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Research article

Prevalence, antibiograms, antibiotic resistance genes, and virulence genes of *Arcobacter butzleri* isolated from healthy pigs in mid-northeastern Thailand

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Abstract

Pigs can have *Arcobacter butzleri*. However, information on *A. butzleri* in Thai pigs remains scarce. This work aimed to survey *A. butzleri* in healthy pigs and assess their antimicrobial susceptibility, potential transferrable antimicrobial resistance (AMR) genes, and virulence-associated genes (VAGs). Cross-sectional fecal samples of 203 pigs from 18 farms were cultured and molecularly identified. *A. butzleri* prevalence in all pigs was (31/203; 15.3%): nursery (0/8; 0%), finisher (27/144; 18.8%), and sow (4/51; 7.8%). The total farm *A. butzleri* prevalence was 50%: nursery (0/2: 0%), finisher (8/14: 57.1%), and sow (2/9: 22.2%) farms. From the 10 antibiotic disks evaluated, the isolates were mostly sensitive to imipenem (96.8%), tetracycline (83.9%), streptomycin (67.7%), and amoxycillin/clavulanic acid (54.8%); however, they were mostly resistant to cefotaxime (98.6%), sulbactam/cefoperazone (71%), ampicillin (67.7%), enrofloxacin (48.4%), and fosfomycin (42.9%) and were neither sensitive nor resistant to erythromycin. Most multidrug resistance patterns in this study were in four to six classes. Three isolates resisted all 10 antibiotics. However, only the *TetO* gene was detected in one isolate, whereas ESBLs (*SHV, CTX-M*, and *TEM*), PMQRs (*qnrA, qnrS, qnrB, oqxAB*, and *aac(6')-Ib-cr)*, *ermB*, and *mefA* genes were not found in any isolates. The rankings of VAGs presented in the isolates were *ciaB* (100%), *mviN* (97%), *pldA* (93%), *tlyA* (90%), *cj1349* (90%), *cadF* (83%), *hecB* (10%), *hecA* (7%), and *irgA* (0%), and most isolates carried six VAGs (77%). *A. butzleri* is present in healthy pigs, and this database is the first to show *A. butzleri* VAG and AMR genes in Thai pigs.

Keywords: *Arcobacter butzleri,* Pig, Prevalence, Resistance gene, Virulence gene

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INTRODUCTION

Arcobacter is the second genus in the Campylobacteriaceae family (Vandamme et al., 1991). Historically, *Arcobacter* began with case-control studies in which *Campylobacter*-like organisms or aerotolerant *Campylobacter* were found in the organs of aborted bovine and porcine fetuses (Ellis et al., 1978, 1977). A later study has indicated that *Arcobacter* is frequently isolated from aborted pig organs, whereas no other abortifacient pathogens have been discovered (On et al., 2002). However, the role of *Arcobacter* as a pathogen in swine and cattle remains unclear. In contrast, these bacteria have been recovered from the placenta and/or amniotic fluids of healthy cattle and pigs (Ellis et al., 1977, 1978). Furthermore, *Arcobacter* has been detected in gastric ulcer lesions as well as in the normal stomachs of slaughter pigs in Brazil (de Oliveira et al., 2010). In all regard, livestock are certainly *Arcobacter* reservoirs for human diseases.

Arcobacter research has increased significantly since the International Commission on Microbiological Specifications for Foods designated *Arcobacter* as a critical threat to human health in 2002 (Iwu et al., 2021). Bacteria in the genus *Arcobacter* are discovered in various sources, including animals, humans, meat products, seafood, vegetables, and water (Soma Sekhar et al., 2018; Kim et al., 2019; Shrestha et al., 2019; Aydin et al., 2020; Brückner et al., 2020). Some species of *Arcobacter* are considered to be an emerging food poisoning etiology in humans (Iwu et al., 2021). In particular, *Arcobacter butzleri* frequently causes diseases in humans (Jiménez-Guerra et al., 2020) and is prevalent in the food chain (Ferreira et al., 2019), especially in swine carcasses (Gobbi et al., 2018). Even though other countries have been continuously reporting *Arcobacter* in pigs (Gobbi et al., 2018; Soma Sekhar et al., 2018; Vicente-Martins et al., 2018; Kim et al., 2019), information regarding this bacterium in swine in Thailand remains limited. This work aimed to isolate and molecularly identify (based on genus-specific 16S rRNA gene and 23S rDNA sequences) *A. butzleri* from healthy swine and assess their antibiotic susceptibility, antimicrobial resistance (AMR) genes, and virulence-associated genes (VAGs). A recent study has revealed *A. butzleri* with VAGs in human diarrheal stools in Western Thailand (Kietsiri et al., 2021). The discovery of VAGs may initially indicate the pathogenicity of *A. butzleri*. *A. butzleri* is found in the pig's gut, and potentially transferable AMR genes are highly relevant because they can be exchanged between gut microbiomes. The findings of this study on *A. butzleri* prevalence, AMR characteristics, and virulence factors will be useful in developing a database of this bacterium in Thai swine and AMR surveillance systems.

MATERIALS AND METHODS

Sample collection and animal ethics

The sample size was calculated using the ready function in Open-Source Epidemiologic Statistics for Public Health software version 3.01 (OpenEpi, http://www.openepi.com/). The "allowable error" and "alpha" values were chosen to be 5% and 0.95, respectively. As a result, the minimal sample size

of 163 was estimated considering the expected prevalence of 12% *Arcobacter* detection in pig's feces by the cultural method (Mohan et al., 2014).

The samples were collected between June and October 2020. Feces were aseptically collected directly from an individual swine rectum. The animal protocol was approved by the Animal Research Ethics Committee of Khon Kaen University (IACUC-KKU 53/2563). The samples were kept in sterile plastic bags, chilled, and transported to the laboratory within 6 h. A total of 203 fecal samples from healthy pigs were collected from 18 backyard farms (wherever possible), primarily in the Thai provinces of Khon Kaen and some districts of Udon Thani and Maha Sarakham. The fecal samples consisted of nursery pigs (n = 8) from 2 farms, finishers (n = 144) from 14 farms, and sows (n = 51) from 9 farms. The nursery pigs are 1–3 months old, finishers are 4–6 months old, and sows are breeding female pigs.

Bacterial isolation and identification

Approximately 5 g of each fecal sample was added to 45 mL of *Arcobacter* enrichment broth (Oxoid, Hampshire, UK) with a charcoal cefoperazone desoxycholate agar (CCDA) selective supplement (SR0155E; Oxoid). The inoculum was incubated aerobically at 30 °C for 48–72 h. Approximately 200 µL of the pre-enriched inoculum was passively filtered on 0.45 µM cellulose acetate membrane paper (G.E. Healthcare, Japan) attached to the surface of 5% bovine blood-CCDA-tryptic soy agar (MERCK, Germany). After filter removal, the agar plates were incubated at 30 °C under aerobic conditions for 48–72 h. Presumptive *Arcobacter* colonies are gram negative, spiral or helical rods in shape, and urease and H_2S negative; the colonies also hydrolyze indoxyl acetate, produce oxidase, and are motile, with a single polar unsheathed flagellum (Fernandez et al., 2015). The pure culture was collected, tested for antibiotic susceptibility, and kept at −80 °C for further molecular analysis.

Polymerase chain reaction (PCR) targeting *A. butzleri*

Pure colonies of active-grown *Arcobacter* on blood agar were washed and pelleted with sterile distilled water. The suspension was then boiled at 100 °C for 10 min, snap chilled on ice for 20 min, and centrifuged (Sigma, Germany) at 14,725 rpm for 5 min. The supernatants were used as DNA templates for the PCR assays. The reference strain included in all analyses was *A. butzleri* DMST 19755 (obtained from the Department of Public Health, Ministry of Science, Thailand). The PCR mixture without the DNA template was used as the negative control.

