Microbiol. Biotechnol. Lett. (2023), 51(4), 390–402
http://dx.doi.org/10.48022/mbl.2306.06010
pISSN 1598-642X eISSN 2234-7305

MBL
Microbiology and Biotechnology Letters

Virulence and Antimicrobial Resistance Gene Profiling of *Salmonella* Isolated from Swine Meat Samples in Abattoirs and Wet Markets of Metro Manila, Philippines

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Received: June 26, 2023 / Revised: September 28, 2023 / Accepted: October 6, 2023

Salmonella are Gram-negative pathogenic bacteria commonly found in food animals such as poultry and swine and potentially constitute risks and threats to food safety and public health through transmissible virulence and antimicrobial resistance (AMR) genes. Although there are previous studies in the Philippines regarding genotypic and phenotypic AMR in _Salmonella_, there are very few on virulence and their associations. Hence, this study collected 700 _Salmonella_ isolates from swine samples in abattoirs and wet markets among four districts in Metro Manila and characterized their genotypic virulence and β-lactam AMR profiles. Gene frequency patterns and statistical associations between virulence and _bla_ genes and comparisons based on location types (abattoirs and wet markets) and districts were also determined. High prevalence (>50%) of virulence genes was detected encompassing _Salmonella_ pathogenicity islands (SPIs) 1-5 suggesting their pathogenic potential, but none possessed plasmid-borne virulence genes _spvR_ and _spvC_. For _bla_, bla_{TEM} was detected with high prevalence (>45%) and revealed significant associations to four SPI genes, namely, _avrA_, _hilA_, _mgtC_, and _spi4R_, which suggest high resistance potential particularly to β-lactam antibiotics and relationships with pathogenicity that remain mechanistically unestablished until now. Lastly, comparisons of location types and districts showed variations in gene prevalence suggesting effects from environmental factors throughout the swine production chain. This study provides vital data on the genotypic virulence and AMR of _Salmonella_ from swine in abattoirs and wet markets that suggest their pathogenicity and resistance potential for policymakers to implement enforced surveillance and regulations for the improvement of the Philippine swine industry.

Keywords: **Antimicrobial resistance genes, association, prevalence, _Salmonella_, virulence genes**

Introduction

Salmonella are one of the four major causes of diarrheal diseases worldwide; while they often result in mild cases, they can also induce life-threatening infections [1]. Nonetheless, they have been classified as class 3 according to damage-response frameworks wherein these pathogens cause damage to host regardless of their immune condition [2]. Transmission is primarily by fecal-oral route especially from food animals such as swine and poultry, and eventually leads to complex pathogenicity programs involving diverse virulence gene expressions and effector protein functions for colonization, invasion, and proliferation in host cells [3]. *Salmonella* virulence genes are currently found in up to 23 known *Salmonella* pathogenicity islands (SPIs); however, only SPIs 1–5 are well-distributed and documented across different serovars [4]. In the Philippines, studies predominantly report incidences along with serogroups and serovars of *Salmonella* [5–8], which was observed to be

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the leading known microbiological cause of food poisoning from 2005 to 2018 [8]. However, there are only few studies on *Salmonella* virulence genes in the Philippines, with few genes such as *spvC* [6, 7] and *invA*, which was used as a confirmatory test for *Salmonella* identification in these studies [5].

Antimicrobial resistance (AMR) is the ability of microorganisms to survive exposure to antimicrobials that were originally effective in treating infections caused by these microorganisms [9]. While intrinsic resistance to antimicrobials, which involves inherent properties in bacteria, is common, other mechanisms such as selective pressure and horizontal gene transfer from resistant strains can also facilitate the emergence and dissemination of AMR [10]. In 2010, the global antimicrobial consumption in food animal industries, excluding aquaculture, was estimated at 63,151 tons, predicting to increase by 67% by 2030, with Asia potentially increasing by up to 129% [11]. In 2017, food animal antimicrobial consumption was estimated at 93,309 tons, with Asia consuming the largest percentage [12]. In the US alone, almost 90% of antimicrobials are for growth promotion and infection prevention purposes rather than for therapy [13]. In addition, many antimicrobials in this industry and clinical settings are similar or the same [14]. Accordingly, it is predicted that by 2050, AMR would cause 10 million deaths per year, highest in Asia [15]. *Salmonella* are also reservoirs for AMR, carrying transmissible resistance genes, and labeled as serious threats by the Centers for Disease Control and Prevention (CDC) [16]. High phenotypic resistances including multidrug resistance (MDR) or non-susceptibility to at least one drug from three or more classes of antibiotics, were observed in *Salmonella* isolated from slaughtered swine in abattoirs [17–19] and raw chicken from wet markets [20] within Metro Manila, Philippines. In genotypic studies, Calayag *et al.* [19] also reported high prevalence of some β-lactamase (*bla*) and quinolone resistance (*qnr*) genes in *Salmonella* from slaughtered swine. Since the discovery of penicillin, β-lactam antibiotics continue to be developed and used in different settings [13]. These also allowed for diversification of *bla* genes into several subtypes, which among the most common especially in *Enterobacteriaceae* are TEM (bla_{TEM}), SHV (bla_{SHV}), and CTX-M ($bla_{CTX\text{-}M}$) [21]. Some subtypes can confer and transmit resistance to third generation cephalosporins and aztreonam, also known as extended-spectrum β-lactamases (ESBLs) [22]. The CDC also listed ESBL-producing *Enterobacteriaceae* as critical priorities posing a serious threat worldwide [16].

