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RESEARCH ARTICLE

Comparison of *Salmonella* spp. contamination and antimicrobial resistance patterns between two pig slaughterhouse models

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Abstract

Objective: To compare the *Salmonella* spp. contamination and antimicrobial resistance (AMR) patterns between two pig slaughterhouse models.

Materials and Methods: Three hundred sixty pig fecal samples and 360 pork samples were collected from one Hazard Analysis and Critical Control Point (HACCP) standard slaughterhouse and one poor hygiene practice (PHP) slaughterhouse (180 pig fecal samples and 180 pork samples were collected from each slaughterhouse) in Khon Kaen province during 2017-2018 (n=720). All samples were purified and serotype identified. The positive Salmonella spp. isolates were then tested for their antimicrobial susceptibility against ampicillin, ciprofloxacin, chloramphenicol, nalidixic acid, streptomycin, sulfamethoxazole-trimethoprim, tetracycline, and colistin.

Results: TThere were 230 Salmonella spp. isolates purified from the total of samples (n=720); 39.17% Salmonella spp. were isolated from pig fecal samples (141/360) and 24.72% from pork (89/360). The fecal and pork isolates from the HACCP slaughterhouse were 30.56% (110/360) and 6.39% (23/360), whereas the fecal and pork isolates from PHP slaughterhouse were 8.61% (31/360) and 18.33% (66/360), respectively. The most predominant serotype was the Enterica type I 4,[5],12:i:-.

Conclusion: After the statistical analyzed between two slaughterhouses, the results demonstrated that the HACCP standard slaughterhouse could reduce the proportion of serotype numbers of *Salmonella* spp., and stop some AMR spreading from pigs to pork, but the PHP slaughterhouse increased the proportion of positive *Salmonella* and serotype numbers from pigs to pork.

Keywords: Antimicrobial resistance, *Salmonella* spp., Hazard Analysis and Critical Control Point slaughterhouse, poor hygiene practice slaughterhouse

Introduction

Antimicrobials are widely used for human clinical treatment, animal therapy, and even added into animal fodder to promote economic animal growth. The frequency of using antimicrobial agents in animals is much higher than in humans. Interestingly, $2.5*10^7$ pounds of antimicrobials are consumed for nontherapeutic purposes for economic animals, which are consumed eight times higher than for human clinical treatment (Landers et al., 2012). Many previous studies demonstrated that the antimicrobial resistant *Escherichia coli* could enter into the food chain via the contaminated meat products during slaughtering, no matter with the fed foods for cattle containing antimicrobial growth promoter or not (Alexander et al., 2010). Moreover, McCrackin confirmed that farm antimicrobial pressure could increase the bacteria colonization of animals after the prevalence of antimicrobial resistant *Campylobacter* spp. was analyzed (McCrackin et al., 2016). It has been believed that antimicrobial resistance (AMR) pathogens at the table are generated from the antimicrobials used in farm animals (Hummel et al., 1986; Van et al., 2008). In contrast, no direct connection of the antimicrobial resistant bacteria from farm to table was proposed (Alexander et al., 2010). Therefore, evaluation the procedure on AMR pathogens spread in the food industry chain would help us to figure out how to stop AMR spreading.

The slaughtering step is the most crucial part of the food chain. A good slaughtering process could stop the pathogens spreading generated from farms. For example, the Hazard Analysis and Critical Control Point (HACCP) management system was introduced to produce safe meat products for consumers on controlling the biological, chemical, and physical hazards of meat, through monitoring and organizing the whole procedure. This management system was accepted after the USDA's Food Safety and Inspection Service (FSIS) announced the rule in 1996 for chicken products. There were 190,000 cases less during 1996-2000 (Williams & Ebel, 2012). However, the systematic review reported the disadvantages of this system on evaluating the effectiveness of different HACCP slaughterhouses (Wilhelm et al., 2011). On the other hand, the poor hygiene practice (PHP) level slaughterhouses would be in a more dangerous condition than HACCP slaughterhouses due to the lack of proper hygiene practices (Fasanmi et al, 2018).