The primers BUTZ-F and ARCO-R (Houf et al., 2000) targeting the 16S rRNA gene of *A. butzleri* were used in the PCR analysis. The reaction mixtures contained 5 µL of 10X PCR buffer (Thermo Fisher Scientific, USA), 3 µL of 25 mM $MgCl_2$ (Thermo Fisher Scientific), 1 µL of 10 mM of each deoxyribonucleotide triphosphate (Thermo Fisher Scientific), 0.5 µL of 10 µM of each primer (Integrated DNA Technologies, Singapore), 0.25 µL of 5 units of *Taq* DNA polymerase (Thermo Fisher Scientific), and 5 µL of DNA template and were adjusted to 50 µl with DNase-free H_2O (Invitrogen, USA). Sample amplifications were operated in a MyCycler PCR machine (Bio-Rad, USA). The program consisted of pre-heating at 94 °C for 4 min, followed by 35

cycles of denaturation at 94 °C for 45 s, annealing at 61°C for 45 s, extension at 72 °C for 30 s, and final extension at 72 °C for 5 min. The PCR products were visualized by electrophoresis in 3% agarose gel (Vivantis, Malaysia), stained with ethidium bromide, and inspected under a UV transilluminator (Vilber Lourmat, France).

Confirmation of *A. butzleri* by 23S rDNA sequences

The same set of DNA used in the initial *A. butzleri* species identification was used for the PCR amplification of *Arcobacter* genus-based 23S rRNA using the primers and conditions of the published protocol (Bastyns et al., 1995). The total volume of the PCR mixture was 20 µL, which included 2 µL of 10X *Taq* buffer (Thermo Fisher Scientific), 1.2 µL of 25 mM $MgCl_2$ (Thermo Fisher Scientific), 0.4 µL of 10 mM of each deoxyribonucleotide triphosphate (Thermo Fisher Scientific), 1 µL of 10 µM of each primer (Integrated DNA Technologies), 0.25 µL of 5 units of *Taq* DNA polymerase (Thermo Fisher Scientific), 2 µL of DNA template, and 12.15 µL of DNase-free H_2O (Invitrogen). After pre-heating at 95 °C for 3 min, 34 cycles of denaturation at 94 °C for 60 s, annealing at 61 °C for 60 s, extension at 72 °C for 60 s, and final extension at 72 °C for 3 min. The amplicons with a size of 331 bp were further analyzed using their partial nucleotide sequences by the barcode-tagged sequencing method with the NGS-based innovative sequencing platform (Celemics, Korea), and the 23S rDNA sequences were compared with those in the GenBank database (http://www.ncbi.nim.nih.gov) to confirm *A. butzleri* identification.

Antimicrobial susceptibility

The antibiotic susceptibility of the *A. butzleri* isolates was determined using the Kirby-Bauer disk diffusion method following the Clinical and Laboratory Standard Institute guidelines (CLSI, 2020), and the interpretation was compared to *Campylobacter jejuni* and *Enterobacteriaceae* for fosfomycin (FOS) interpretation (Wayne, 2020). The 10 antibiotic disks (Oxoid, UK) were enrofloxacin (ENR), FOS, ampicillin (AMP), amoxycillin/clavulanic acid (AMC), streptomycin (STR), cefotaxime (CTX), sulbactam/cefoperazone (SCF), imipenem (IPM), tetracycline (TET), and erythromycin (ERY). In brief, 100 µL of active-grown *Arcobacter* culture, which was quantified to 0.5 standard McFarland (approximately 10^8 CFU/mL in phosphate-buffered saline) before use, was spread on blood agar plates. The antibiotic disks were aseptically placed on the surface of the agar plates and incubated at 30 °C under aerophilic conditions for 24–48 h. *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 259223 were included for quality control, and their appropriate mediums and growth conditions were used.

Detection of AMR genes

The frozen stored bacterial isolates were regrown on blood agar, and a few colonies from the second subcultured bacteria were subjected to DNA extraction using the GF-1 bacterial DNA extraction kit (Vivantis, Malaysia). The DNA quality was checked before using the DNA in multiplex and/or uniplex PCR assays. The goal of this study was to look for plasmid-mediated AMR genes in *A. butzleri* isolates. Extended-spectrum beta-lactamases

(ESBLs) (*SHV, CTX-M*, and *TEM*), plasmid-mediated quinolone resistance; plasmid-mediated quinolone resistances (PMQRs) (*qnrA, qnrB, qnrC, qnrD, qnrS, oqxAB*, and *aac(6')-Ib-cr)*, macrolide resistance (*ermB* and *mefA*), and TET (*TetO*) groups were also included in the AMR gene analysis. The standard reference genes were generously provided by Associate Professor Dr. Anusak Kerdsin. Our known *A. skirrowii ermB*- and *TetO*-positive genes, BV457 and BV458, were also included in the PCR assays. Excluding *mefA*, *qnrC*, and *qnrD* for references, all standard AMR genes were included and compared in the PCR assays. All primers were purchased from Integrated DNA Technologies, Inc. (United States). The *Taq* polymerases were 2X ViRed *Taq* master mix in all reactions (Vivantis, Malaysia).

Multiplex PCR for *ermB* and *mefA* gene detection, as well as uniplex PCR for *TetO*, were performed according to the procedures of Nagai et al. (2001). PMQR multiplex PCR was performed according to the procedures of Ciesielczuk et al. (2013). Finally, ESBL multiplex PCR was performed according to the procedures of Monstein et al. (2007). Table 1 lists the primers used in the reaction. In each PCR reaction, a total volume mixture of 15 µL was used with similar programs of 30–35 cycles (denature at 95 °C for 30 s and extension at 72 °C for 90 s), with the exception of 30 s of annealing at temperatures of 53 °C for *ermA* and *mefA*, 55 °C for *TetO* and ESBLs, and 63 °C for PMQRs. If a specific size of the PCR product appears in the multiplex PCR, a uniplex PCR is performed, and the amplicons were submitted for sequencing.

Table 1 Primers for AMR gene detection and standard reference genes in this study.

Target gene	Primer	Primer sequence (5'-3')	Product size (bp)	Reference	Standard gene reference in the assay*
ermB	erm(B)-F	CGTACCTTGGATATTCACCG	224	Nagai et al. (2001)	*Streptococcus suis* 41948; *A. skirrowii* BV 457**
	erm(B)-R	GTAAACAGTTGACGATATTCTCG			*Streptococcus suis* 23139
mefA	mef(A)-F	CTGTATGGAGCTACCTGTCTGG	402	Nagai et al. (2001)	no reference
	mef(A)-R	CCCAGCTTAGGTATACGTAC			
TetO	TetO-F	AACTTAGGCATTCTGGCTCAC	515	Nagai et al. (2001)	*Streptococcus suis* 41948; *A. skirrowii* BV 458**
	TetO-R	TCCCACTGTTCCATATCGTCA			*Streptococcus suis* 23139
qnrA	qnrA-F	CAGCAAGAGGATTTCTCACG	630	Ciesielczuk et al. (2013)	*E. coli* C95A+B
	qnrA-R	AATCCGGCAGCACTATTACTC			
qnrB	qnrB-F	GGCTGTCAGTTCTATGATCG	488	Ciesielczuk et al. (2013)	*E. coli* C57B
	qnrB-R	GATCAACGATGCCTGGTAG			
qnrC	qnrC-F	GCAGAATTCAGGGGTGTGAT	118	Ciesielczuk et al. (2013)	no reference
	qnrC-R	AACTGCTCCAAAAGCTGCTC			
qnrD	qnrD-F	CGAGATCAATTTACGGGGAATA	581	Cavaco et al. (2009)	no reference
	qnrD-R	AACAAGCTGAAGCGCCTG			
qnrS	qnrS-F	GCAAGTTCATTGAACAGGGT	428	Cattoir et al. (2007)	*E. coli* C5914
	qnrS-R	TCTAAACCGTCGAGTTCGGCG			
oqxAB	oqxAB-F	CCGCACCGATAAATTAGTCC	313	Ciesielczuk et al. (2013)	*E. coli* C54
	oqxAB-R	GGCGAGGTTTTGATAGTGGA			
aac(6')-Ib-cr	aac(6')-Ib-cr-F	TTGGAAGCGGGGACGGAC	260	Wareham et al. (2010)	*E. coli*
	aac(6_)-Ib-cr-R	ACACGGCTGGACCATA			
SHV	SHV.SE	ATGCGTTATATTCGCCTGTG	747	Monstein et al. (2007)	*Klebsiella pneumoniae*
	SHV.AS	TGCTTTGTTATTCGGGCCAA			
CTX-M	CTX-M-U1	ATGTGCAGYACCAGTAARGTKATGGC	593	Monstein et al. (2007)	*E. coli*
	CTX-M-U2	TGGGTRAARTARGTSACCAGAAYCAGCGG			
TEM	TEM-164.SE	TCGCCGCATACACTATTCTCAGAATGA	445	Monstein et al. (2007)	*E. coli*
	TEM-165.AS	ACGCTCACCGGCTCCAGATTTAT			

