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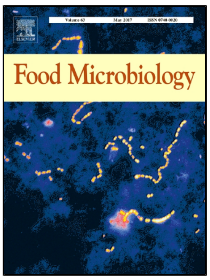

Molecular characterization and antimicrobial resistance of *Salmonella enterica* from swine slaughtered in two different types of Philippine abattoir

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Highlights:

- Incidence of *Salmonella enterica* in tonsils and jejunum of slaughtered swine was determined from two types of abattoirs.
- No significant difference between the incidences of *S. enterica* contamination in both types of abattoirs.
- Most samples were contaminated with *S. enterica* serogroup O:3,10.
- Most isolates were non-susceptible to nitrofurantoin, trimethoprim/sulfamethoxazole, and ampicillin.

Molecular characterization and antimicrobial resistance of *Salmonella enterica* from swine slaughtered in two different types of Philippine abattoir

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Abstract

Salmonella enterica is a well-known pathogen commonly acquired from the consumption of contaminated food. It has been estimated to affect millions of humans and cause hundreds of thousands of deaths per year globally. Pork, one of the most commonly consumed meats worldwide, has been identified as one of the main sources of human salmonellosis. In this study, we aimed to detect and characterize *S. enterica* from slaughtered swine and generate antimicrobial profiles of select isolates. Tonsils and jejunum with mesenteric lymph nodes (MLN) were collected from a total of 240 swine from eight abattoirs (five accredited and three locally registered abattoirs) across Metro Manila. *S. enterica* were isolated using conventional culture methods and confirmed by PCR amplification of the *invA* gene. Isolates were further characterized based on somatic antigen by multiplex PCR. We report that there is no significant difference (P = 0.42) between the incidences of *S. enterica* in swine slaughtered in accredited (44.0%) and in locally registered abattoirs (46.7%). Most samples were contaminated with *S. enterica* under serogroup O:3,10. Antimicrobial susceptibility testing of 183 isolates using the VITEK® 2 system revealed high resistance to ampicillin (67.8%) and trimethoprim/sulfamethoxazole (80.3%). Multidrug-resistance was found in 124 (67.8%) isolates.

Keywords: abattoir, antimicrobial resistance, *Salmonella enterica*, swine

1. Introduction

Pork is one of the most commonly consumed meats worldwide (OECD, 2015; FAO, 2014). In 2015, world consumption of pork was 118,230 metric tons. In the Philippines alone, 1,006 metric tons of pork was consumed in the same year. Next to chicken, pork has been consistently the most consumed meat in the Philippines since 2000 (OECD, 2015). Consequently, the swine industry has been the biggest contributor in Philippine livestock production since 1980, and total hog production in the Philippines has been increasing since (CountrySTAT Philippines, 2016). Pork, both raw and processed, is considered as one of the main sources of salmonellosis in humans (de Freitas Neto et al., 2010). Several outbreaks of *Salmonella* from pork have been reported worldwide. In 2008, an outbreak of *S.* Typhimurium was identified in Denmark, Norway, and Sweden. It was later confirmed that pork from Denmark was the source of *S.* Typhimurium (Bruun et al., 2009). This case demonstrates the potential of *Salmonella* to cause international outbreaks due to meat export and import. Outbreaks of *S.* Typhimurium have also been reported in Sydney in 1995 (Delpech et al., 1998) and in Denmark in 2010 (Kuhn et al., 2013). From 1998-2008, pork has been implicated to 37 *Salmonella* outbreaks in the United States (US) (Jackson et al., 2013). More recently in 2015, another *Salmonella* outbreak from pork occurred in five states in the US (CDC, 2015).

The risk of *S. enterica* contamination in pork increases along with pork production chain and peaks during slaughter at the abattoir (Arguello et al., 2012; Gomes-Neves et al., 2012). It is therefore necessary that abattoirs maintain proper sanitation and high levels of hygiene to prevent the spread of the pathogen. In the Philippines, abattoirs are regulated either by the national or the local government. Abattoirs that have adequate facilities and operational

procedures are "accredited" by the national government, while those that have failed to meet the standards are regulated by the local government (locally registered).

In the Philippines, routine testing of *Salmonella* is often limited to culture techniques (Ng and Rivera, 2015). Application of molecular methods rarely occurs, and is limited only to genus or species identification. Serogrouping is almost never conducted. Molecular characterization often starts with PCR-based detection methods and then followed by subtyping (Adzitey et al., 2013). PCR-based detection of *Salmonella* commonly targets the *invA* gene, and its sensitivity and specificity have been validated by several studies (Chiu and Ou, 1996; Malorny et al., 2003; Rahn et al., 1992). The *invA* gene is unique to *Salmonella* and plays a critical role in the invasion of epithelial cells (Galán and Curtiss, 1991; Rahn et al., 1992). It encodes a highly conserved protein component of the *Salmonella* type III secretion system which translocates bacterial proteins across the cell membrane and into the cytoplasm of the host cell (Lilic et al., 2010). The *invA* gene is found in almost all strains of *Salmonella* which makes it a suitable detection marker. Molecular subtyping of *Salmonella* begins with serogrouping. Multiplex PCR-based serogrouping is most commonly employed, and is based on the sequence of the somatic (O) antigen, the outermost component of the lipopolysaccharide layer in Gram-negative bacteria. In *Salmonella,* a considerable amount of diversity is found in O antigens which contribute to its antigenic diversity (Fitzgerald et al., 2003).