Swine are among common reservoir hosts for *Salmonella* [23]. Developing countries, including the Philippines, contribute to 60% of the world's swine production [24]. However, approximately more than 70% of Philippine swine production involves backyard farms [25], which can be defined as ≤10 sows [26]. In the study of Calayag *et al.* [18], higher incidences of *Salmonella enterica* were detected in locally registered abattoirs than in accredited abattoirs with high MDR among isolates. Hence, the current study collected *Salmonella* isolates from various swine samples in abattoirs and wet markets of four districts in Metro Manila and characterized virulence and AMR gene profiles using multiplex PCR. This study aimed to determine the pathogenicity and resistance potential of *Salmonella* that can help in drafting policies, laws, and guidelines in the improvement of the Philippine swine industry.

Materials and Methods

Meat sample collection

Sample collection was conducted in abattoirs and wet markets of four districts of Metro Manila, Philippines, namely, Northern, Eastern, Southern, and Capital. For abattoirs, the tonsils and jejunum of freshly slaughtered swine were aseptically collected using sterilized scissors and forceps as described by Ng and Rivera [17] and Calayag *et al.* [18]. While for wet markets, various swine meat samples such as ground pork, pork chop, pork shoulder *(kasim)*, cured pork *(tocino)*, pork sausage *(longganisa)*, meat ball, ham, *menudo* cut, and pork riblets *(buto-buto)* were collected from different market stalls. Meat samples obtained were then placed in clean zip-lock bags and into a cooler with ice before transporting to the laboratory for immediate processing. Ethical review and approval were waived for this study due to informed consent obtained from the Philippine National Meat Inspection Service. Animal slaughter and evisceration were performed according to national regulations. Informed consent was also obtained from veterinarians in charge of the abattoirs for sample collection.

Enrichment and isolation of *Salmonella*

Following standardized protocols [19], each meat sample bag was aseptically opened, and 25 g of meat sample was sliced with sterilized scissors and forceps and weighed in sterilized aluminum foil. Samples were then transferred in 225 ml of buffered peptone water (BPW) (BD Diagnostics System, USA) contained in Whirl-Pak® bags (Nasco, USA), followed by agitation for 2 min and incubation at 37℃ for 18–24 h. A 100 µl of resulting BPW cultures was then transferred to 10 ml Rappaport Vassiliadis (RV) broth (BD Diagnostics System) and incubated at 42℃ for 18–24 h. Subsequently, 10 µl of resulting RV cultures was streaked into xylose lysine deoxycholate (XLD) agar (BD Diagnostics System) and incubated at 37℃ for 18–24 h. Colonies that grew with typical phenotypic characteristics (black colonies on red media) on XLD were then subcultured to nutrient agar (NA) (BD Diagnostics System) and incubated at 37℃ for 18–24 h before DNA extraction and other downstream processes.

DNA extraction

DNA extraction was performed through a boil-lysis method as previously described [18, 19]. Then, 2–3 colonies of presumptive *Salmonella* in NA were suspended in 50 µl 1× Tris-EDTA buffer and subjected to boiling temperature at 100℃ for 10 min. The resulting suspension was then subjected to centrifugation at 2,656 ×*g* for 5 min, and the supernatant containing the DNA was collected for *Salmonella* confirmation and virulence and AMR genes detection.

Molecular confirmation of *Salmonella*

Primer sequences, amplification product size, and reference for *invA* gene PCR can be found in Table 1. DNA extracts were subjected to *invA* gene detection that is generally considered as a genetic marker for *Salmonella* confirmation due to their wide distribution across *Salmonella* serovars [27]. Following previously established protocols [5, 18, 28], each PCR reaction mixture was 12.5 µl in volume and contained 6.25 µl of 2× GoTaq Green Master Mix (Promega, USA), 4.25 µl of nuclease-free water, 0.5 µl of *invA* forward and reverse primers at 10 µM concentrations, and 1 µl of DNA extract. For the PCR, the protocol was indicated as 95℃ for 2 min (initial denaturation), then 30 cycles of three steps, namely, 95℃ for 30 s (denaturation), 60℃ for 30 s (annealing), and 72℃ for 30 s (extension), and finally, 72℃ for 5 min

Table 1. Primers, sequences and amplification product size for virulence genes.