* Standard reference genes obtained from Associate Professor Dr. Anusak Kerdsin
**Our previously identified *A. skirrowii* BV457-*ermB* and *A. skirrowii* BV458-*TetO* positive genes

Determination of VAGs

RNA from *A. butzleri* isolates was extracted from the bacterial cell pellet using the GF-1 total RNA extraction kit (Vivantis) and converted to DNA using the Viva cDNA synthesis kit (Vivantis) for VAG analysis. The cDNA samples were stored at −80 °C after quality and quantity checks using a spectrophotometer (BioDrop, UK). The nine VAGs evaluated in this study were *cadF*, *ciaB*, *cj1349*, *hecA*, *hecB*, *irgA*, *mviN*, *pldA*, and *tlyA*. A single-gene PCR assay used the previously designed primers (Douidah et al., 2012) and PCR conditions (Kietsiri et al., 2021). Table 2 shows the primer pairs for the assay and the target sizes. Some of the resulting amplicons were sequenced and used as the standard in subsequent assays. The reaction mixture (25 µL) contained final concentrations of 1X of 10X *Taq* buffer (Thermo Fisher Scientific), 0.2 mM of 10mM dNTP (Thermo Fisher Scientific), 0.1 µM of each 10µM primer (Integrated DNA Technologies), 1.25 units of 5U *Taq* gold polymerase (Invitrogen), 1.5 mM of 25mM $MgCl_2$ (Thermo Fisher Scientific), and 2 µL of genomic DNA. The PCR program included initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 45 s; annealing for 45 s at 53 °C for *ciaB, cj1349, mviN, pldA*, and *tlyA* genes, 55 °C for *cadF* and *hecB* genes, and 56 °C for *hecA* and *irgA* genes; and extension at 72 °C for 3 min. The amplicons were visualized using electrophoresis.

Table 2 Primers as of (Douidah et al., 2012) used for virulence-associated gene detection in this study.

Target gene	Virulence factor	Primer sequence (5′ to 3′)	Product size (bp)
cadF	Fibronectin-binding protein	TTACTCCTACACCGTAGT	283
		AAACTATGCTAACGCTGGTT	
ciaB	Invasion antigen B	TGGGCAGATGTGGATAGAGCTTGGA	284
		TAGTGCTGGTCGTCCCACATAAAG	
cj1349	Fibronectin-binding protein	CCAGAAATCACTGGCTTTTGAG	659
		GGGCATAAGTTAGATGAGGTTCC	
hecA	Filamentous hemagglutinin	GTGGAAGTACAACGATAGCAGGCTC	537
		GTCTGTTTTAGTTGCTCTGCACTC	
hecB	Hemolysin activation protein	CTAAACTCTACAAATCGTGC	528
		CTTTTGAGTGTTGACCTC	
irgA	Iron acquisition	TGCAGAGGATACTTGGAGCGTAACT	437
		GTATAACCCCATTGATGAGGAGCA	
mviN	Peptidoglycan biosynthesis	TGCACTTGTTGCAAAACGGTG	294
		TGCTGATGGAGCTTTTACGCAAGC	
pldA	Phospholipase A	TTGACGAGACAATAAGTGCAGC	293
		CGTCTTTATCTTTGCTTTCAGGGA	
tlyA	Hemolysin	CAAAGTCGAAACAAAGCGACTG	230
		TCCACCAGTGCTACTTCCTATA	

RESULTS

Using specific primers for *A. butzleri*, 31 isolates were initially identified as *A. butzeri*. Figure 1 shows a photograph of the PCR amplicons (401 bp) indicating *A. butzleri*. The same result was achieved when the genus-based 23S rRNA was amplified. The 23S rDNA sequences of the 31 isolates were compared to the GenBank database, resulting in *A. butzleri* identification. Table 3 displays the accession numbers and similarity rates of the GenBank reference isolates. Details of the samples analyzed, rates of *A. butzleri* detection, and isolate identifications in each farm and age group are shown in Table 4. A total of 18 farms were included in the study, with some farms having one, two, or all age groups. Pig production sizes ranged from one to fifty heads. One farm raised all three age groups, another raised only nursery pigs and sows, four farms raised only finishers and sows, three farms only had sows, and nine farms only raised finishers. All eight nursery pigs from two farms tested negative for *A. butzleri*; in one farm, both nursery pigs and sows tested negative; however, in another farm, finishers and sows carried *A. butzleri* but the nursery pigs did not. Most *A. butzleri* isolates were recovered from the finishers. From 203 fecal samples of healthy pigs, 31 pigs (15.3%; 95%CI: 10.6-20.9) carried *A. butzleri* (Table 5). The prevalence in each age group: *A. butzleri* detection rates in finishers and sows were 18.8% (95%CI: 13.2-25.9) and 7.8% (95%CI: 3.1-18.5), respectively. Considering at least one positive pig as a positive farm, pig samples from nine (50%, 95%CI: 29.0-70.9) of 18 farms were positive for *A. butzleri*; finishing farms yielded 57.1% (95%CI: 32.6-78.6), and sow farms yielded 22.2% (95%CI: 6.3-54.7) positive rates (Table 5).

Figure 1 Photograph of polymerase chain reaction amplicons indicating *A. butzleri* (401 bp). M: Molecular weight marker (1.5 Kb). Numbers 1 to 7 represent the isolate samples. Pos: Positive control; *A. butzleri* DMST 19755. Neg: negative control.

Table 3 GenBank accession numbers of the AMR gene, VAG, and 23S rDNA sequences of the isolates in this study.