Thereby, the AMR pathogens spreading from slaughterhouses should be quantified evaluated, in order to provide the information to avoid the AMR pathogen risk. *Salmonella* spp. was chosen as the indicator for slaughterhouses evaluation. Because it is one of the four global chief causes of the 550 million diarrheal diseases announced yearly by the World Health Organization. Furthermore, Thailand was suffered under this pathogen, the 73.2% pork from wet markets was contaminated by *Salmonella* spp. (Patchanee et al, 2016). The two hygiene level pig slaughterhouses were analyzed because the pig industry provides the most consumed meat for the world, and the pig and pork could shed a large amount *Salmonella* spp. In this study, the samples from pig feces and pork from one HACCP slaughterhouse and one PHP slaughterhouse were collected and analyzed.

Materials and Methods

Sample collection

To investigate the associate relationship of the antimicrobial resistance *Salmonella* spp. between pigs and pork from two different hygiene level slaughterhouses, three hundred and sixty pig fecal and 360 pork samples were collected from two slaughterhouses in Khon Kaen province, Thailand from 2017 to 2018 (n=720). The two slaughterhouses are one Hazard Analysis and Critical Control Point (HACCP) standard slaughterhouse (180 pig fecal and 180 pork samples from 6 farms), and one poor hygiene practice (PHP) slaughterhouse (180 pig fecal and 180 pork samples from 12 farms). The HACCP slaughterhouse was certified by the Department of Livestock Development (DLD), and the PHP slaughterhouse was registered with the local DLD office. The fecal samples were collected by sterilized rectal swab, the pork samples of HACCP slaughterhouse were collected by the sterilized swabs covered on the primal cuts after the final wash; the pork samples of PHP slaughterhouse were also collected at the primal cuts (there is no final wash step in this PHP slaughtering procedure). All samples were stored in an ice box for transportation, then tests were performed immediately. The study was reviewed and approved by the Institutional Animal Care and Use Committee of Khon Kaen University, No. IACUC-KKU-13/62.

Salmonella isolation

All samples were isolated for *Salmonella* spp., following the ISO standard 6579-1:2002 (ISO, 2002), and biochemical tested by Triple Sugar Iron agar (Becton Dickinson, Franklin Lakes, NJ) and Motility Indole Lysine medium (Becton Dickinson, Franklin Lakes, NJ). *Salmonella* spp. isolation was performed at the laboratory of Khon Kaen University Veterinary Teaching Hospital.

Serotype identification

All sample O (somatic) and H (flagellar) antigens were characterized by agglutination method in WHO National Salmonella and Shigella Center for serotype identification, and the serotype was assigned according to the Kauffmann–White scheme (Grimont et al., 2007).

Antimicrobial susceptibility test

All the *Salmonella* spp. isolates were analyzed for antimicrobial susceptibility against ampicillin (AMP) 10 µg, ciprofloxacin (CIP) 5 µg, chloramphenicol (CHL) 30 µg, nalidixic acid (NA) 30 µg, streptomycin (STR) 10 µg, sulfamethoxazole/trimethoprim (SXT) 25 µg, tetracycline (TET) 30 µg and colistin (COT) 10 µg by disk diffusion test (BD Diagnostics, Sparks, MD), following the Clinical and Laboratory Standards Institute guidelines (CLSI, 2014).

Statistical analysis

The significant differences between two slaughterhouses of: 1) the proportion of positive *Salmonella*, 2) numbers of serotype, and 3) antimicrobial susceptibilities, were statistical analyzed by software SPSS version 17.0 (SPSS Inc, Chicago, USA), using Pearson's chi-square test.