Gene	Primer	Sequence	Product size (bp)	Reference
invA	*invA*	F: 5′-ACAGTGCTCGTTTACGACCTGAAT-3′	244	[27]
	invA	R: 5′-AGACGACTGGTACTGATCTAT-3′		
avrA	*avrA*	F: 5′-GTTATGGGACGGAACGACATCGG-3′	385	[29]
	avrA	R: 5′-ATTCTGCTTCCCGCCGCC-3′		
hilA	*hilA*	F: 5′-CTGCCGCAGTGTTAAGGATA-3′	497	[29]
	hilA	R: 5′-CTGTCGCCTTAATCGCATCGT-3′		
sseC	*sseC*	F: 5′-TATGGTAGGTGCAGGGGAAG-3′	121	[30]
	sseC	R: 5′-CTCATTCGCCATAGCCATTT-3′		
mgtC	*mgtC*	F: 5′-TGACTATCCAATGCTCCAGTGAAT-3′	655	[31]
	mgtC	R: 5′-ATTTACTGGCCGCTATGCTGTTG-3′		
SPI4	*SPI4*	F: 5′-GATATTTATCAGTCTATAACAGC-3′	1,269	[32]
	SPI4	R: 5′-ATTCTCATCCAGATTTGATGTTG-3′		
pipB	*pipB*	F: 5′-TAATGTGCCACATACAGGTAACGC-3′	789	[33]
	pipB	R: 5′-TTCTGGAGGATGTCAACGGGTG-3′		
spvC	*spvC*	F: 5′-ACTCCTTGCACAACCAAATGCGGA-3′	571	[27]
	spvC	R: 5′-TGTCTTCTGCATTTCGCCACATCA-3′		
spvR	*spvR*	F: 5′-ATGGATTTCATTAATAAAAAATTA-3′	894	[34]
	spvR	R: 5′-TCAGAAGGTGGACTGTTTCAGTTT-3′		

(final extension). Isolates that were positive for *invA* gene were considered as confirmed *Salmonella* and were then subjected to molecular detection for virulence and AMR genes.

Multiplex PCR detection of virulence genes

Primer sequences, amplification product sizes, and references for virulence genes detection can be found in Table 1. Confirmed *Salmonella* isolates were further subjected to genotypic virulence analysis using multiplex PCR optimized in a previous study [35]. A total of eight virulence genes, excluding *invA* (previously used for *Salmonella* confirmation), were detected among isolates. Representing SPIs 1–5 were *avrA* and *hilA* for SPI 1, *sseC* for SPI 2, *mgtC* for SPI 3, *spi4R* for SPI 4, and *pipB* for SPI 5, whereas two genes, namely, *spvC* and *spvR*, are plasmid-borne genes. For multiplex PCR of *avrA*, *sseC*, *mgtC*, and *pipB* genes, the protocol involved 94℃ for 4 min (initial denaturation), then 35 cycles of three steps, namely, 94℃ for 1 min (denaturation), 58℃ for 2 min (annealing), and 72℃ for 2 min (extension), and finally, 72℃ for 5 min (final extension). For multiplex PCR of *hilA* and *spvR* genes, as well as singleplex of *spvC* gene, the protocol involved 95℃ for 3 min (initial denaturation), then 34 cycles of three steps, namely, 95℃ for 30 s (denaturation), 50℃ for 30 s (annealing), and 72℃ for 30 s (extension), and finally, 72℃ for 5 min (final extension). For singleplex PCR of *spi4R* gene, the protocol involved 94℃ for 4 min (initial denaturation), then 34 cycles of three steps, namely, 94℃ for 1 min (denaturation), 58℃ for 1 min (annealing), and 72℃ for 2 min (extension), and finally, 72℃ for 5 min (final extension). Each multiplex PCR reaction mixture was 12.5 µl in volume and contained 6.25 µl 5× MyTaq HS Red Mix (Bioline, UK), variable amounts of nuclease-free water depending on the volume of primers, 0.25 µl of forward and reverse primers at 10 µM concentrations, and 2 µl of DNA template. Singleplex PCR reactions contained identical mixtures as *invA* gene PCR except for *spi4R* that contained 20 µM primer concentrations. For positive controls, *S. enterica* subsp. *enterica* ATCC (American Type Culture Collection) serovars from Kwik-Stik (Microbiologics, USA) were used. Namely, Typhimurium (ATCC 14028) and Enteritidis (ATCC 13076) for *avrA*, *sseC*, *mgtC*, *pipB*, and *spi4R*, whereas Choleraesuis (ATCC 7001) for *hilA*, *spvC*, and *spvR*.

Multiplex PCR of AMR genes

Primer sequences, amplification product sizes, and references for AMR genes detection can be found in Table 2. Confirmed *Salmonella* isolates were also subjected to genotypic AMR analysis using multiplex PCR. A total of three AMR genes, all β-lactam resistance encoding genes, were detected among isolates, namely, bla_{TEM}, $bla_{\text{CTX-M}}$, and bla_{SHV}. Each multiplex PCR reaction mixture was 12.5 µl in volume and contained 6.25 µl 5× MyTaq HS Red Mix (Bioline), 2.75 µl of nuclease-free water, 0.25 µl of forward and reverse primers wherein bla_{TEM} primers at 10 µM, $bla_{\text{CTX-M}}$ and bla_{SHV} at 30 µM, and 2 µl of DNA template. Following previous studies [19, 36], multiplex PCR protocol for *bla* genes involved 95℃ for 3 min (initial denaturation), then 30 cycles of three steps, namely, 95℃ for 30 s (denaturation), 60℃ for 30 s (annealing), and 72℃ for 1 min (extension), and finally, 72℃ for 10 min (final extension). For positive controls, *Klebsiella pneumoniae* (ATCC 700603) was used for bla_{SHV}, whereas in in-house laboratory controls from Calayag *et al.* [19], positive samples were used for bla_{TEM} and $bla_{\text{CTX-M}}$.

Data analysis

Descriptive statistics using Fisher's exact test through SPSS version 26 (IBM) was used to find significant associations between virulence and AMR genes among

Table 2. Primers, sequences and amplification product size for antimicrobial resistance genes.