No.	Isolate ID	From (Pig)	Species/ gene	Reference isolate No. in the GenBank	% Similarity	Accession No.
1	KK328	Finishing	*A. butzleri*	*Arcobacter butzleri* strain ED-1 23S ribosomal RNA gene, complete sequence	100	OQ396365
2	KK329	Finishing	*A. butzleri*	*Arcobacter butzleri* strain ED-1 23S ribosomal RNA gene, complete sequence	100	OQ396366
3	KK330	Finishing	*A. butzleri*	*Arcobacter butzleri* strain ED-1 23S ribosomal RNA gene, complete sequence	100	OQ396367
4	KK331	Finishing	*A. butzleri*	*Arcobacter butzleri* strain ED-1 23S ribosomal RNA gene, complete sequence	100	OQ396368
5	KK338	Finishing	*A. butzleri*	*Arcobacter butzleri* strain ED-1 23S ribosomal RNA gene, complete sequence	100	OQ396369
6	KK341	Finishing	*A. butzleri*	*Arcobacter butzleri* strain ED-1 23S ribosomal RNA gene, complete sequence	100	OQ396370
7	KK342	Finishing	*A. butzleri*	*Arcobacter butzleri* strain ED-1 23S ribosomal RNA gene, complete sequence	100	OQ396371
8	KK343	Finishing	*A. butzleri*	*Arcobacter butzleri* strain ED-1 23S ribosomal RNA gene, complete sequence	100	OQ396372
9	KK345	Finishing	*A. butzleri*	*Arcobacter butzleri* strain ED-1 23S ribosomal RNA gene, complete sequence	100	OQ396373
10	KK349	Finishing	*A. butzleri*	*Arcobacter butzleri* strain ED-1 23S ribosomal RNA gene, complete sequence	100	OQ396374
11	KK351	Finishing	*A. butzleri*	*Arcobacter butzleri* strain ED-1 23S ribosomal RNA gene, complete sequence	100	OQ396375
12	KK352	Finishing	*A. butzleri*	*Arcobacter butzleri* strain ED-1 23S ribosomal RNA gene, complete sequence	100	OQ396376
13	KK353	Finishing	*A. butzleri*	*Arcobacter butzleri* strain ED-1 23S ribosomal RNA gene, complete sequence	100	OQ396377
14	KK354	Finishing	*A. butzleri*	*Arcobacter butzleri* strain ED-1 23S ribosomal RNA gene, complete sequence	100	OQ396378
15	KK356	Finishing	*A. butzleri*	*Arcobacter butzleri* strain ED-1 23S ribosomal RNA gene, complete sequence	100	OQ396379
16	KK357	Finishing	*A. butzleri*	*Arcobacter butzleri* strain ED-1 23S ribosomal RNA gene, complete sequence	100	OQ396380
17	KK358	Finishing	*A. butzleri*	*Arcobacter butzleri* strain ED-1 23S ribosomal RNA gene, complete sequence	100	OQ396381
18	KK359	Finishing	*A. butzleri*	*Arcobacter butzleri* strain ED-1 23S ribosomal RNA gene, complete sequence	100	OQ396382
19	KK360	Finishing	*A. butzleri*	*Arcobacter butzleri* strain ED-1 23S ribosomal RNA gene, complete sequence	100	OQ396383
20	KK361	Finishing	*A. butzleri*	*Arcobacter butzleri* strain ED-1 23S ribosomal RNA gene, complete sequence	100	OQ396384
21	KK362	Sow	*A. butzleri*	*Arcobacter butzleri* strain ED-1 23S ribosomal RNA gene, complete sequence	100	OQ396385

Table 3 GenBank accession numbers of the AMR gene, VAG, and 23S rDNA sequences of the isolates in this study. (Cont.)

No.	Isolate ID	From (Pig)	Species/ gene	Reference isolate No. in the GenBank	% Similarity	Accession No.
22	KK364	Sow	*A. butzleri*	*Arcobacter butzleri* strain ED-1 23S ribosomal RNA gene, complete sequence	100	OQ396386
23	KK365	Sow	*A. butzleri*	*Arcobacter butzleri* strain ED-1 23S ribosomal RNA gene, complete sequence	100	OQ396387
24	KK375	Finishing	*A. butzleri*	*Arcobacter butzleri* strain ED-1 23S ribosomal RNA gene, complete sequence	99.3	OQ396388
25	KK377	Finishing	*A. butzleri*	*Arcobacter butzleri* strain ED-1 23S ribosomal RNA gene, complete sequence	100	OQ396389
26	KK417	Finishing	*A. butzleri*	*Arcobacter butzleri* strain ED-1 23S ribosomal RNA gene, complete sequence	100	OQ396390
27	KK424	Finishing	*A. butzleri*	*Arcobacter butzleri* strain ED-1 23S ribosomal RNA gene, complete sequence	100	OQ396391
28	KK432	Finishing	*A. butzleri*	*Arcobacter butzleri* strain ED-1 23S ribosomal RNA gene, complete sequence	100	OQ396392
29	KK485	Finishing	*A. butzleri*	*Aliarcobacter butzleri* strain ED-1 chromosome, complete genome	97.7	OQ396393
30	KK486	Sow	*A. butzleri*	*Arcobacter butzleri* strain ED-1 23S ribosomal RNA gene, complete sequence	100	OQ396394
31	KK509	Finishing	*A. butzleri*	*Arcobacter butzleri* strain ED-1 23S ribosomal RNA gene, complete sequence	100	OQ396395
32	KK417	Finishing	*TetO*	*Niallia circulans strain* FDAARGOS_783 chromosome, complete genome (ID number: CP053989.1)	89.0	2676145*
33	KK365	Sow	*cadF*	*Arcobacter butzleri* 7h1h	98.9	2676155*
34	KK365	Sow	*ciaB*	*Aliarcobacter butzleri* strain P1100	93.8	2676168*
35	KK365	Sow	*cj1349*	*Aliarcobacter butzleri* JV22	99.1	2676172*
36	KK365	Sow	*hecA*	*Arcobacter butzleri* strain NCTC12481	97.0	2676188*
37	KK365	Sow	*hecB*	*Aliarcobacter butzleri* strain ED-1	99.2	2676212*
38	KK365	Sow	*mviN*	*Aliarcobacter butzleri* JV22	99.2	2676220*
39	KK365	Sow	*pldA*	*Aliarcobacter butzleri* JV22	98.9	2676228*
40	KK365	Sow	*tlyA*	*Aliarcobacter butzleri* strain ED-1	100	2676235*

*Submission ID

Table 4 Number of samples analyzed, *A. butzleri* detection rates, and isolate identifications in each farm (18 farms, 203 pigs).

Farm No.	Analyzed samples (n)				Positive No. (%)	95% CI	n (Isolate ID)	
	Nursery	Finisher	Sow	Total			Finisher	Sow
1	-	4	-	4	4 (100)	39.8−100	4 (KK328, KK329, KK330, KK331)	-
2	-	6	-	6	0	0	-	-
3	-	11	-	11	5 (45.5)	16.8−76.6	5 (KK338, KK341, KK342, KK343, KK345)	-
4	-	8	-	8	6 (75.0)	34.9−96.8	6 (KK349, KK351, KK352, KK353, KK354, KK356)	-
5	-	5	-	5	5 (100)	47.8−100	5 (KK357, KK358, KK359, KK360, KK361)	-
6	4	10	9	23	5 (21.7)	7.5−43.7	2 (KK375, KK377)	3 (KK362, KK364, KK365)
7	-	33	-	33	3 (9.1)	1.9−24.3	3 (KK417, KK424, KK432)	-
8	-	8	5	13	0	0	-	-
9	-	8	-	8	0	0	-	-
10	-	10	-	10	1 (10)	0.3−44.5	1 (KK485)	-
11	-	-	4	4	1 (25.0)	0.6−80.6	-	1 (KK486)
12	4	-	2	6	0		-	-
13	-	3	6	9	1 (11.1)	0.3−48.3	1 (KK509)	-
14	-	6	2	8	0	0	-	-
15	-	6	10	16	0	0	-	-
16	-	-	3	3	0	0	-	-
17	-	-	10	10	0	0	-	-
18	-	26	-	26	0	0	-	-
Total	8	144	51	203	31 (15.3)	10.6−20.9	-	-

Table 5 The prevalence of *A. butzleri* in pigs of different age groups and farms.