Results and Discussion

There were 39.17% *Salmonella* spp. isolated from pig fecal samples (141/360), and 24.72% from pork (89/360). The isolates from pig fecal were significantly higher than from pork, indicating the proportion of positive *Salmonella* was decreased after the slaughtering process in this investigation. According to the results of the two slaughterhouses, the fecal isolates 30.56% (110/360) significantly higher than the pork isolates 6.39% (23/360) in the HACCP slaughterhouse ($P<0.05$); the fecal isolates 8.61% (31/360) significantly lower than the pork isolates 18.33% (66/360) in the PHP slaughterhouse ($P<0.05$) (Table 1). The proportion of positive *Salmonella* in the HACCP slaughterhouse (30.56% fecal isolates and 6.39% pork isolates) was significantly different with the proportion of positive *Salmonella* in PHP slaughterhouse (8.61% fecal isolates and 18.33% pork isolates) ($P<0.05$) revealing the HACCP slaughterhouse could reduce the proportion of positive *Salmonella* from pig fecal to pork, but the PHP slaughterhouse could increase the proportion of positive *Salmonella*.

The predominant serotypes were Enterica type I 1,4,[5],12:i:-, Rissen, Kedougou, Anatum, Weltevreden, and Bovismorbificans. The most one was Enterica type I 1,4,[5],12:i:-, at up to 42.17% (97/230). It was reported as one of the most frequent serotypes in EU (EFSA, 2017) and in Thailand (Hendriksen et al., 2009). This serotype causes 37.4% pig and 22% pork cases in 2017 EU (EFSA, 2018). However, the *S.* Enterica type I 1,4,[5],12:i:- contribute almost half of all isolates in this study, and it is much higher than the report in Chiang Mai (9%) (Patchanee et al., 2016), indicating the prevalence in Thailand is increasing fast. The others: Rissen, Kedougou, Anatum, and Weltevreden belonged to the top 10 common serotypes of patients in Thailand (Hendriksen et al., 2009). Besides, the *S.* Bovismorbificans exhibited an increasing prevalence, with outbreaks in many countries from 2014 to 2017 (Knoblauch et al., 2015; Brandwagt et al., 2018) revealing a dynamic main serotype of *Salmonella* in the world.

Furthermore, the numbers of serotype from HACCP slaughterhouse (9 serotypes from pigs and 3 from pork) were significantly different with the PHP slaughterhouse (10 serotypes from pigs and 14 from pork) ($P<0.05$). The HACCP slaughterhouse reduced the number of serotypes, but the PHP slaughterhouse increased the numbers of serotypes (Table 1). It indicates the HACCP could stop some *Salmonella* subtypes spreading, but the PHP contribute to more *Salmonella* subtypes spreading.

Table 1. The *Salmonella* spp. serotypes from two different hygiene level slaughterhouses.

Pig fecal isolates			
Serotypes	HACCP slaughterhouse	PHP slaughterhouse	Total rate of pig (Number, %)
S. Enterica type I 1,4,[5],12:i:-	55	3	58, 41.13%
S. Rissen	15	9	24, 17.02%
S. Kedougou	14	-	14, 9.93%
S. Anatum	-	6	6, 4.26%
S. Bovismorbificans	9	-	9, 6.38%
S. Brunei	-	1	1, 0.71%
S. Cannstatt	-	1	1, 0.71%
S. Derby	4	-	4, 2.84%
S. Krefeld	1	-	1, 0.71%
S. Lexington	-	1	1, 0.71%
S. Saintpaul	-	1	1, 0.71%
S. Stanley	-	4	4, 2.84%
S. Typhimurium	1	-	1, 0.71%
S. Weltevreden	-	3	3, 2.13%
S. Enterica 3,10:eh:-	-	2	2, 1.42%
S. Enterica 4,12:-:-	10	-	10, 7.09%
S. Enterica 4,5,12:-:1,2	1	-	1, 0.71%
total	110(9 serotypes)	31(10 serotypes)	141
Pork isolates			
Serotype	HACCP slaughterhouse	PHP slaughterhouse	Total rate of pork (Number, %)
S. Enterica type I 1,4,[5],12:i:-	14	25	39, 43.82%
S. Rissen	-	11	11, 12.36%
S. Aberdeen	-	2	2, 2.25%
S. Anatum	-	9	9, 10.11%
S. Bovismorbificans	1	2	3, 3.37%
S. Bredeneyi	-	1	1, 1.12%
S. Brunei	-	2	2, 2.25%
S. Cannstatt	-	2	2, 2.25%
S. Derby	-	1	1, 1.12%
S. Hvittingfoss	-	1	1, 1.12%
S. Krefeld	-	1	1, 1.12%
S. Stanley	-	1	1, 1.12%
S. Typhimurium	-	1	1, 1.12%
S. Weltevreden	-	7	7, 7.87%
S. Enterica 4,12:-:-	8	-	8, 8.99%
total	23(3 serotypes)	66(14 serotypes)	89

