevalence in this study is higher than the prevalence of ESBL-EC in pigs reported in other Asian countries such as Thailand (44.4%),[5] China (43.2%),[20] Taiwan (19.7%),[21] and South Korea (4.98%).[22] Appropriate interventions should be implemented to lower this level considering the public health implication of ESBL-EC. In the Netherlands, it has been shown that the prevalence among swine farms decreased from 27% in 2011 to 13% in 2013 when antimicrobial usage was restricted.[23]

The isolates fit the characteristics of ESBL described by Pitout and Laupland.[2] They are resistant to cefuroxime, ceftazidime, ceftriaxone, and cefepime. They are still sensitive to cefoxitin, imipenem, and meropenem. Their resistance is related to their ability to produce ESBLs that can hydrolyze the β-lactam ring. A 100% resistance rate to ceftazidime, ceftriaxone, and cefepime was also reported.[24] In contrast, Michael *et al.* reported only 9.3% resistance to

Table 5. Distribution of Extended-Spectrum β-Lactamase Genes Among Extended-Spectrum β-Lactamase-Producing *Escherichia coli* Isolates ($N=48$) from Pig Farms

Genotype patterns	*Rectal swab*	*Boot swab*	*%*
$bla_{\text{CTX-M-1}} + bla_{\text{CTX-M-15}} + bla_{\text{CTX-M-2}} + bla_{\text{CTX-M-8}} + bla_{\text{CTX-M-9}} + bla_{\text{TEM}} + bla_{\text{SHV}}$	0	1	2.08
$bla_{\text{CTX-M-1}} + bla_{\text{CTX-M-15}} + bla_{\text{CTX-M-8}} + bla_{\text{TEM}}$	1	0	2.08
$bla_{\text{CTX-M-1}} + bla_{\text{CTX-M-15}} + bla_{\text{CTX-M-8}} + bla_{\text{TEM}} + bla_{\text{SHV}}$	3	4	14.58
$bla_{\text{CTX-M-1}} + bla_{\text{CTX-M-15}} + bla_{\text{SHV}}$	1	0	2.08
$bla_{\text{CTX-M-1}} + bla_{\text{CTX-M-15}} + bla_{\text{TEM}}$	2	0	4.17
$bla_{\text{CTX-M-1}} + bla_{\text{CTX-M-15}} + bla_{\text{TEM}} + bla_{\text{SHV}}$	3	1	8.33
$bla_{\text{CTX-M-1}} + bla_{\text{CTX-M-2}} + bla_{\text{CTX-M-8}} + bla_{\text{SHV}}$	1	0	2.08
$bla_{\text{CTX-M-1}} + bla_{\text{CTX-M-8}} + bla_{\text{CTX-M-9}} + bla_{\text{TEM}} + bla_{\text{SHV}}$	1	0	2.08
$bla_{\text{CTX-M-1}} + bla_{\text{CTX-M-8}} + bla_{\text{TEM}}$	5	0	10.42
$bla_{\text{CTX-M-1}} + bla_{\text{CTX-M-8}} + bla_{\text{TEM}} + bla_{\text{SHV}}$	4	1	10.42
$bla_{\text{CTX-M-1}} + bla_{\text{CTX-M-9}} + bla_{\text{TEM}} + bla_{\text{SHV}}$	2	0	4.17
$bla_{\text{CTX-M-1}} + bla_{\text{TEM}}$	2	1	6.25
$bla_{\text{CTX-M-1}} + bla_{\text{TEM}} + bla_{\text{SHV}}$	2	2	8.33
$bla_{\text{CTX-M-15}} + bla_{\text{CTX-M-9}} + bla_{\text{TEM}} + bla_{\text{SHV}}$	1	0	2.08
$bla_{\text{CTX-M-1}} + bla_{\text{CTX-M-9}} + bla_{\text{TEM}} + bla_{\text{SHV}}$	1	0	2.08
$bla_{\text{CTX-M-8}} + bla_{\text{CTX-M-9}} + bla_{\text{TEM}}$	0	1	2.08
$bla_{\text{CTX-M-8}} + bla_{\text{CTX-M-9}} + bla_{\text{TEM}} + bla_{\text{SHV}}$	1	0	2.08
$bla_{\text{CTX-M-8}} + bla_{\text{TEM}}$	0	1	2.08
$bla_{\text{CTX-M-8}} + bla_{\text{TEM}} + bla_{\text{SHV}}$	0	1	2.08
$bla_{\text{CTX-M-9}} + bla_{\text{TEM}}$	1	0	2.08
$bla_{\text{CTX-M-1}}$	0	1	2.08
$bla_{\text{CTX-M-8}}$	1	0	2.08
$bla_{\text{CTX-M}}$	1	0	2.08
bla_{TEM}	1	0	2.08
Total	34	14	100

ceftazidime.[25] As the cephalosporins were found to be ineffective in the study area, it is practical for swine farms to stop using them.