Monitoring the antimicrobial susceptibility of *Salmonella* is important in determining the choice of treatment for salmonellosis in pigs and to shed light on the resistant *Salmonella* that may be transferred to humans (Van der Wolf et al., 1999). In the Philippines, clinical non-typhoid *Salmonella* resistant to ciprofloxacin and ceftriaxone has been generally increasing since

2005. In 2014, 21.6% and 13.0% of non-typhoid *Salmonella* isolates were resistant to ciprofloxacin and ceftriaxone, respectively (RITM, 2014).

The objective of the present study was to estimate the incidence of *S. enterica* from freshly slaughtered swine in selected accredited and locally regulated abattoirs in Metro Manila, Philippines. Specifically, this study aimed to (i) detect and isolate *S. enterica* from the tonsils and jejunum with mesenteric lymph nodes (MLN) of slaughtered swine using both culture and PCR-based methods, (ii) determine the serogroup of *S. enterica* isolates using a PCR-based characterization of the somatic antigen, (iii) generate antimicrobial profiles of selected *S. enterica* using VITEK® 2, and (iv) compare the incidences of *S. enterica* contamination in swine slaughtered in accredited and in locally registered abattoirs.

2. Materials and Methods

2.1 Sample collection

A total of 240 swine from five accredited and three locally registered abattoirs in Metro Manila were analyzed in the study. These establishments were located in the four districts of Metro Manila: Capital District (1), Eastern Manila (1), Northern Manila (4), and Southern Manila (2). Thirty hogs, all coming from the same farm, were randomly selected for sample collection from each abattoir. Upon evisceration, tonsil tissues and a 15-cm segment of the jejunum including its contents and MLN were collected from each hog using sterile scissors and forceps. The samples were transferred into sterile plastic bags and chilled in a cooler during transport to the laboratory. All samples were processed immediately upon arrival at the laboratory.

2.2 Bacterial enrichment and isolation

Twenty-five grams each of tonsil tissue and intestinal samples were weighed in sterile aluminum foil. The samples were minced and transferred to 225 mL buffered peptone water (BPW), agitated for 2 min, and incubated at 35°C for 18-24 h. For the selective enrichment of *S. enterica*, 100 µL of pre-enriched *S. enterica* in BPW was inoculated in 10 mL Rappaport-Vassiliadis broth, and then incubated at 42°C for 18-24 h. After incubation, broth cultures were streak-plated onto brilliant green agar (BGA) and xylose lysine deoxycholate agar (XLD), and then incubated at 35°C for 18-24 h. Pink to red colonies in BGA and clear to pinkish red colonies (with or without black center) in XLD were considered presumptive *S. enterica* and were subjected to PCR-based identification.

2.3 DNA extraction

Presumptive *S. enterica* were grown on nutrient agar overnight at 35°C. After incubation, 3-5 colonies were harvested and suspended in 100 µL TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0). Cell suspensions were heated at 100°C for 10 min, and then cooled to room temperature. Afterwards, cell suspensions were pelleted by centrifugation at 6000 rpm for 5 min. The supernatant was collected and then transferred into a new tube.

2.4 PCR-based identification and characterization of S. enterica *isolates*

PCR-based identification of *S. enterica* targeting the *invA* gene was performed as previously described by Ng and Rivera (2015) with the following modifications: annealing at 60°C for 30 s and extension at 72C° for 30s. For *Salmonella* O-serogrouping, a multiplex PCR-based assay was adapted from Soguilon-Del Rosario and Rivera (2015). In the present study, the latest serogroup designation was used: O:4 (B), O:7 (C_1), O:8 (C_2-C_3), O:9 (D_1-D_4), and O:3,10 (E_1). For each run, a no template control (NTC) and a positive control, *S.* Typhimurium UPCC 1360, was included. Amplicons were analyzed using agarose gel electrophoresis. Briefly, 5 µL

amplicons were loaded into 2% agarose gels containing 10,000× SYBR® Safe DNA Gel Stain (1:10,000). Amplicons were allowed to separate at 100 V for 45 min, and then viewed in a gel documentation system.