The positive pig feces source *Salmonella* spp. isolates from the HACCP slaughterhouse were resistant to AMP (76.36%), CHL (11.82%), STR (60.00%), SXT (17.27%) and TET (85.45%), while the isolates from pork were AMP (95.65%), S (95.65%) and TET (95.65%). From the PHP slaughterhouse, the pig feces source were resistant to AMP (61.29%), CHL (3.23%), STR (29.03%), SXT (38.71%) and TET (64.52%), while from the pork source were AMP (69.70%), CHL (1.52%), STR (45.45%), SXT (15.15%) and TET (69.70%) (Figure 1). Based on the results of antimicrobial susceptibility test, the ampicillin, streptomycin and tetracycline were the most common resistance antimicrobial agents of the 8 type agents. This shows the most common resistance is similar to the reports in Thailand (AMP, STR, and TET) (Chotinun et al., 2014; Patchanee et al., 2016). A proper policy is needed to reduce these three antimicrobials application in pigs. Additionally, the SXT need to be monitored, due to the increasing resistance ratio (38.17%) compared to the previous reports 15.40% in pork (Angkititrakul et al., 2005).

Then, the resistance ratio of 8 antimicrobial agents was statistically analyzed by Pearson's chi-square test. The CHL and SXT resistance rates of pig isolates significantly higher than pork, but the others antimicrobial agents were not significantly different between pig fecal and pork isolates ($p>0.05$) in HACCP slaughterhouse. It demonstrated the HACCP slaughterhouse could decrease some AMR spreading from farms to retails. In the PHP slaughterhouse: the S resistance rates of pig fecal isolates significantly lower than pork; the SXT resistance rates of pig fecal isolates significantly higher than pork; the others were not significantly different. It indicates that the PHP slaughterhouse posed no influence on AMR spreading from farms to retails.

The most frequent multiple drug resistance (MDR) pattern of total isolates was AMP-STR-TET, which was 51.30% of total positive isolates (118/230). The next submajor resistance pattern CHL-TET was 5.22% of total (12/230). The rest MDR pattern were 5.65%, including AMP-STR-SXT-TET, AMP-SXT, AMP-STR, AMP-CHL-TET, AMP-CHL-SXT-TET, and NA-STR. The most common MDR pattern AMP-STR-TET, revealed that these AMP, STR, and TET were highly applied to pig industry in Thailand (Chotinun et al., 2014; Patchanee et al., 2016). The MDR isolates (including AMP-STR-TET, AMP-SXT-TET, CHL-TET, and other multiple resistance isolates) was 73.44% (169/230) indicating a serious MDR *Salmonella* spp. condition in the pig industry (Figure 2).