Group	Analyzed samples (n)		Positive samples (%)	95% CI		Positive samples (%)	95% CI	
	Pigs	Farms	Pigs	Lower Limit	Upper Limit	Farms	Lower Limit	Upper Limit
Nursery	8	2	0	0	0	0	0	0
Finisher	144	14	27 (18.8)	13.2	25.9	8 (57.1)	32.6	78.6
Sow	51	9	4 (7.8)	3.1	18.5	2 (22.2)	6.3	54.7
All pigs	203	18	31 (15.3)	10.6	20.9	9 (50.0)	29.0	70.9

Susceptibility by ranking order of all isolates was IPM (96.8%), TET (83.9%), STR (67.7%), AMC (54.8%), FOS (39.3%), ENR (32.3%), AMP (22.6%), SCF (12.9%), ERY (19.4%), and CTX (3.2%). In contrast, the isolates exhibited antibiotic resistance to CTX (98.6%), SCF (71%), AMP (67.7%), ENR (48.4%), FOS (42.9%), STR (32.3%), ERY (29.0%), AMC (22.6%), TET (12.9%), and IPM (3.2%). IPM, STR, and CTX had clear sensitive or resistant results, whereas ERY (51.6%), AMC (22.6%), ENR (19.4%), FOS (17.9%), SCF (16.1%), AMP (9.7%), and TET (3.2%) fell into intermediate susceptibility. The isolates were neither sensitive nor resistant to ERY (Figure 2). *A. butzleri* isolates showed antibiotic resistance against one to ten drugs. Three isolates (KK364, KK417, and KK424) were resistant to all 10 antibiotics. TET resistance was found in seven isolates (KK352, KK359, KK361, KK362, and three all-drug resistant isolates). Another 10 isolates included three all-drug resistance, and seven other isolates (KK331, KK351, KK353, KK354, KK485, KK486, and KK509) exhibited CTX and AMC resistance, with 5/10 and 7/10 being SCF and ENR resistant, respectively. However, 7 of these 10 isolates were IPM susceptible. Most isolates showed multidrug resistance (MDR) patterns against four to six antibiotic classes (Table 6). Four (12.9%) isolates were insensitive to one antibiotic: CTX ($n = 3$) and STR ($n = 1$). Two antibiotic class resistance patterns (12.9%) were CTX-SCF ($n = 2$) and STR-CTX ($n = 2$). Three MDR patterns (9.7%) were AMP-AMC-CTX ($n = 2$) and AMP-CTX-SCF ($n = 1$). Four MDR (16.1%) patterns were ENR-AMP-CTX-SCF ($n = 1$), ENR-AMP-STR-CTX ($n = 1$), FOS-CTX-SCF-TET ($n = 1$), ENR-STR-CTX-ERY ($n = 1$), and AMP-AMC-CTX-SCF ($n = 1$). Five MDR patterns (19.4%) were ENR-FOS-AMP-CTX-SCF ($n = 3$), FOS-AMP-CTX-SCF-ERY ($n = 1$), ENR-AMP-CTX-SCF-ERY ($n = 1$), and FOS-AMP-AMC-CTX-SCF ($n = 1$). Six MDR patterns (16.1%) were ENR-AMP-STR-CTX-SCF-ERY ($n = 2$), ENR-AMP-CTX-SCF-TET-ERY ($n = 1$), ENR-FOS-AMP-CTX-SCF-TET ($n = 1$), and ENR-FOS-AMP-STR-CTX-SCF ($n = 1$). Seven MDR patterns (6.5%) were ENR-FOS-AMP-AMC-CTX-SCF-ERY ($n = 1$) and ENR-FOS-AMP-AMC-STR-CTX-SCF ($n = 1$). Eight MDR patterns (6.5%) were FOS-AMP-STR-CTX-SCF-IPM-TET-ERY ($n = 1$) and ENR-FOS-AMP-AMC-STR-CTX-SCF-ERY ($n = 1$).

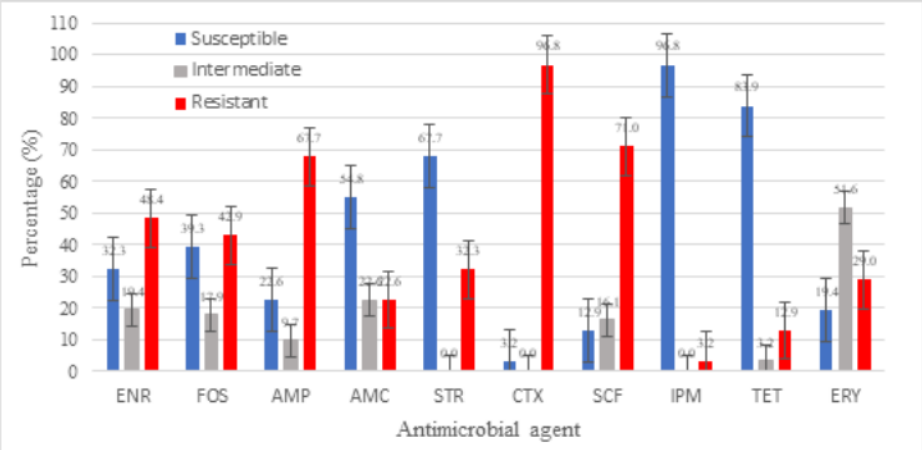

Figure 3 Antibiotic sensitivity of the *A. butzleri* isolates (n = 31). ENR: enrofloxacin, FOS: fosfomycin, AMP: ampicillin, AMC: amoxycillin/clavulanic acid, STR: streptomycin, CTX: cefotaxime, SCF: sulbactam/cefoperazone, IPM: imipenem, TET: tetracycline, ERY: erythromycin.

Table 6 Patterns of antibiotic resistance in *A. butzleri* isolates (n = 31) against ten antibiotics.

No. of Drugs	Resistance Pattern (n)	No. of Isolate	Resistance (%)	95% CI
1	CTX (3), STR (1)	4	12.9	3.6 - 29.8
2	CTX-SCF (2), STR-CTX (2)	4	12.9	3.6 - 29.8
3	AMP-AMC-CTX (2), AMP-CTX-SCF (1)	3	9.7	2.0 - 25.8
4	ENR-AMP-CTX-SCF (1) ENR-AMP-STR-CTX (1) FOS-CTX-SCF-TET (1) ENR-STR-CTX-ERY (1) AMP-AMC-CTX-SCF (1)	5	16.1	5.1 - 33.7
5	ENR-FOS-AMP-CTX-SCF (3) FOS-AMP-CTX-SCF-ERY (1) ENR-AMP-CTX-SCF-ERY (1) FOS-AMP-AMC-CTX-SCF (1)	6	19.4	7.1 - 37.5
6	ENR-AMP-STR-CTX-SCF-ERY (2) ENR-AMP-CTX-SCF-TET-ERY (1) ENR-FOS-AMP-CTX-SCF-TET (1) ENR-FOS-AMP-STR-CTX-SCF (1)	5	16.1	5.1 - 33.7
7	ENR-FOS-AMP-AMC-CTX-SCF-ERY (1) ENR-FOS-AMP-AMC-STR-CTX-SCF (1)	2	6.5	0.8 - 21.4
8	FOS-AMP-STR-CTX-SCF-IPM-TET-ERY (1) ENR-FOS-AMP-AMC-STR-CTX-SCF-ERY (1)	2	6.5	0.8 - 21.4

ENR: enrofloxacin, FOS: fosfomycin, AMP: ampicillin, AMC: amoxycillin/clavulanic acid, STR: streptomycin, CTX: cefotaxime, SCF: sulbactam/cefoperazone, IPM: imipenem, TET: tetracycline, ERY: erythromycin

Given that one isolate (KK432) lost viability and could not be sustained for DNA extraction, only 30 isolates were included in the AMR gene analysis. In the multiplex PCR assays, no ESBL, PMQR, *ermB*, or *mefA* genes were detected in any of the isolates. Figure 3 shows that no PCR product of the three ESBL genes was found in any of the samples tested, whereas the positive control showed size-specific bands in the 3% agarose gel: *SHV*-747bp, *CTX-M*-593bp, and *TEM*-445bp. Similarly, none of the seven PMQR gene products were found in any of the 30 samples examined; however, clear bands with specific sizes of *qnrA*-630bp, *qnrS*-428bp, *qnrB*-488bp, *oqxAB*-313bp, and *aac(6')-Ib-cr*-260bp appeared (Figure 4). In this experiment, standard controls for the *qnrC, qnrD*, and *mefA* genes were not employed; however, the primers for these genes in the analysis were included. All samples were tested for multiplex *ermB* and *mefA* PCRs, as well as for *TetO* uniplex PCRs. Figure 5 shows the amplified products from the uniplex PCR assays used to detect the *ermB*, *mefA*, and *TetO* genes in some of the isolates. Even though the standard controls *ermB* and *TetO* were clearly visible, only one of the analyzed samples tested positive for the *TetO* gene (Figure 5). The sequence of the *TetO* gene product (758 bp) was compared to available species or chromosomes in the GenBank database, and its sequence was submitted to the web database (Table 3). The blast result yielded 89% similarity to the *Niallia circulans* strain FDAARGOS_783 chromosome, complete genome (ID number: CP053989.1). The following VAGs were found in order of detection among 30 isolates: *ciaB* (100%), *mviN* (97%), *pldA* (93%), *tlyA* (90%), *cj1349* (90%), *cadF* (83%), *hecB* (10%), *hecA* (7%), and *irgA* (0%) (Table 7). Each individual isolate carried one to eight VAGs, with most isolates carrying six VAGs, accounting for 77% of all isolates. Some of the VAG nucleotide sequences from this study were deposited in GenBank (Table 3).