2.5 Antimicrobial susceptibility testing

A total of 183 randomly selected *S. enterica* isolates were subjected to antimicrobial susceptibility testing using the VITEK® 2 Compact system and VITEK® 2 AST-GN70 cards. Each card contains 16 antimicrobials including amikacin, ampicillin, ampicillin/sulbactam, aztreonam, cefazolin, cefepime, ceftriaxone, ciprofloxacin, ertapenem, gentamicin, meropenem, nitrofurantoin, piperacillin/tazobactam, tigecycline, tobramycin, trimethoprim/sulfamethoxazole, and an extended-spectrum ß-lactamase (ESBL) confirmation test. For the antimicrobial susceptibility testing, *S. enterica* isolates were grown in nutrient agar and incubated at 35°C for 18-24 h. Three to four colonies were suspended in 3 mL of 0.45% sterile saline solution using sterile applicator sticks. The turbidity was adjusted to 0.5 McFarland using DensiChek™. Bacterial suspensions and test cards were loaded in a cassette, and then loaded into the VITEK® 2 system. The cards were then automatically filled with the bacterial suspension, sealed, and then incubated at 35.5°C. The duration of the analysis can take around 7-18 h where turbidity is automatically measured at 15-min intervals using kinetic analysis. The VITEK® 2 system applies the most recent breakpoints published by the Clinical Laboratory Standards Institute (CLSI) in interpreting data.

2.6 Data analysis

S. enterica contamination acquired prior to delivery to the abattoir (e.g. from farm or transport) was measured as the number of *Salmonella*-positive jejunum (including contents and MLN). Presence of *S. enterica* in both or either tonsils or jejunum indicates contamination of the

end product. Differences between observed contamination in the jejunum and in the end product were assessed using a paired T-test. The total contaminated end products in accredited and locally registered abattoirs were compared and the values were expressed in percentages. Fisher's exact test was used to determine the significance of the difference between the two types of abattoir. All data analyses were performed using SPSS 16.0. Significance was determined to be $P < 0.05$.

3. Results

3.1 PCR-based detection and characterization of S. enterica *from slaughtered swine*

A total of 333 *S. enterica* isolates were recovered from 108 out of 240 (45.0%) slaughtered swine. Multiple strains of *S. enterica* were isolated from several samples. It is evident that there is an increase in *S. enterica* contamination after slaughter where the number of contaminated end products is always greater than the number of contaminated jejuna regardless of the type of abattoir (Fig. 1). Paired T-test revealed that there is a statistically significant increase in end product contamination in both accredited and locally registered abattoirs ($P > 0.034$ and $P > 0.049$, respectively), suggesting that an external factor contributes to the increase in *S. enterica* contamination. Of the 150 swine from accredited abattoirs (A1-A5), 66 (44.0%) were positive for *S. enterica*. On the other hand, 42 out of 90 (46.7%) swine slaughtered in locally registered abattoirs (L1-L3) were carrying *S. enterica* (Fig. 1). Fisher's exact test revealed that there is no statistical difference between the observed incidences of end product contamination in the two types of abattoirs ($P = 0.42$).

Serogrouping was conducted only on the isolates obtained from 140 tonsil and jejunum samples and revealed that 104 (74.3%) were contaminated with a single serogroup of *S. enterica*.

Most of these samples were contaminated with *S. enterica* under serogroup O:3,10 (51.0%) and O:7 (23.1%). Multiple serogroups were present in 36 (34.6%) samples (Table 1).

3.2 Antimicrobial susceptibility testing

A total of 183 randomly selected *S. enterica* isolates were subjected to antimicrobial susceptibility testing using VITEK® 2. Excluding cefazolin and aminoglycosides, most *S. enterica* isolates were non-susceptible to nitrofurantoin (93.4%), trimethoprim/sulfamethoxazole (80.3%), and ampicillin (70.5%) (Table 2). Non-susceptibility to the late cephalosporins ceftriaxone and cefepime, and the fluoroquinolone ciprofloxacin was also observed. Multidrug-resistance was found in 124 (67.8%) out of 183 isolates (Table 3). While all of the multidrug-resistant isolates were resistant to ampicillin, most of the isolates were resistant to ampicillin, ampicillin/sulbactam, and trimethoprim/sulfamethoxazole. Results of the ESBL test were not shown as there are currently no ESBL breakpoints for *S. enterica* set by the CLSI (2014).

4. Discussion

In swine, *S. enterica* is commonly isolated from the tonsils, digestive tract, lymph nodes, and feces (Ng and Rivera, 2015). Presence of *S. enterica* in the jejunum including its contents and the MLN would indicate that the contamination was acquired before the animals were brought to the abattoir (Botteldoorn et al., 2003). To determine *S. enterica* contamination at the abattoir, tonsils were collected since they are one of the first organs to come in contact upon ingestion of contaminated feedstuff or feces (Ng and Rivera, 2015).