Conclusions

The slaughtering process of food animals at slaughterhouses is reported as one of the important sources of meat product for AMR *Salmonella* spp. spreading (Gillespie et al. 2005). According to the results from this study, the important quantified evaluations were observed that the HACCP standard slaughterhouse could reduce the proportion of numbers of *Salmonella* serotype and some AMR from pigs to pork, but the poor hygiene practice (PHP)

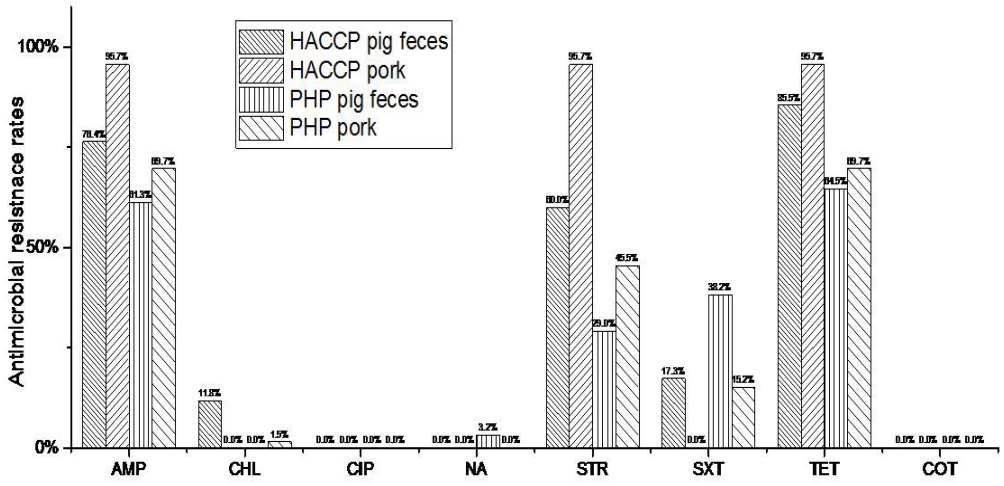


Figure 1. Antimicrobial resistance from the two hygiene level slaughterhouses.

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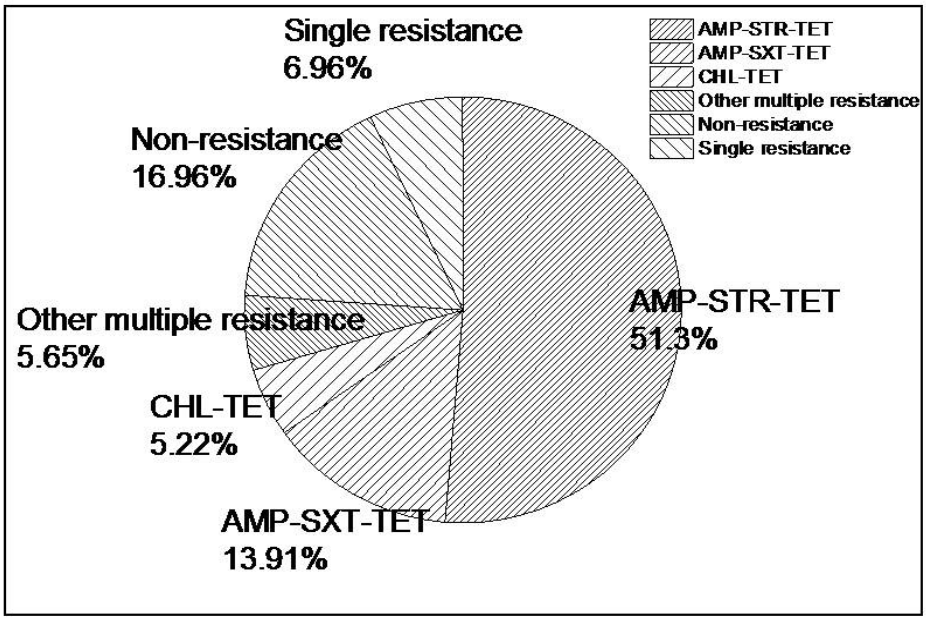


Figure 2. MDR patterns of *Salmonella* spp. from the two slaughterhouses.

slaughterhouse increased the proportion of positive *Salmonella* and numbers of serotypes. The PHP slaughterhouse pose a significantly higher risk of pork contaminated with *Salmonella* spp. than those produced from HACCP. The HACCP standard slaughterhouse is strongly advocated.

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