The observed gap between the level of resistance among the Penams is related to the popularity of the antibiotics. Ampicillin was introduced first in medicine compared with piperacillin. Thus, the first has been used more frequently. The resistance level against amoxicillin/clavulanic acid is close to the report of Gundogan and Avci.[26] The studies of Lee and Yeh observed higher levels at 42% resistance in pigs in Taiwan, whereas only 1.3% was reported by Michael *et al.* in pigs in Germany.[21,25]

The resistance against ciprofloxacin is comparable with the previous report.[27] Fluoroquinolone-resistant ESBL-EC is a concern in human medicine because this family of antibiotics are commonly used for treatment of urinary tract infections. Hence, monitoring and characterization of this plasmid-mediated resistance is of public health importance.[28]

The resistance against gentamicin is comparable with the previous report.[27,29] In contrast, Ugwu *et al.* reported 100% resistance, whereas Gundogan and Avci and Michael *et al.* reported a lower resistance compared with this study.[24–26] Apparently, gentamicin is the most common antibiotic under the aminoglycoside family used within the study area.

High resistance to trimethoprim/sulfamethoxazole was reported previously.[25] Commensal ESBL-EC strains can serve as reservoir of antimicrobial resistance because of high rate of resistance to other groups of antimicrobials (trimethoprim/sulfamethoxazole, ampicillin, ciprofloxacin, and gentamicin).[6]

The ESBL isolates are only sensitive to piperacillin/tazobactam, carbapenems, and amikacin. This observation may indicate that these drugs are not yet popular in the swine industry in contrast to the other antibiotics where resistance was observed. The observed sensitivities provide an opportunity to implement strategies aimed to conserve the effectiveness of these antibiotics. Colistin, despite being a popular antibiotic used in the study area, remains effective against the isolates. However, an alarming 4.2% of the isolates are resistant, which warrants further investigation because colistin-resistant ESBL *E. coli* is a threat to human antimicrobial therapy. Moreover, the observed prevalence of *mcr*-1 gene in this study is 54.17%. In 2015, *mcr* (Mobile COL-R)-1 gene was first described in China in commensal *E. coli* strains from animals. From then on, *mcr-1* gene was reported in enteric bacteria isolated from animals and meat for human consumption in different countries.[19] Carbapenem resistance was not observed in the isolates. Carbapenems are widely used and still considered as the treatment of choice against ESBL-producer bacteremia and its resistance is a newly focused problem in humans.[28,30]

The use of prophylactic antimicrobials was reported to be a risk factor in the occurrence of ESBL-EC in pigs.[24,31,32] The observed phenotypic resistance to β-lactams was confirmed by detecting the β-lactam-encoding genes in the isolates. The results show that the genotypic resistance profile of the isolates is diverse and co-occurrence of multiple genes of $bla_{\text{CTX-M}}$, bla_{SHV}, and bla_{TEM} is common among isolates. The $bla_{\text{CTX-M}}$ is the most common ESBL-encoding gene in this study, which is similar to published studies in pigs.[5,9,20,21,31] Similar to previous reports on pigs, the $bla_{\text{CTX-M-1}}$ is the most prevalent $bla_{\text{CTX-M}}$ variant in this study.[33] Because $bla_{\text{CTX-M-15}}$ is the most common genotype of ESBL-EC in humans, the high prevalence rate of this variant has public health importance.[3]

The $bla_{\text{CTX-M-8}}$ is the second most prevalent CTX-M type among isolates observed in this study. This is an emerging genotype in humans but no report can be found in pigs to date.[34]

The $bla_{\text{CTX-M-9}}$ gene was also previously isolated in a healthy pig.[5,32] In contrast to this study, previous reports have identified $bla_{\text{CTX-M-9}}$ group as the most common genotype in isolates from pigs.[20,35] The $bla_{\text{CTX-M-2}}$ is the least prevalent CTX-M type in isolates of porcine origin, which agrees with studies of Biasino *et al.*[36] No $bla_{\text{CTX-M-25}}$ gene was detected in this study. Either, the gene is not present during the time of study or an improvement can be done in the optimization procedure for the PCR condition employed.