Gene	Primer	Sequence	Product size (bp)	Reference
bla_{TEM}	bla_{TEM}	F: 5′-TCGCCGCATACACTATTCTCAGAATGA-3′	244	[36]
	bla_{TEM}	R: 5′-ACGCTCACCGGCTCCAGATTTAT-3′		
$bla_{\text{CTX-M}}$	$bla_{\text{CTX-M}}$	F: 5′-ATGTGCAGYACCAGTAARGTKATGGC-3′	385	
	$bla_{\text{CTX-M}}$	R: 5′-TGGGTRAARTARGTSACCAGAAYCAGCGG-3′		
bla_{SHV}	bla_{SHV}	F: 5′-ATGCGTTATATTCGCCTGTG-3′	497	
	bla_{SHV}	R: 5′-TGCTTTGTTATTCGGGCCAA-3′		

isolates to determine relationships of pathogenicity and AMR in *Salmonella*. Virulence and AMR gene patterns were visualized using R version 4.0.5 in R studio (R Foundation) with the UpSetR 1.4.0 package to generate intersecting plots and determine frequencies of gene patterns. Genotypic virulence and AMR profiles were determined in frequencies to reveal the general prevalence of these genes across *Salmonella* isolates. Prevalence was also compared based on location types whether abattoir or wet market and Metro Manila districts whether Northern, Eastern, Southern, or Capital.

Table 3. Fisher's exact test on the associations between virulence and AMR genes of *Salmonella* isolates (n = 700).

Virulence and AMR gene associations	Fisher's exact test (two-sided p-value)
bla_{TEM} and *avrA*	**0.001***
bla_{TEM} and *hilA*	**<0.001***
bla_{TEM} and *sseC*	0.404
bla_{TEM} and *mgtC*	**<0.001***
bla_{TEM} and *spi4R*	**<0.001***
bla_{TEM} and *pipB*	0.146

Results

Prevalence and associations of virulence and AMR genes in *Salmonella*

A total of 700 *Salmonella* isolates were collected for DNA extraction and genotypic analysis. High AMR potential for β-lactam antibiotics was also found in this study. For *bla* genes, bla_{TEM} was detected at a high prevalence of 46.43% among *Salmonella* isolates, followed by bla_{SHV} at 1.00% and bla_{CTX-M} with only 0.29% (Fig. 1). For SPIs 1–5 virulence genes, prevalence was more than 50%, whereas no isolates harbored plasmid-borne *spvC* and *spvR* genes, suggesting high pathogenic potential of these *Salmonella* isolates (Fig. 1). From highest to lowest detected virulence genes, *hilA* and *avrA* from SPI 1 were at 68.43% and 65.29%, respectively, followed by *mgtC* (64.71%) from SPI 3, *pipB* (57.29%) from SPI 5, *sseC* (54.29%) from SPI 2, and *spi4R* (54.00%) from SPI 4 (Fig. 1).

Fisher's exact test showed some significant associations ($p < 0.05$) between virulence and AMR genes detected among *Salmonella* isolates (Table 3). AMR gene bla_{TEM} showed associations with virulence genes *avrA*, *hilA*, *mgtC*, and *spi4R*, but not *sseC* and *pipB* (Table 3). In contrast, bla_{CTX-M} and bla_{SHV} were not subjected to this analysis due to their low prevalence across *Salmonella* isolates.

Patterns of virulence and AMR gene prevalence in *Salmonella*

Using the UpSetR package in RStudio to determine intersecting plots in virulence and AMR gene prevalence

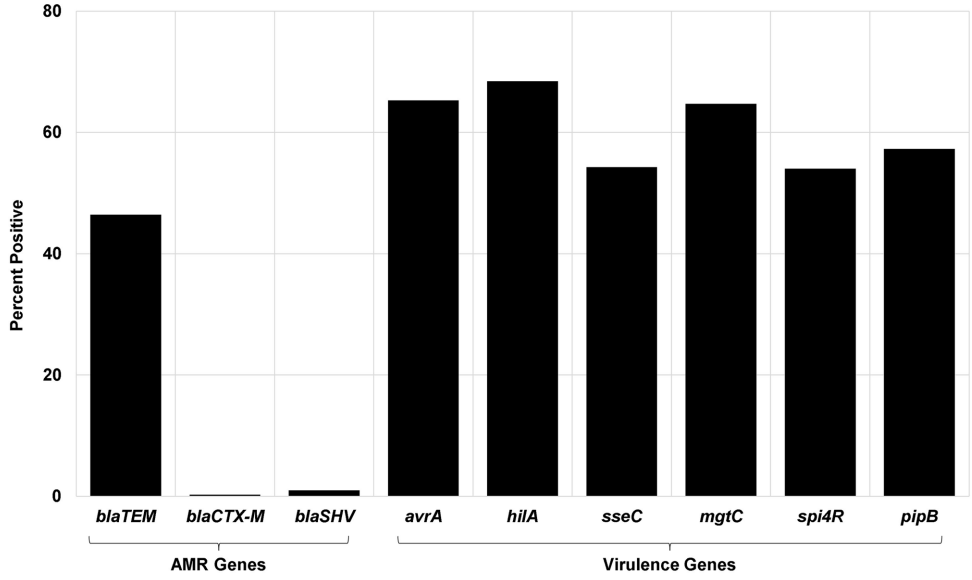

Fig. 1. Prevalence of virulence and AMR genes among *Salmonella* isolates (n = 700).