In this study, 44.0% and 46.7% *Salmonella*-positive pigs were detected from accredited and locally registered abattoirs, respectively. While the incidence of *S. enterica* in swine slaughtered in locally registered abattoirs was higher, the difference was not statistically

significant. Contrastingly, a recent study reported that the incidence of *Salmonella*-positive pigs was significantly higher in locally registered abattoirs than in accredited abattoirs (Ng and Rivera, 2015). This could be due to the difference in sample locations between the two studies. In the Philippines, accreditation of abattoirs is based on the appropriate facilities and operational procedures applied in the establishment. Abattoirs that have not met these standards are supervised by the local government and are only allowed to distribute meat within the city where the establishment is located. The insignificant difference observed between the incidences of end product contamination in the two types of abattoirs could reflect either poor implementation of slaughter policies or contamination acquired during transport. Whichever the case, abattoir accreditation may be disregarded as either type has been found to yield essentially the same number of contaminated end products. Such findings must be addressed because accredited abattoirs are allowed to distribute meat on a national level. The risk that a multi-city *Salmonella* outbreak occurs due to trade, as was observed in the multi-country *Salmonella* outbreak involving Denmark, Norway, and Sweden (Bruun et al., 2009), may not be unlikely.

Because of the risk of resistant bacteria due to the use of antimicrobials on livestock production, numerous efforts have been made to regulate the use of antimicrobial growth promoters (AGPs). Sweden was the first to impose a ban on AGPs in 1986, followed by Denmark (Cogliani et al., 2011). The European Union banned avoparcin in 1997 upon discovery of vancomycin-resistant *Enterococcus* (Cogliani et al., 2011). Remaining AGPs were banned in the European Union in 2006 (Cogliani et al., 2011; Maron et al., 2013). Other countries, such as Mexico, South Korea, Taiwan, and New Zealand, also have a ban on AGPs (Maron et al., 2013; Van Boeckel et al., 2015). However, in the United States, the use of AGPs is only discouraged. While there is no ban on AGPs, the FDA has issued voluntary guidelines to withdraw the use of

medically important antimicrobials in growth promotion (FDA, 2013). In the Philippines, the use of chloramphenicol, carbadox, olaquindox, nitrofurans, and human β-agonist drugs has been banned in livestock production.

To evaluate the antimicrobial susceptibility of select *S. enterica* isolates, we used the highly automated VITEK® 2 system which can be used for identification and antimicrobial susceptibility testing (AST). It has an optical system which combines multichannel fluorimeter and photometer readings and reads each test at 15-minute intervals using kinetic analysis. The accuracy of VITEK® 2 AST for both Gram-positive and Gram-negative bacteria has been evaluated in several studies (Garcia-Garrote et al., 2000; Gherardi et al., 2012; Ligozzi et al., 2002; Ling et al., 2001). In this present study, 183 randomly selected *S. enterica* isolates were subjected to VITEK® 2 AST. Excluding the first generation cephalosporin cefazolin and the aminoglycosides amikacin, gentamicin, and tobramycin, most isolates were non-susceptible to nitrofurantoin (93.4%), trimethoprim/sulfamethoxazole (80.3%), and ampicillin (70.5%) (Table 3). Cefazolin and the aminoglycosides were excluded because they are not clinically effective for *Salmonella* and must not be reported susceptible (CLSI, 2014).

Nitrofurantoin is used to treat urinary tract infections. In contrast to other studies wherein nitrofurantoin non-susceptibility in *Salmonella* from pork appears to be low to moderate (Ibar et al., 2008; Yan et al., 2010), the present study reports a very high rate of non-susceptibility despite its ban on food-producing animals more than a decade ago. This would suggest that pig farmers do not strictly adhere to veterinary policies set by the government. Ampicillin and trimethoprim/sulfamethoxazole are antimicrobials traditionally used to treat invasive non-typhoid salmonellosis (Chen et al., 2013). In the Philippines, these antimicrobials are also used as anti-infective agents in veterinary medicine which could explain the high rates of non-

susceptibility to ampicillin and trimethoprim/sulfamethoxazole. Ampicillin- and trimethoprim/sulfamethoxazole-resistant *Salmonella* have become increasingly common which, in turn, present clinical dilemma in the treatment of invasive salmonellosis (Chen et al., 2013). The emergence of fluoroquinolone and ceftriaxone resistance presents an even bigger problem since these are the drugs of choice for non-typhoid salmonellosis in cases where ampicillin or trimethoprim/sulfamethoxazole is clinically ineffective (Chen et al., 2013). In the present study, non-susceptibility to ciprofloxacin (7.1%) and ceftriaxone (2.2%) was observed. For invasive *Salmonella* infections that are resistant to both ciprofloxacin and ceftriaxone, carbapenems may be the last drug of choice (Chen et al., 2013). However in the present study, non-susceptibility to ertapenem (1.6%) and meropenem (0.5%) was also detected. While these isolates were susceptible to ciprofloxacin, the possibility of acquiring fluoroquinolone resistance due to mutation or horizontal gene transfer should not be overlooked.