The prevalence of bla_{TEM} genotype in this study is similar to the report of Valentina and higher than that reported by Xu *et al*.[20,37] The prevalence of bla_{SHV} gene in the isolates is high in contrast to other reports that bla_{SHV} genotype was not isolated.[5,20] In this study, bla_{SHV} genotype have lower prevalence than $bla_{\text{CTX-M}}$ and bla_{TEM}. This supports the previous reports that $bla_{\text{CTX-M}}$ is more widely distributed and prevalent among ESBL genotypes.

The relationships of swine isolates from human isolates were established in earlier studies, suggesting a potential zoonotic transmission.[7–9] Fecal contaminations in pork during slaughter, processing, and selling of pork products can result in zoonotic transmission.[36,38] Although boot swab samples had lesser number of ESBL-EC isolates than fecal samples, it is not statistically different from the prevalence on fresh fecal samples (Table 2). Identification of ESBL-EC in farm premises and pig housing could result in possible transmission to farm personnel and in the surrounding community as previously reported.[8]

Most of the isolates from pigs and boot swabs carry more than two $bla_{\text{CTX-M}}$ groups. Coexistence of more than two CTX-M β-lactamases in the same isolate is frequent in this study and has been previously reported.[5,21,25] Because CTX-M has homologous regions, this coexistence of different CTX-M β-lactamases can be expected, which may result in the development of recombinant enzymes.[39] Multiple genotypes in single isolate could be suspected to result in difficulty in treating the infections they could cause because phenotypic ESBL expression is more likely to ensue.

Several investigators have reported the co-occurrence of different β-lactamase genes in the same isolates.[39] The $bla_{\text{CTX-M}}$- and bla_{TEM} are the most common ESBL genotypes among our isolates that agrees with other studies.[5,20] To our knowledge, there was no previous report of co-resistance of different ESBL genotypes among isolates from pigs and humans in the Philippines except in broilers.[10] Despite the reduced expression of one or two of ESBL genes, the occurrence of multiple ESBL resistance genes in a single strain could maintain its resistance to β-lactamases.

The authors also observed that four isolates were negative to the universal $bla_{\text{CTX-M}}$ primers but were positive to other $bla_{\text{CTX-Mgroup}}$ genes. Likewise, there is one isolate that yielded positive result to the universal $bla_{\text{CTX-M}}$ primer but negative to all $bla_{\text{CTX-Mgroups.}}$ In addition, one isolate was negative to the $bla_{\text{CTX-M-1}}$ gene but was positive to its subtype, $bla_{\text{CTX-M-15}}$. Development and use of multiplex PCR is suggested to minimize such problems. There are also two isolates not identified by Vitek as ESBL-EC but confirmed through CDT. This is supported by the study of Garrec *et al.* that showed higher sensitivity for CDT (97%) than Vitek (80%).[40] However, one possible reason for the negative ESBL test result is the coexistence of AmpC beta lactamase in the isolate where ESBL is masked by the high-level expression of AmpC-type enzymes.[41] The coexistence of ESBL and AmpC beta lactamases in one single isolate has been well documented in many previous studies.[26,36,38] Thus, it is possible that some of our isolates may have coexistence of ESBL and AmpC beta lactamases. Cefoxitin resistance, an indicator for AmpC enzymes, occurred in 25% of isolates in our study. Hence, in future studies, we recommend that AmpC detection be included as well.

PCR amplification of $bla_{\text{CTX-M}}$ alone and without sequencing of ESBL-EC isolates mostly provides enough support that a $bla_{\text{CTX-M}}$ gene causes the expressed phenotype in CDT. Nevertheless, further sequencing analysis is recommended for bla_{TEM} and bla_{SHV} to discriminate between the non-ESBL parent enzymes and different variants of TEM or SHV.[2]

This study presents baseline data on the prevalence of ESBL-EC in swine farms in Luzon, Philippines. In addition, the occurrence of this resistant bacterium in healthy pigs and their pens pose a great risk of transmission to the workers, environment, and other animals with access to pigs and pig manure. Regulations and policies on antimicrobial usage should be strictly implemented and monitored while further surveillance studies should be conducted on other high pig-producing regions in the Philippines. Isolation and phylogenetic evaluation of this bacterium in farm workers, pork, and other possible fomites will improve the understanding on the epidemiology of this resistant bacterium.

The presented data confirmed that ESBL-EC is highly prevalent in healthy pigs and pig farms in the study area. The isolates are multidrug resistant with diverse combinations and belong to the three main ESBL genotypes, $bla_{\text{CTX-M}}$-, bla_{TEM}, and bla_{SHV}. The varied genotypic resistance profile of the isolates implies the diversity of ESBL-EC present in pigs that warrants further typing to detect possible presence of mutated and new subtype circulating in the country.

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