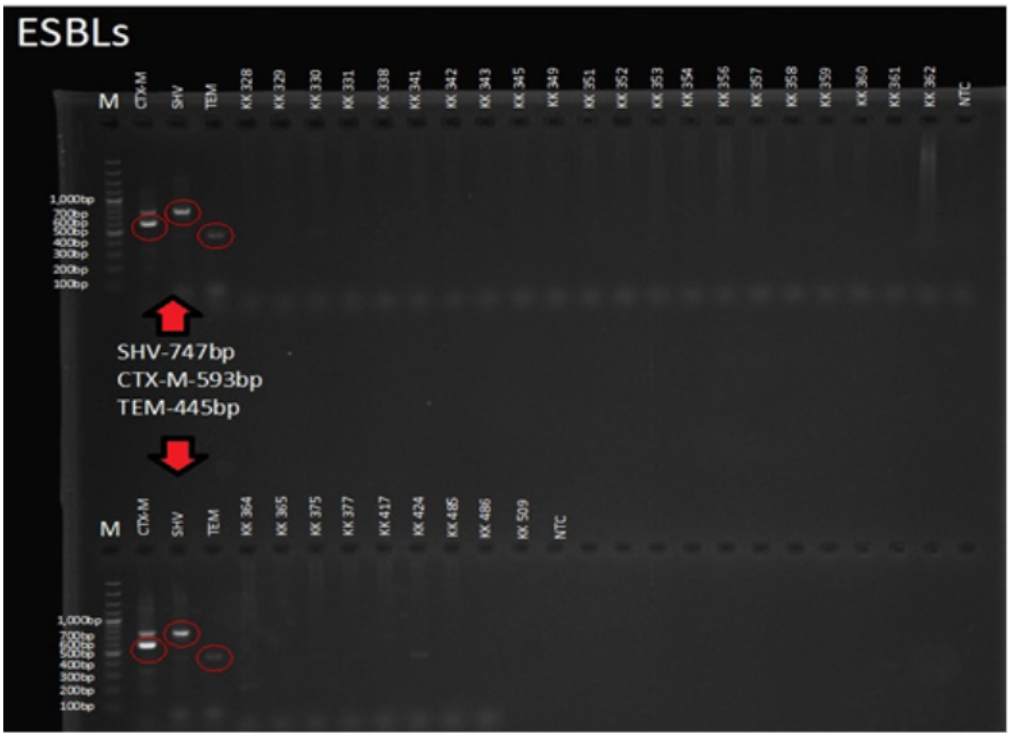

Figure 3 The agarose gel picture demonstrates that there was not any PCR product of the three ESBLs genes found in all 30 samples analyzed, whereas positive control showed size-specific bands; *SHV*-747bp, *CTX-M*-593bp, and *TEM*-445bp. NTC: negative control.

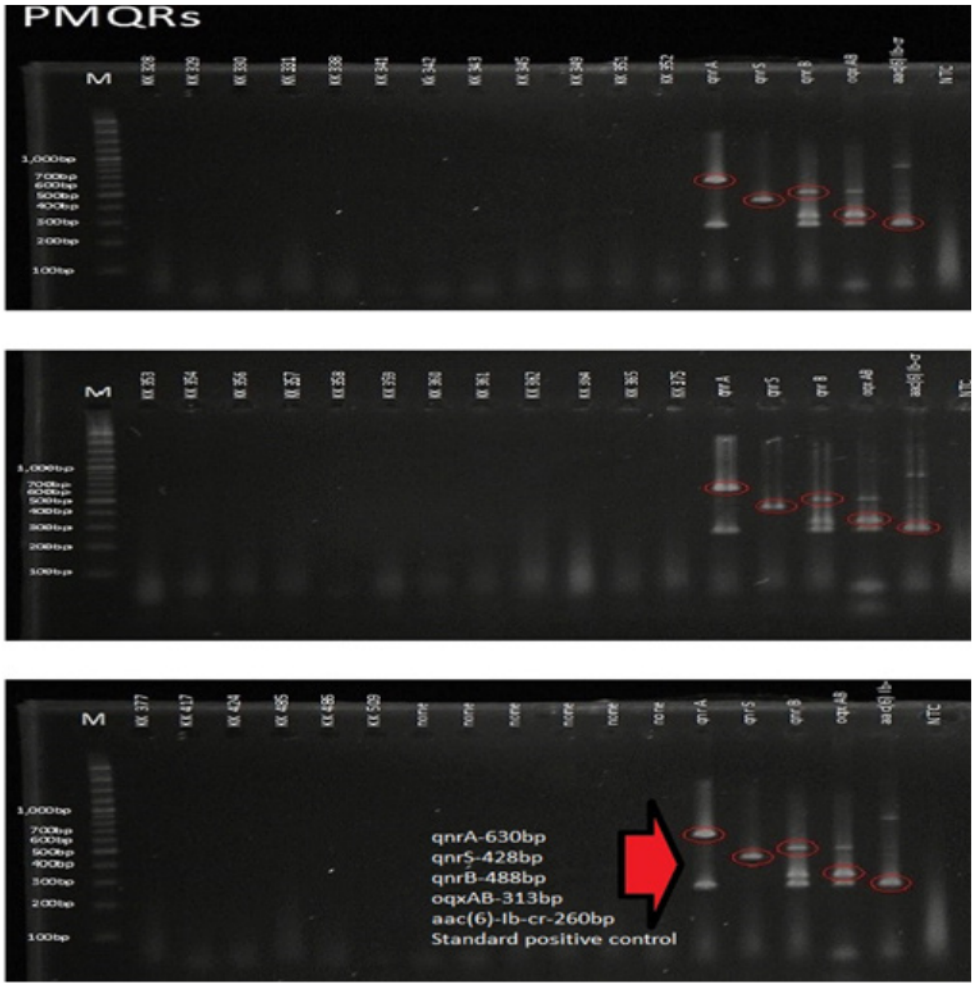

Figure 4 The agarose gel picture demonstrates negative result of the seven PMQRs gene products in all 30 samples analyzed, but clear bands with specific sizes of *qnrA*-630bp, *qnrS*-428bp, *qnrB*-488bp, *oqxAB*-313bp, and *aac(6')-Ib-cr*-260bp standard references did appear. In this study, there was no *qnrC* and *qnrD* for the references. NTC: negative control.