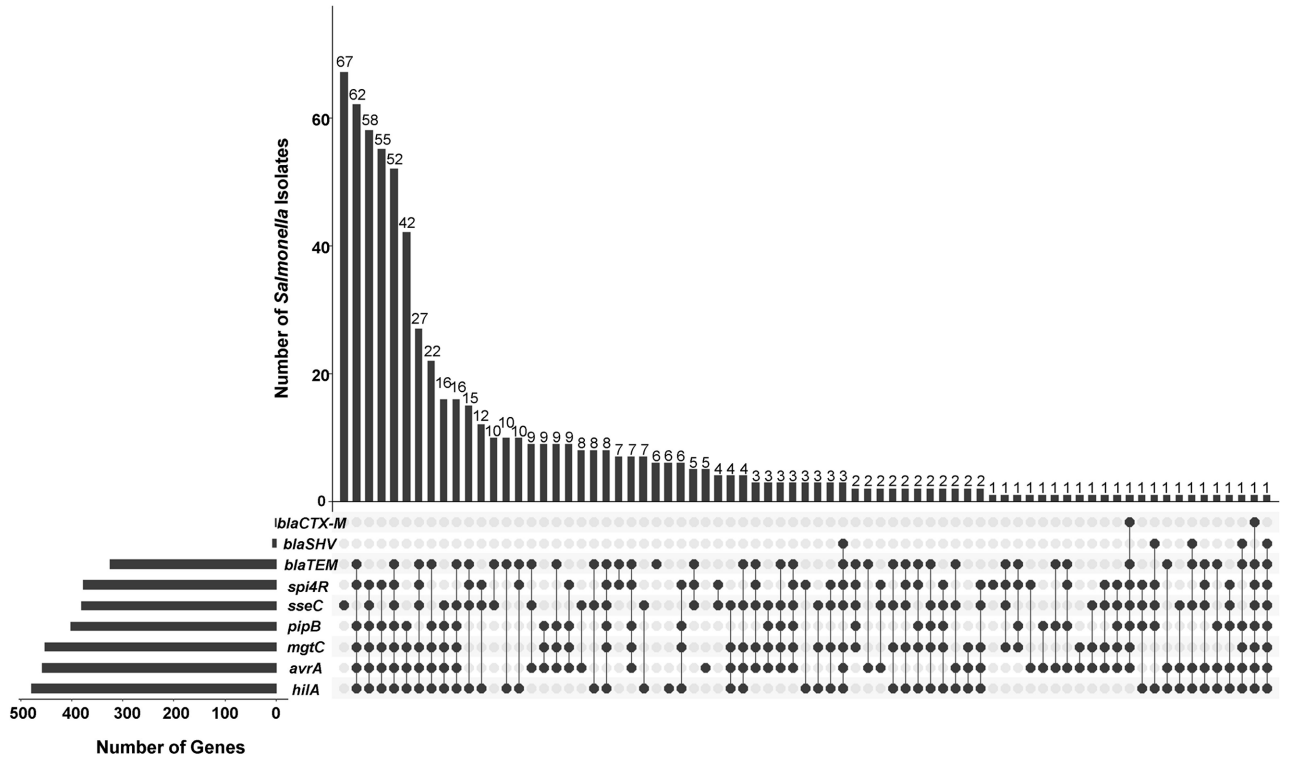

Fig. 2. Patterns and intersections of virulence and AMR genes among *Salmonella* isolates (n = 700) using UpSetR 1.4.0 package R version 4.0.5 in R studio.

across *Salmonella*, the highest intersecting pattern (8.86%) with *bla* and SPI genes involved bla_{TEM}, *avrA*, *hilA*, *mgtC*, *spi4R*, and *pipB*, whereas if including *sseC*, it was 7.43% of the isolates (Fig. 2). Interestingly, the highest pattern (9.57%) involved *sseC* alone with no other virulence or AMR genes, whereas the third highest pattern (8.29%) possessed all six SPIs 1–5 genes, namely, *avrA*, *hilA*, *sseC*, *mgtC*, *spi4R*, and *pipB* (Fig. 2). Among the seven bla_{SHV} positive isolates, six (85.71%) also possessed bla_{TEM} gene, whereas all two bla_{CTX-M} positive isolates also possessed bla_{TEM}, suggesting association, and potential relationship of *bla* genes in *Salmonella* and other *Enterobacteriaceae* (Fig. 2). In addition, three isolates positive for both bla_{TEM} and bla_{SHV} possessed *avrA*, *hilA*, *sseC*, *mgtC*, and *spi4R* (Fig. 2). Similarly, the two bla_{TEM} and bla_{CTX-M} positive isolates also possessed multiple SPIs 1–5 virulence genes (Fig. 2). Collectively, these data suggest not only pathogenic and AMR potential but also interactions or associations between pathogenicity and AMR in *Salmonella*.