5. Conclusion

The incidence rates of *S. enterica* in swine end products from both types of abattoirs in the present study appeared to be high. Several factors can be considered to contribute to end product contamination such as farms or origin of the animals, transport, or the abattoir itself. While the abattoir has been identified as the peak of *S. enterica* contamination (Arguello et al., 2012), we cannot exclude the possibility that contamination was acquired during transport from the farm to the abattoir. Collecting swabs from transport trucks and at different points in the slaughtering process (e.g. lairage, dehairing, evisceration) must be done in order to determine exactly where the greatest risk of contamination occurs. Nevertheless, our results show that high rates of *S. enterica* contamination occur regardless of the abattoir accreditation. On the other

hand, the results of the antimicrobial susceptibility assay suggest that antimicrobial use in swine farms be closely monitored. While nitrofurantoin has long been banned for use in veterinary practices in the Philippines, a high rate of non-susceptibility to the agent was still observed suggesting that efforts in the regulation of nitrofurantoin use by farmers was not effective. The observed non-susceptibility to ciprofloxacin and ceftriaxone, although low, is also alarming. Monitoring the antimicrobial susceptibility of bacterial isolates from livestock animals is therefore necessary to determine the effects of the withdrawal of AGPs in the livestock industry and to identify emerging resistant strains in the pork production chain.

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References

Adzitey, F., Huda, N., Ali, G.R.R., 2013. Molecular techniques for detecting and typing of bacteria, advantages and application to foodborne pathogens isolated from ducks. Biotech. 3, 97-107.

Arguello, H., Carvajal, A., Collazos, J.A., García-Feliz, C., Rubio, P., 2012. Prevalence and serovars of *Salmonella enterica* on pig carcasses, slaughtered pigs and the environment of four Spanish slaughterhouses. Food Res. Int. 45, 905-912.

294 Botteldoorn, N., Heyndrickx, M., Rijpens, N., Grijspeerdt, K., Herman, L., 2003. *Salmonella* on
295 pig carcasses: Positive pigs and cross contamination in the slaughterhouse. J. Appl.
296 Microbiol. 95, 891-903.
297 Bruun, T., Sørensen, G., Forshell, L., Jensen, T., Nygård, K., Kapperud, G., Lindstedt, B.,
298 Berglund, T., Wingstrand, A., Petersen, R., 2009. An outbreak of *Salmonella*
299 Typhimurium infections in Denmark, Norway and Sweden, 2008. Eurosurveillance 14.
300 Centers for Disease Control and Prevention, 2015. Multistate outbreak of multidrug-resistant
301 *Salmonella* I 4,[5],12:i:- and *Salmonella* Infantis infections linked to pork (Final
302 Update).http://www.cdc.gov/salmonella/pork-08-15/index.html (accessed 15.02.16)
303 Chen, H.-M., Wang, Y., Su, L.-H., Chiu, C.-H., 2013. Nontyphoid *Salmonella* infection:
304 Microbiology, clinical features, and antimicrobial therapy. Pediatr. Neonatol. 54, 147-
305 152.
306 Chiu, C.-H., Ou, J.T., 1996. Rapid identification of *Salmonella* serovars in feces by specific
307 detection of virulence genes, *invA* and *spvC*, by an enrichment broth culture-multiplex
308 PCR combination assay. J. Clin. Microbiol. 34, 2619-2622.
309 Clinical and Laboratory Standards Institute, 2014. Performance Standards for Antimicrobial
310 Susceptibility Testing; Twenty-Fourth Informational Supplement. CLSI document M100-
311 S24. Clinical and Laboratory Standards Institute, Wayne, PA.
312 Cogliani, C., Goossens, H., Greko, C., 2011. Restricting antimicrobial use in food animals:
313 Lessons from Europe. Microbe 6, 274-279.
314 CountrySTAT Philippines, 2016. Livestock and Poultry: Volume of
315 Production.http://countrystat.psa.gov.ph/?cont=10&pageid=1&ma=B20PNVLP
316 (accessed 15.02.16)

de Freitas Neto, O., Penha Filho, R., Barrow, P., Berchieri Junior, A., 2010. Sources of human non-typhoid salmonellosis: A review. Rev. Bras. Cienc. Avic. 12, 1-11.

Delpech, V., McAnulty, J., Morgan, K., 1998. A salmonellosis outbreak linked to internally contaminated pork meat. Aust N Z J Public Health 22, 243-246.

Fitzgerald, C., Sherwood, R., Gheesling, L.L., Brenner, F.W., Fields, P.I., 2003. Molecular analysis of the *rfb* O antigen gene cluster of *Salmonella enterica* serogroup O:6,14 and development of a serogroup-specific PCR assay. Appl. Environ. Microbiol. 69, 6099-6105.

Food and Agricultural Organization of the United Nations, 2014. Sources of Meat. http://www.fao.org/ag/againfo/themes/en/meat/backgr_sources.html (accessed 16.02.16)

Galán, J.E., Curtiss, R., 1991. Distribution of the *invA*, *-B*, *-C*, and *-D* genes of *Salmonella* Typhimurium among other *Salmonella* serovars: *invA* mutants of *Salmonella* Typhi are deficient for entry into mammalian cells. Infect. Immun. 59, 2901-2908.

Garcia-Garrote, F., Cercenado, E., Bouza, E., 2000. Evaluation of a new system, VITEK 2, for identification and antimicrobial susceptibility testing of Enterococci. J. Clin. Microbiol. 38, 2108-2111.