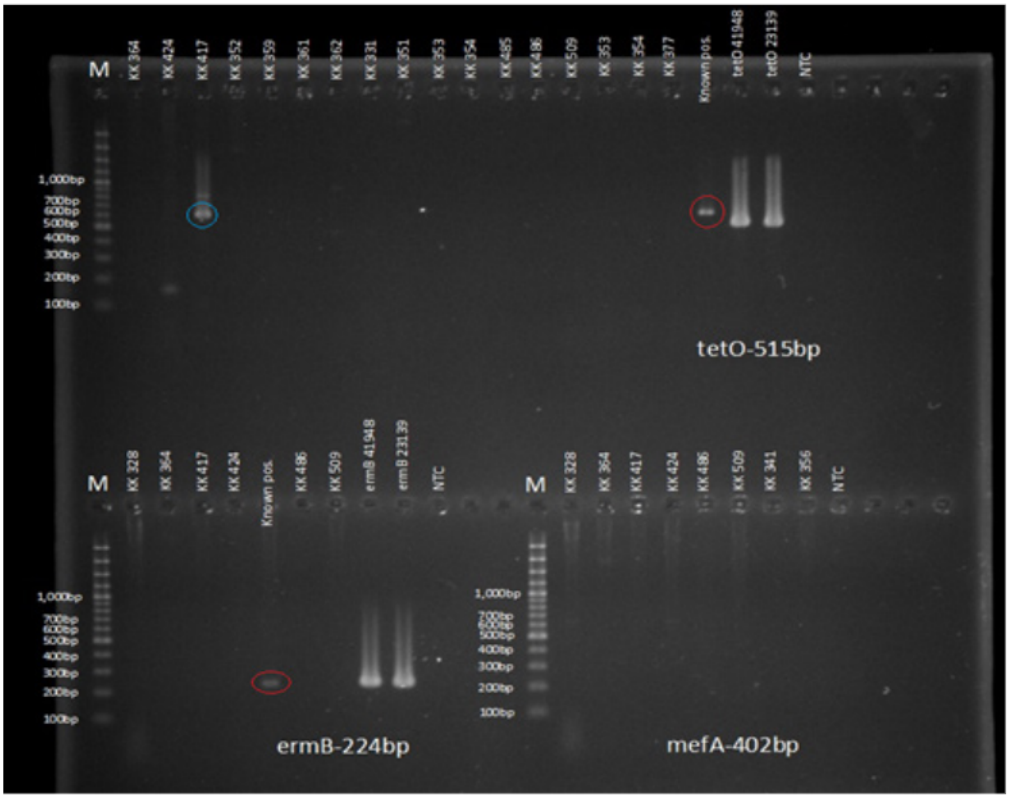

Figure 5 A photograph demonstrates negative/positive amplified products from uniplex PCR assays for *ermB, mefA*, and *TetO* gene in some of the isolates. In this study, one isolate (KK417) and our *TetO* known positive (*A. skirrowii* BV457) had the specific band for *TetO*, and in the picture, *ermB* and *TetO* standard controls and our *ermB* known positive (*A. skirrowii* BV458) were clearly visible. There was no *mefA* standard control in the assay. NTC: negative control, known pos.: known positive.

Table 7 Result of virulence gene detection in *A. butzleri* isolates (n = 30) in this study.

No.	Isolate ID	*cadF*	*ciaB*	*cj1349*	*hecA*	*hecB*	*irgA*	*mviN*	*pldA*	*tlyA*
1	KK328	-	*ciaB*	*cj1349*	-	*hecB*	-	*mviN*	*pldA*	*tlyA*
2	KK329	*cadF*	*ciaB*	*cj1349*	-	*hecB*	-	*mviN*	*pldA*	*tlyA*
3	KK330	*cadF*	*ciaB*	*cj1349*	-	-	-	*mviN*	*pldA*	*tlyA*
4	KK331	*cadF*	*ciaB*	*cj1349*	-	-	-	*mviN*	*pldA*	*tlyA*
5	KK338	*cadF*	*ciaB*	*cj1349*	-	-	-	*mviN*	*pldA*	*tlyA*
6	KK341	*cadF*	*ciaB*	*cj1349*	-	-	-	*mviN*	*pldA*	*tlyA*
7	KK342	*cadF*	*ciaB*	*cj1349*	-	-	-	*mviN*	*pldA*	*tlyA*
8	KK343	*cadF*	*ciaB*	*cj1349*	-	-	-	*mviN*	*pldA*	*tlyA*
9	KK345	*cadF*	*ciaB*	*cj1349*	-	-	-	*mviN*	*pldA*	*tlyA*
10	KK349	*cadF*	*ciaB*	*cj1349*	-	-	-	*mviN*	*pldA*	*tlyA*
11	KK351	*cadF*	*ciaB*	*cj1349*	-	-	-	*mviN*	*pldA*	*tlyA*
12	KK352	*cadF*	*ciaB*	*cj1349*	-	-	-	*mviN*	*pldA*	*tlyA*
13	KK353	*cadF*	*ciaB*	*cj1349*	-	-	-	*mviN*	*pldA*	*tlyA*
14	KK354	-	*ciaB*	*cj1349*	-	-	-	*mviN*	*pldA*	*tlyA*
15	KK356	*cadF*	*ciaB*	*cj1349*	-	-	-	*mviN*	*pldA*	*tlyA*
16	KK357	*cadF*	*ciaB*	*cj1349*	-	-	-	*mviN*	*pldA*	*tlyA*
17	KK358	*cadF*	*ciaB*	*cj1349*	-	-	-	*mviN*	*pldA*	*tlyA*
18	KK359	*cadF*	*ciaB*	*cj1349*	-	-	-	*mviN*	*pldA*	*tlyA*
19	KK360	*cadF*	*ciaB*	*cj1349*	-	-	-	*mviN*	*pldA*	*tlyA*
20	KK361	*cadF*	*ciaB*	*cj1349*	-	-	-	*mviN*	*pldA*	*tlyA*
21	KK362	*cadF*	*ciaB*	-	-	-	-	*mviN*	*pldA*	*tlyA*
22	KK364	*cadF*	*ciaB*	*cj1349*	-	-	-	*mviN*	*pldA*	*tlyA*
23	KK365	*cadF*	*ciaB*	*cj1349*	*hecA*	*hecB*	-	*mviN*	*pldA*	*tlyA*
24	KK375	*cadF*	*ciaB*	*cj1349*	-	-	-	*mviN*	*pldA*	*tlyA*
25	KK377	*cadF*	*ciaB*	*cj1349*	-	-	-	*mviN*	*pldA*	*tlyA*
26	KK417	-	*ciaB*	*cj1349*	*hecA*	-	-	*mviN*	*pldA*	-
27	KK424	*cadF*	*ciaB*	*cj1349*	-	-	-	*mviN*	*pldA*	*tlyA*
28	KK485	-	*ciaB*	-	-	-	-	*mviN*	-	-
29	KK486	*cadF*	*ciaB*	*cj1349*	-	-	-	*mviN*	*pldA*	*tlyA*
30	KK509	-	*ciaB*	-	-	-	-	-	-	-
	Total (%)	25 (83)	30 (100)	27 (90)	2 (7)	3 (10)	0 (0)	29 (97)	28 (92)	27 (90)

DISCUSSION

Thus far, 29 species from the *Arcobacter* genus have been reported (Pérez-Cataluña et al., 2018). Conducting more advocacy research on *Arcobacter* species diversity in swine would be interesting. When using multiplex PCR assays, three species, *A. butzleri, A. skirrowii,* and *A. cryaerophilus*, were found individually or in combination in pig feces (Soma Sekhar et al., 2018). The presumptive cultured *Arcobacter* was identified as *A. butzleri* in our study using the PCR assay as described by Houf et al. (2000) and compared to the type strain DMST 19755. A definitive agreement was established on the species identification of the culture isolates between the initial *A. butzleri* indication and the subsequent genus-specific 23S rRNA gene. As a result, this procedure inevitably excluded other species of *Arcobacter*. Nevertheless, *A. butzleri* is the

most common species of *Arcobacter* infection in humans and pigs (Whiteduck-Léveillée et al., 2015; Brückner et al., 2020). The prevalence of *A. butzleri* in pigs can be used to support a surveillance database for an emerging zoonotic *Arcobacter*.

The number of pigs and farms was relatively limited due to farm closures due to African swine fever. Therefore, only willing small-holding farms in the rural community participated. However, pig numbers fulfilled the statistical requirements. This study revealed a high prevalence of *A. butzleri* in pig farms in mid-northeastern Thailand. Half of the farms investigated in this study were positive for *A. butzleri.* The true incidence of infection in pigs may be higher than this finding because *A. butzleri* is difficult to culture due to its metabolic inert characteristics and the presence of various competitive bacteria in the feces (Fanelli et al., 2020). At present, standard measures for *Arcobacter* diagnosis have not been established; however, bacterial culture remains the standard method (Ramees et al., 2017; Gobbi et al., 2018). Even though direct molecular diagnosis (i.e., real-time PCR) of *Arcobacter* from stool samples yields a high recovery rate (Yamauchi et al., 2020), live bacteria are important for further antibiogram evaluation and gene analysis. Therefore, the present study used culture-based identification. Furthermore, fecal samples contain inhibitors that can impair DNA or RNA extraction success. For instance, a direct PCR method has lower sensitivity than the modified gram staining method for detecting *Arcobacter* in a human stool sample (Dermani et al., 2017).