Virulence and AMR gene prevalence based on location types

Comparing abattoirs with 436 isolates to wet markets with 264 isolates, some differences were observed for virulence and AMR gene prevalence (Fig. 3). For virulence genes, *avrA*, *hilA*, *mgtC*, *spi4R*, and *pipB* generally showed higher prevalence in abattoir isolates than in wet market isolates, whereas *sseC* was higher (61.36%) in wet market *Salmonella* isolates than in abattoirs (50.00%) (Fig. 3). *hilA* showed the highest difference, followed by *mgtC* between location types, which suggest possible variations in the incidence and potential pathogenicity of *Salmonella* across these settings. For *bla* genes, bla_{TEM} prevalence was higher in abattoir (50.69%) than in wet market (39.39%) isolates (Fig. 3). The two bla_{CTX-M} positive isolates all originated from abattoirs, whereas bla_{SHV} prevalence was slightly higher in wet markets (1.52%) than in abattoirs (0.69%) (Fig. 3). These suggest high AMR potential against β-lactam antibiotics in abattoirs and wet markets considering

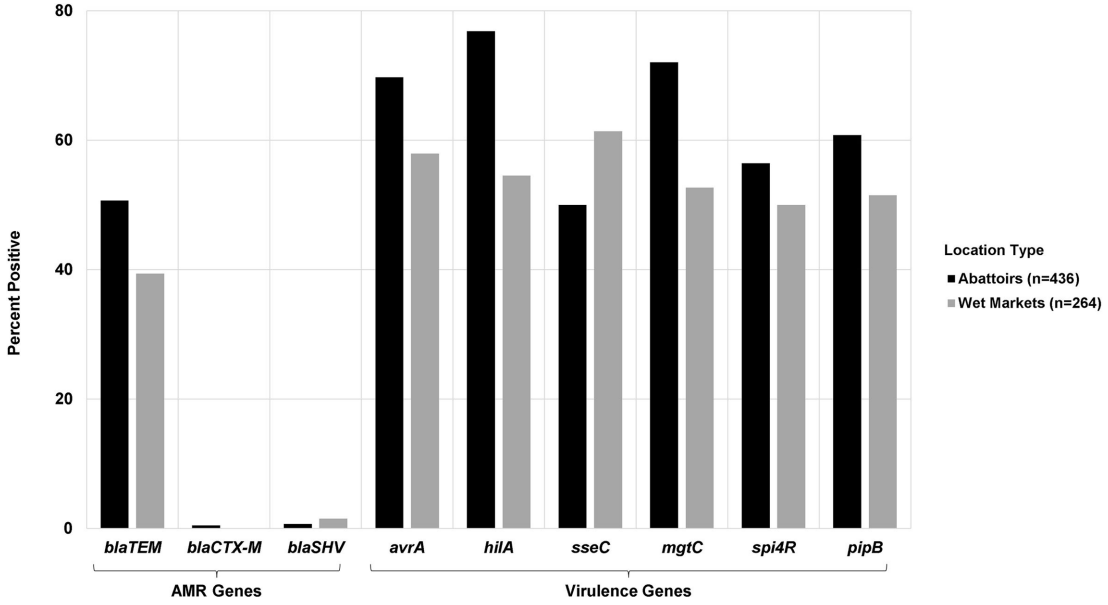

Fig. 3. Comparison of virulence and AMR gene prevalence across abattoirs and wet market *Salmonella* isolates.

that bla_{TEM} was generally widely distributed among *Salmonella* across location types.

Virulence and AMR gene prevalence based on Metro Manila districts

Comparing the four Metro Manila districts, namely, Northern (n = 197), Eastern (n = 178), Southern (n = 184), and Capital (n = 141), some differences were also observed for virulence and AMR gene prevalence across *Salmonella* (Fig. 4). For virulence genes, the most prevalent in *avrA* gene was among Capital isolates (76.60%), *hilA* among Southern isolates (82.07%), *sseC*

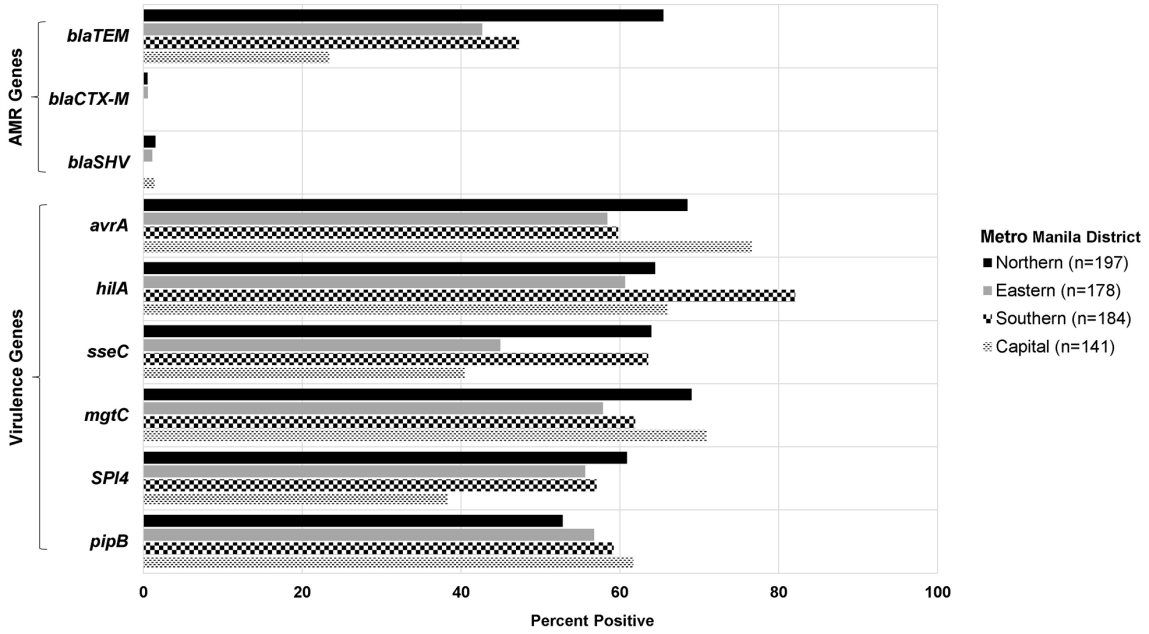

Fig. 4. Comparison of virulence and AMR gene prevalence across four Metro Manila districts (Northern, Eastern, Southern, Capital) isolates.