Gherardi, G., Angeletti, S., Panitti, M., Pompilio, A., Di Bonaventura, G., Crea, F., Avola, A., Fico, L., Palazzo, C., Sapia, G.F., 2012. Comparative evaluation of the VITEK-2 Compact and Phoenix systems for rapid identification and antibiotic susceptibility testing directly from blood cultures of Gram-negative and Gram-positive isolates. Diagn. Microbiol. Infect. Dis. 72, 20-31.

338 Gomes-Neves, E., Antunes, P., Tavares, A., Themudo, P., Cardoso, M.F., Gärtner, F., Costa,
339 J.M., Peixe, L., 2012. *Salmonella* cross-contamination in swine abattoirs in Portugal:
340 Carcasses, meat and meat handlers. Int. J. Food Microbiol. 157, 82-87.
341 Ibar, M., Vigo, G., Pineyro, P., Caffer, M., Quiroga, P., Perfumo, C., Centron, D., Giacoboni, G.,
342 2008. Serovars of *Salmonella enterica* subspecies *enterica* and its antimicrobial
343 resistance in slaughterhouse pigs. Rev. Argent. Microbiol. 41, 156-162.
344 Jackson, B.R., Griffin, P.M., Cole, D., Walsh, K.A., Chai, S.J., 2013. Outbreak-associated
345 *Salmonella enterica* serotypes and food commodities, United States, 1998-2008. Emerg.
346 Infect. Dis. 19, 1239-1244.
347 Kuhn, K., Sørensen, G., Torpdahl, M., Kjeldsen, M., Jensen, T., Gubbels, S., Bjerager, G.,
348 Wingstrand, A., Porsbo, L., Ethelberg, S., 2013. A long-lasting outbreak of *Salmonella*
349 Typhimurium U323 associated with several pork products, Denmark, 2010. Epidemiol.
350 Infect. 141, 260-268.
351 Ligozzi, M., Bernini, C., Bonora, M.G., de Fatima, M., Zuliani, J., Fontana, R., 2002. Evaluation
352 of the VITEK 2 System for identification and antimicrobial susceptibility testing of
353 medically relevant Gram-positive cocci. J. Clin. Microbiol. 40, 1681-1686.
354 Lilic, M., Quezada, C.M., Stebbins, C.E., 2010. A conserved domain in type III secretion links
355 the cytoplasmic domain of InvA to elements of the basal body. Acta Crystallogr. D Biol.
356 Crystallogr. 66, 709-713.
357 Ling, T.K., Tam, P., Liu, Z., Cheng, A.F., 2001. Evaluation of VITEK 2 rapid identification and
358 susceptibility testing system against Gram-negative clinical isolates. J. Clin. Microbiol.
359 39, 2964-2966.

360 Malorny, B., Hoorfar, J., Bunge, C., Helmuth, R., 2003. Multicenter validation of the analytical
361 accuracy of *Salmonella* PCR: Towards an international standard. Appl. Environ.
362 Microbiol. 69, 290-296.
363 Maron, D.F., Smith, T.J., Nachman, K.E., 2013. Restrictions on antimicrobial use in food animal
364 production: An international regulatory and economic survey. Global. Health 9.
365 Research Institute for Tropical Medicine, 2014. Antimicrobial Resistance Surveillance Program:
366 2014 Data Summary Report.
367 Ng, K.C.S., Rivera, W.L., 2015. Multiplex PCR–based serogrouping and serotyping of
368 *Salmonella enterica* from tonsil and jejunum with jejunal lymph nodes of slaughtered
369 swine in Metro Manila, Philippines. J. Food Prot. 78, 873-880.
370 Organization for Economic Cooperation and Development, 2015. Meat consumption (indicator).
371 https://data.oecd.org/agroutput/meat-consumption.htm#indicator-chart (accessed
372 16.02.16)
373 Rahn, K., De Grandis, S., Clarke, R., McEwen, S., Galan, J., Ginocchio, C., Curtiss, R., Gyles,
374 C., 1992. Amplification of an *invA* gene sequence of *Salmonella* Typhimurium by
375 polymerase chain reaction as a specific method of detection of *Salmonella*. Mol. Cell.
376 Probes 6, 271-279.
377 Soguilon-Del Rosario, S.A., Rivera, W.L., 2015. Incidence and molecular detection of
378 *Salmonella enterica* serogroups and *spvC* virulence gene in raw and processed meats
379 from selected wet markets in Metro Manila, Philippines. Int. J. Philipp. Sci. Tech. 8, 52-
380 55.
381 US Food and Drug Administration, 2013. Guidance for Industry #213: New Animal Drugs and
382 New Animal Drug Combination Products Administered in or on Medicated Feed or