Many previous studies have concentrated on market-weight pigs because they are a potential source of *Arcobacter* contamination in food chains. Given the uneven number of samples from different age groups, this current result cannot compare significant differences among pig ages. Most likely, a high number of finisher samples were evaluated, and most *A. butzleri* isolates were retrieved from finishing pigs. *A. butzleri* detection rates between finishers and sows were relatively similar, but the prevalence of finishing farms appeared higher than that of sow farms. In this study, only a small number of nursery pigs were included, and all were negative for *A. butzleri*. The conclusion that nursery-age pigs do not carry *A. butzleri* cannot be established. However, notably, *A. butzleri* was not detected in the nursery of a farm where the finishers and sows tested positive. Future investigations and specific experimental designs for this point are needed to clarify *A. butzleri*'s occurrence in nursery pigs. Finishing pigs endowed 23.3% *A. butzleri*, according to Soma Sekhar et al. (2018). Our findings showed that *A. butzleri* was present at 15.3% in the intestinal tracts of adult pigs, and 50% of the study farms were positive. Finishing pigs and finishing farms showed 18.8% and 57.1% *A. butzleri* positive rates, respectively. These carrier pigs can spread *A. butzleri* to other pigs and their surroundings, as well as potentially contaminate pig carcasses. Humans may contact *A. butzleri* infections orally, that is, by eating contaminated pork; however, no firm evidence of this theory has been reported in Thailand. Nevertheless, caution is advised because *A. butzleri* has been found in 74% of raw meat and poultry samples sold in local markets in Western Thailand (Bodhidatta et al., 2013).

Antibiotic sensitivity varies between bacterial isolates recovered from different environments; the results herein are relevant to pigs in mid-northeastern Thailand. *A. butzleri* isolates in this study were mostly sensitive to IPM, TET, STR, and AMC but mostly resistant to CTX, SCF, AMP, ENR,

and FOS. Our results agree with the study on *A. butzleri* isolated from seafood, which was completely resistant to vancomycin, cephalothin, cefoxitin, and sulphamethizole and susceptible to polymyxin-B, kanamycin, STR, gentamicin, TET, and IPM (Jasim et al., 2021). While ciprofloxacin has been suggested as an appropriate antibiotic for the treatment of *Arcobacter* (*A. butzleri, A. cryaerophilus*, and *A. lanthieri*) infections in humans (Brückner et al., 2020), *A. butzleri* isolates in our study were relatively resistant to ENR. Ciprofloxacin and ENR are fluoroquinolones and are widely used in chicken productions (Verma et al., 2020), but ENR is a drug approved for use in animals and a common drug used in pigs in Thailand (Lekagul et al., 2020). Cephalosporin resistance is most likely due to the initial culturing method, in which the isolation medium contains cefoperazone. The conventional antibiotics used for *Arcobacter* and *Campylobacter* culture media are cefoperazone, amphotericin, and teicoplanin (Kim et al., 2019). Furthermore, while carbapenems, such as IPM, are prohibited in food-producing animals, other beta-lactams, such as ceftiofur, are widely used (Mollenkopf et al., 2018). Carbapenems are typically used in treating ESBL-positive *E. coli* or beta-lactamase-producing bacteria in clinic (Mahmoud et al., 2020). Notably, beta-lactamase inhibitors, such as clavulanic acid combined with amoxycillin but not sulbactam combined with cefoperazone, were able to overcome most *A. butzleri* isolates in this study. FOS is a class of antibiotics known as phosphonic antibiotics, which are rarely used in pigs. Resistance to FOS develops frequently during therapy, making it unsuitable for the long-term treatment of severe infections in humans (Ito et al., 2017). *A. butzleri* isolates in this experiment were resistant to FOS. *A. butzleri* isolated from patients with gastroenteritis in Belgium showed low sensitivity to AMP (9%) but had a high susceptibility rate to ciprofloxacin (87%), ERY (78%), gentamicin (99%), TET (89%), and doxycycline (76%) (Van den Abeele et al., 2016). In contrast, in this study, *A. butzleri* isolates in pigs were neither sensitive nor resistant to ERY.

Currently, AMR genes in *Arcobacter* are poorly understood. This study focused on the potentially transferable AMR genes of *A. butzleri* obtained from pigs. Given that *A. butzleri* is found in the pig's gut, horizontal antibiotic resistance gene exchange is a concern. Resistance to third-generation cephalosporins in gram-negative bacteria (particularly *E. coli* derived from pigs) is linked to plasmid-mediated ESBL genes, specifically *CTX-M, SHV*, or *TEM* (Hammerum et al., 2014; Vlieghe et al., 2015). Ten of the 31 isolates tested positive for CTX and AMC resistance, with five and seven being SCF and ENR resistant, respectively. However, ESBLs (*SHV, CTX-M*, and *TEM*), PMQRs (*qnrA, qnrS, qnrB, oqxAB,* and *aac(6')-Ib-cr*), *ermB*, and *mefA* genes were not found in any isolates. The insensitivity of *A. butzleri* to antibiotics may be mediated by other mechanisms under other genes. Another seven isolates phenotypically exhibited TET resistance, but only one possessed the *TetO* gene. The *TetO* sequence's similarity to the GenBank database was relatively low, which could be attributed to the variable annealing temperature used in amplifying the gene. *TetO* and two other genes, *TetW* and *Tet32*, are mosaic TET resistance genes, which are a subset of genes encoding ribosomal protection proteins. As a result, its sequences were only 89% similar. *TetO* was discovered in *A. butzleri*, which is significant because it is found in various environmental organisms (Petrocchi-Rilo et al., 2021).

A. butzleri pathogenicity can be predicted using VAGs. The following VAGs were found in 30 isolates in order of detection: *ciaB* (100%), *mviN* (97%), *pldA* (93%), *tlyA* (90%), *cj1349* (90%), *cadF* (83%), *hecB* (10%), *hecA* (7%), and *irgA* (0%). Each isolate had one to eight VAGs, with 77% of all isolates having six VAGs. Even though each *A. butzleri* isolate obtained from pigs inherited many VAGs, no abnormal clinical signs were observed in pigs. *HecA, hecB*, and *irgA* have been proposed as *Arcobacter* pathogenic virulence markers for rapid diagnosis in human diarrheal stools (Kietsiri et al., 2021). None of the pig isolates possessed all three genes found in our study. However, more research is needed to clarify the pathogenicity of *A. butzleri*s in pigs.

CONCLUSIONS

Healthy pigs in mid-northeastern Thailand that carry *A. butzleri* inherited one to eight VAGs but had no abnormal clinical signs. Future research is warranted to determine the pathogenicity of *A. butzleri* in pigs. Most *A. butzleri* isolates were mostly resistant to four to six antibiotic classes. Three isolates were resistant to all 10 antibiotics evaluated. However, only the *TetO* gene was detected in one isolate, whereas ESBLs (*SHV, CTX-M*, and *TEM*), PMQRs (*qnrA, qnrS, qnrB, oqxAB, and aac(6')-Ib-cr*), *ermB*, and *mefA* genes were not found in any of the tested isolates. The findings indicate a relatively high prevalence of *A. butzleri* in pig farms, as well as the first database for possible transferable AMR genes and VAGs in *A. butzleri* derived from pigs in Thailand.

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AUTHOR CONTRIBUTIONS

NC: data curation, formal analysis, methodology, writing-original draft, KD: conceptualization, data curation, formal analysis, funding acquisition, project administration, validation, writing- review & editing, TN: methodology, writing-review & editing, SN: methodology, writing-review & editing.

CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

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