http://dx.doi.org/10.48022/mbl.2306.06010

among Northern isolates (63.96%), *mgtC* among Capital isolates (70.92%), *spi4R* among Northern isolates (60.91%), and *pipB* among Capital isolates (61.70%) (Fig. 4). These suggest that isolates from these locations have higher pathogenic potential and thus require further surveillance. For *bla* genes, the most prevalent in bla_{TEM} and bla_{SHV} was found in Northern isolates (65.48% and 1.52%, respectively), whereas the two bla_{CTX-M} positive isolates were found in Eastern and Northern districts (Fig. 4). These suggest that *Salmonella* isolated from the Northern district showed the highest AMR potential among other districts.

Discussion

The high prevalence of virulence and *bla* genes detected among *Salmonella* isolated from swine in this study suggests their high pathogenicity and AMR potential that pose a significant threat to the Philippine swine industry and throughout the value chain. *hilA* was the most commonly detected among virulence genes at 68.43% which is generally at lower rates than most studies [37–39]. *hilA*, as the highest, has a pivotal role in *Salmonella* pathogenicity as a master transcriptional regulator for the expression of invasion-associated genes [40]. Another study of *Salmonella* from chicken feces in South Africa showed very low prevalence (9%) of *hilA* [41]. However, *hilA* prevalence may be affected by differences in serovars as can be observed in the study of Thung *et al.* [42], which showed less prevalence among serovars Agona and Newport. *hilA* was followed by *avrA* and *mgtC* showing lower prevalence but still at > 60%, which was in contrast to a study in Egypt that showed only 30% detection rates in chicken meats [43]. However, many studies report higher prevalence (> 90%) of *avrA* and *mgtC* that can also differ based on *Salmonella* serovars [29, 44–46]. *avrA* has been associated with several functions from host immune inhibition [47–49], intracellular survival of *Salmonella* [50], chronic infections and carcinogenesis [51]. Zou *et al.* [52] and Suez *et al.* [53] reported the variable presence of *avrA* among *Salmonella* strains. The presence of *avrA* and *spvC* has also been associated with systemic *Salmonella* serovars such as Pullorum and Gallinarum [54]. *mgtC* facilitates intramacrophage proliferation by maintaining ATP homeostasis during phagosome acidification and repression of cellulose production [55]. Mutation experiments involving the *mgt* locus have also resulted in growth impairment of *Salmonella* in-host and non-host environments [56]. Among virulence genes, < 60% are *pipB*, *sseC*, and *spi4R*. Thi *et al.* [57] reported a *pipB* gene prevalence of only < 50% and *mgtC* of < 40% among *Salmonella* from swine. In contrast, Fazl *et al.* [30] reported 100% prevalence of *pipB* and *sseC* among *Salmonella* Typhimurium from humans and poultry in Iran. Another study of *Salmonella* from stool samples of children with diarrhea in Iraq showed < 2% *sseC* gene occurrence present only in one serovar Typhi isolate [58]. *pipB* encodes for a type III secretion system 2 (TTSS 2) released effector protein with involvement for enteric, but not systemic salmonellosis [33, 59, 60]. Meanwhile, *sseC* encodes a translocon protein with a role in the intracellular survival [61, 62]. *spi4R* gene has been reported at higher prevalence (> 90%) by studies of *Salmonella* from clinical and environmental samples [31, 63], whereas among *Salmonella* from broiler chickens, *spi4R* was not present in serovar Infantis, which was suggested to be due to deletion or mutation [64]. Meanwhile, *spi4R* was predicted to encode for the type I secretion system in *Salmonella* [65]. The lack of *spvC* and *spvR* virulence genes among *Salmonella* isolates in this study was also reflected by several reports of either very low or no prevalence in other studies [45, 63, 66], which may be due to their plasmid-borne attribute and their presence primarily in systemically disseminated *Salmonella* serovars [67, 68].

The high prevalence of bla_{TEM} (46.43%) over other *bla* genes, namely, bla_{CTX-M} and bla_{SHV} in this study was also reflected in other studies such as in India and Nigeria and household pets from Thailand and humans from Egypt [44, 46, 66, 69]. Correlations of bla_{TEM} with resistance to ampicillin, a widely used β-lactam antibiotic worldwide, have been previously established and associated with selective pressure [44, 70]. This suggests the possible anthropological contributions that require proper surveillance and regulation. Although, this study detected lower incidences of bla_{CTX-M} and bla_{SHV} which contrasts to reports of prevalence up to 80% among *Salmonella* Enteritidis and Typhimurium from chickens and rats [41], these two *bla* genes are often attributed to ESBL phenotypes [71]. It is plausible that this may be due to higher prices and subsequently less common usage of higher cephalosporins that may potentially

correspond [66]. Nonetheless, their presence may be associated with phenotypic β-lactam resistance including ESBLs, thus posing a threat to public health [72–74]. The co-carriage of bla_{TEM} and bla_{CTX-M} has also been reflected in other studies that may indicate ESBL phenotypes [19].