383 Drinking Water of Food-Producing Animals: Recommendations for Drug Sponsors for
384 Voluntarily Aligning Product Use Conditions with GFI
385 #209.http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforceme
386 nt/GuidanceforIndustry/UCM299624.pdf (accessed 20.02.16)
387 Van Boeckel, T.P., Brower, C., Gilbert, M., Grenfell, B.T., Levin, S.A., Robinson, T.P., Teillant,
388 A., Laxminarayan, R., 2015. Global trends in antimicrobial use in food animals. Proc.
389 Natl. Acad. Sci. USA 112, 5649-5654.
390 Van der Wolf, P., Bongers, J., Elbers, A., Franssen, F., Hunneman, W., Van Exsel, A., Tielen,
391 M., 1999. *Salmonella* infections in finishing pigs in The Netherlands: Bacteriological
392 herd prevalence, serogroup and antibiotic resistance of isolates and risk factors for
393 infection. Vet. Microbiol. 67, 263-275.
394 Yan, H., Li, L., Alam, M.J., Shinoda, S., Miyoshi, S.-i., Shi, L., 2010. Prevalence and
395 antimicrobial resistance of *Salmonella* in retail foods in northern China. Int. J. Food
396 Microbiol. 143, 230-234.

398 Table 1. *S. enterica* isolates recovered from Philippine swine, classified into serogroups.

Serogroup	Accredited slaughterhouse		LRA		Total[b]
	Jejunum	Tonsil	Jejunum	Tonsil	
Single serogroups					104
O:2	0	0	0	0	0
O:4	2	2	0	2	6
O:7	11	12	0	1	24
O:8	2	0	0	0	2
O:9	2	2	0	0	4
O:3,10	14	14	11	14	53
Other[a]	3	0	6	6	15
Multiple serogroups					36
O:4, O:7	0	0	1	0	1
O:4, O:3,10	0	2	1	1	4
O:7, O:3,10	0	2	1	0	3
O:8, O:3,10	1	1	0	0	2
O:4 + other[a]	4	1	2	0	7
O:7+ other[a]	1	2	0	0	3
O:3,10 + other[a]	1	5	1	3	10
O:4, O:7, O:3,10	2	0	0	0	2
O:4, O:3,10 + other[a]	0	3	0	1	4

399 LRA- locally registered abattoirs

400 [a]Other than serogroups O:2, O:4, O:7. O:8, O:9, and O:3,10

401 [b]Only *S. enterica* isolates from 140 tonsils and jejunum samples were typed.

403 Table 2. Non-susceptibility of *S. enterica* isolates (n=183) recovered from swine.

Antimicrobial	Abbreviation	Non-susceptible isolates		
		Intermediate	Resistant	Total
Ampicillin	Am	5 (2.7%)	124 (67.8%)	129 (70.5%)
Ampicillin/Sulbactam	Am/Su	37 (20.2%)	79 (43.2%)	116 (63.4%)
Piperacillin/Tazobactam	Pi/Tz	1 (0.6%)	1 (0.6%)	2 (1.2%)[a]
Cefazolin	Cf	0 (0.0%)	183 (100.0%)	183 (100.0%)[b]
Ceftriaxone	Cx	0 (0.0%)	4 (2.2%)	4 (2.2%)
Cefepime	Cp	0 (0.0%)	1 (0.5%)	1 (0.5%)
Aztreonam	Az	1 (0.5%)	1 (0.5%)	2 (1.0%)
Ertapenem	Er	1 (0.5%)	2 (1.1%)	3 (1.6%)
Meropenem	Me	0 (0.0%)	1 (0.5%)	1 (0.5%)
Amikacin	Ak	0 (0.0%)	183 (100.0%)	183 (100.0%)[b]
Gentamicin	Ge	0 (0.0%)	183 (100.0%)	183 (100.0%)[b]
Tobramycin	To	0 (0.0%)	183 (100.0%)	183 (100.0%)[b]
Ciprofloxacin	Ci	3 (1.6%)	10 (5.5%)	13 (7.1%)
Tigecycline	Ti	6 (3.3%)	3 (1.6%)	9 (4.9%)
Nitrofurantoin	Ni	94 (51.4%)	77 (42.1%)	171 (93.4%)
Trimethoprim/Sulfamethoxazole	Tr/Sx	0 (0.0%)	147 (80.3%)	147 (80.3%)

404 [a]Only 179 isolates were tested on piperacillin/tazobactam.

405 [b]As per CLSI guidelines, all *Salmonella* must be reported non-susceptible to first and second
406 generation cephalosporins (cefazolin) and aminoglycosides (amikacin, gentamicin, tobramycin)
407 (CLSI, 2014).

409 Table 3. Resistance patterns of multidrug-resistant *S. enterica* (n=124) recovered from swine.

Number of isolates	Resistance pattern[a, b]
2	Am, Am/Su, Ci
2	Am, Am/Su, Ni
43	Am, Am/Su, Tr/Sx
1	Am, Ci, Ni
1	Am, Ci, Tr/Sx
18	Am, Ni, Tr/Sx
2	Am, Am/Su, Ci, Tr/Sx
24	Am, Am/Su, Ni, Tr/Sx
1	Am, Am/Su, Cx, Ni, Tr/Sx
2	Am, Am/Su, Ci, Ni, Tr/Sx
1	Am, Am/Su, Cx, Ti, Ni, Tr/Sx
1	Am, Am/Su, Cx, Er, Ti, Ni, Tr/Sx
1	Am, Am/Su, Pi/Tz, Cx, Cp, Az, Er, Me Ti, Ni, Tr/Sx

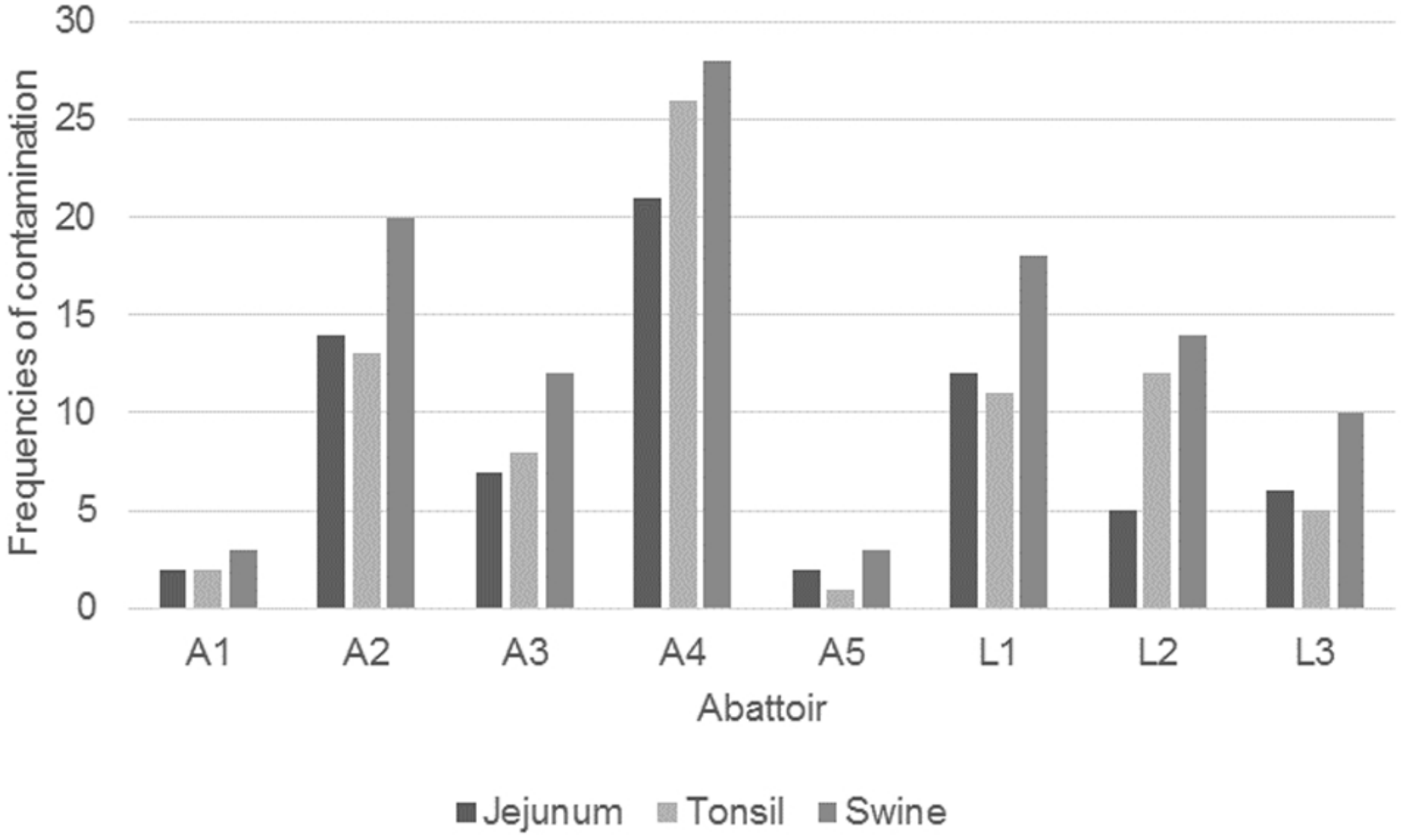
410 [a]Am, ampicillin; Am/Su, ampicillin/ sulbactam; Ci, ciprofloxacin; Ni, nitrofurantoin; Tr/Sx,
411 trimethoprim/ sulfamethoxazole; Cx, ceftriaxone; Ti, tigecycline; Er, ertapenem; Pi/Tz,
412 piperacillin/tazobactam; Cp, cefepime; Az, aztreonam

413 [b]Resistance to cefazolin, amikacin, gentamicin, and tobramycin not shown

415 Figure caption

416 Fig. 1. *S. enterica* contamination frequencies of jejunum (including contents) and MLN, tonsils,

417 and end products in the different abattoirs.


30
25
20
15
10
5
0
Frequencies of contamination
A1
A2
A3
A4
A5
L1
L2
L3
Abattoir
Jejunum
Tonsil
Swine