Associations between virulence and AMR have long baffled researchers due to their complex relationships. This phenomenon is supported by the notion of MDR bacteria such as *Escherichia coli* ST131 several other bacteria such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Staphylococcus aureus* causing extensive infections [75, 76]. With a multitude of virulence genes [4, 77, 78], additional roles aside from conferring virulence factors and facilitating host-pathogen interactions may exist. This study revealed four significant associations involving bla_{TEM} AMR gene and *Salmonella* virulence genes *avrA*, *hilA*, *mgtC*, and *spi4R*. Various *Salmonella* serovars from clinically diarrheic humans in Egypt showed associations of several virulence genes, namely, *invA*, *avrA*, *ssaQ*, *mgtC*, *siiD*, *sopB,* and *bcfC,* with chloramphenicol resistance phenotype [79]. Meanwhile, Higgins *et al.* [80] also revealed strong associations of several virulence genes, including *avrA*, *mgtC*, and *spvC*, to phenotypic MDR status but not to single AMR status among clinical *Salmonella* isolates. Positive correlations were also detected between several virulence and AMR genes, namely, *orgA* and bla_{PSE1}, *spaN* and *tolC* with *cmlA*, and *tolC* with *sul1* in *Salmonella* from dogs and cats [66]. Treatment of MDR *Salmonella* Typhimurium with chloramphenicol and tetracyclines upregulated *hilA*, *prgH*, and *invF* genes that subsequently increased invasiveness, but other antibiotics such as ampicillin and streptomycin had no effect [81, 82]. Plasmid-borne virulence genes *spvC* and *spvR* have also been demonstrated to correlate with various AMR and even MDR phenotypes [45, 83, 84]. An *in silico* study has also evaluated associations of mobile genetic elements that annotate AMR, virulence genes, and plasmids in *Salmonella* [85]. Interestingly, hybrid plasmids containing both AMR and virulence gene sequences have been found in *Salmonella* [86]. However, negative relationships or lack of associations between virulence and AMR in *Salmonella* have also been found. Some examples include nitrofurantoin resistance and virulence gene *bapA* [84] and lack of statistical associations between *mgtC*, *avrA*, *ssaQ*, and *sopB* to all AMR phenotypes in *Salmonella* from swine in Argentina [45]. Hence, the relationship of virulence and AMR in *Salmonella* remains complex and requires further elucidation.

External factors can affect *Salmonella* prevalence and diversity that subsequently contributes to their virulence and AMR potential. *Salmonella* can contaminate meat products throughout the entire food animal production chain that is affected by environmental aspects whether sanitation, transport, processing, or handling, including areas or sites of breeding, slaughter, and retail [42, 87, 88]. All gene prevalence, except for *sseC* and bla_{SHV}, were higher in abattoir than in wet market swine isolates, and comparisons of four Metro Manila districts also showed drastic differences in virulence and AMR gene prevalence, which illustrate variations. In comparison, the pork production chain in China showed no significant difference in the number of ARGs possessed by *Salmonell*a from abattoirs and markets; however, there were differences in the types of genes identified [89]. Variations across the broiler production chain in Trinidad and Tobago have also been observed, from the types of *Salmonella* serovars detected, AMR genes particularly and virulence factors with serovars also contributing to differences [90]. Meanwhile, comparison of intensive and backyard farm swine production in Argentina showed that while no significant difference was observed for virulence genes profile, higher AMR phenotypes among *Salmonella* were observed in intensive than in backyard farming, suggesting risks in animal production [45].

In conclusion, the virulence and AMR gene profiles of *Salmonella* from swine samples in abattoirs and wet markets in Metro Manila districts obtained in this study provide valuable insights to support the need for further research and constant surveillance of the entire swine production chain as well as inform policymakers to enforce and improve farming standards, antimicrobial usage, as well as processing and retail monitoring and regulations. High prevalence of virulence genes reported in this study corroborates with other studies indicating pathogenic potential and genetic diversity among *Salmonella* and supports the wide distribution of SPIs 1–5 virulence genes. High detection rates for bla_{TEM} suggest significant AMR potential for β-lactams, which are among the most utilized antibiotics worldwide, which

poses a concern to therapeutics and public health. Some statistical associations and frequency patterns between virulence and AMR genes in *Salmonella* were also detected that add to the growing repertoire of relationships among pathogenicity and AMR in pathogens that remain complicated and unestablished. Lastly, drastic variations in virulence and AMR gene prevalence were observed comparing abattoirs and markets as well as among Metro Manila districts that suggest contributions of external factors throughout the swine production chain that needs proper surveillance as they remain significant threats to the Philippine swine industry and consumer health.

Acknowledgments

The authors acknowledge Paolo D.G. Mendoza, Camille Andrea R. Flores, and Alyzza Marie B. Calayag for the technical support. This study was supported financially by the Department of Agriculture-Biotechnology Program Office (DABIOTECH-R1808) of the Philippines.

Conflict of Interest

The authors have no financial conflicts of interest to declare.

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http://dx.doi.org/10.48022/mbl.2306.06010