

## CHAPTER V

### DISCUSSION AND CONCLUSIONS

#### 5.1 Prevalence of *Campylobacter* spp.

The prevalence of *Campylobacter* spp. on chicken meat at retail in our study was 47.5 % (95% CI 38.31-56.81). This was comparable to a study in Thailand conducted in poultry meat at retail that showed a *Campylobacter* prevalence of 52% (Vindigni et al., 2007). In addition, other Asian countries such as Vietnam, Japan, Korea, Iran and Pakistan reported *Campylobacter* prevalence of 31%, 58.8%, 66.6%, 62.8% and 48.0% in poultry meats, respectively, which was also close with our result (Luu et al., 2006) (Suzuki and Yamamoto, 2009). In combination with the countries reported prevalences, Asia has 60.3% *Campylobacter* prevalence in poultry meats at retail. This is similar with countries in North America and Western Europe where they reported an average prevalence rate of 63.8 % and 56%, respectively. However, in countries in Middle and South America, Oceania and Africa, 82.3%, 90.4% and 73.1% were reported average prevalence rate of *Campylobacter* in poultry meats at retail, respectively. In contrast, countries in Former Soviet Union and Eastern Europe reported an average of 19.1% prevalence rate (Suzuki and Yamamoto, 2009). The determined prevalence of *Campylobacter* in chicken meat at retail in this study was, so far to our knowledge, the first reported in the Philippines.

As reported by Suzuki and Yamamoto (2009), a majority of retail poultry meats and by-products in most of the countries in the world were contaminated with *Campylobacter* spp. *C. jejuni* was frequently the dominant *Campylobacter* species isolated from retail poultry and *C. coli* was less frequently isolated, even though the ratio of *C. coli* to *C. jejuni* was significantly different among the countries. Nonetheless, *Campylobacter coli* was the most commonly determined *Campylobacter*

species in this study which was similar with the previous study in the Philippines (Magistrado et al., 2001). In Thailand, *C. coli* was also the leading *Campylobacter* species isolated from retail poultry where they determined 54.4% compared to 32.3% *C. jejuni* and 14.2% other *Campylobacter* species (Meeyam et al., 2004, Padungtod and Kaneene, 2005, Tangvacharin et al., 2005). South Africa also published in a study that *C. coli* was the major *Campylobacter* species identified at retail poultry which has 37.5% compared to 28.1% *C. jejuni* and 34.4% other *Campylobacter* species (van Nierop et al., 2005). On the other hand, other countries in the world reported that *C. jejuni* was the most commonly isolated *Campylobacter* in retail poultry. (Suzuki and Yamamoto, 2009).

## **5.2 Multiple comparisons of *Campylobacter* spp. prevalences among the four local wet markets**

There was a significant difference in the prevalence of *Campylobacter* spp. isolated between Cabanatuan, having the highest, and Guimba, having the lowest. Cabanatuan wet market, being the capital of the province of Nueva Ecija, is 5.5 times (P-value=0.005) more likely to have *Campylobacter* spp. contamination in chicken breast skin compared to Guimba. Moreover, non-overlapping in 95% CI prevalence between Cabanatuan and Guimba indicates that there is significant difference between the two areas as presented in Table 7. This could be probably due to various sources of chicken meat supplying the retail chain in Cabanatuan which increases the chance of greater risk of contamination compared with the others which might have less sources of suppliers. Nonetheless, further investigation is needed to validate this observation and identify the possible risk factors that could contribute to the significant difference of higher number of contaminated chicken with *Campylobacter* spp. at retail in Cabanatuan compared to Guimba. This may include the sources of chicken and the practices at wet markets such as storage, mixing of meats, among others. No significant difference was observed between Guimba and the wet markets in the two other areas.

### **5.3 Polymerase Chain Reaction (PCR) method for *C. jejuni* and *C. coli* confirmation, identification and differentiation**

Though presence of *Campylobacter* spp. was determined and confirmed by standard conventional microbiological method according to 9.4 of ISO 10272-1:2005(E), further test to confirm was performed by PCR of *hipO* gene, which is specific for *Campylobacter jejuni* at 323 bp amplicon size and of *glyA* gene for *Campylobacter coli* at 126 bp amplicon size. PCR method was proven to be reliable. Targeting also the said genes were suitable to verify, identify and differentiate the two *Campylobacter* species. Although, the standard hippurate hydrolysis test was mentioned under 9.5 ISO 10272-1:2005(E) to use for species identification between *C. jejuni* and *C. coli*, it was reported in various studies that this test is not always consistent (Botteldoorn et al., 2008, Ronner and Lindmark, 2007). The PCR method was found to be more sensitive, accurate and rapid than the conventional microbiological culture method according to various studies (Boonmar et al., 2007, Botteldoorn et al., 2008, Meeyam et al., 2004).

### **5.4 Quantitative load of *Campylobacter* spp. in chicken meat at retail**

With the 53% positive samples having high contamination of *Campylobacter* on the chicken skin (MPN =  $\infty$ ; lower confidence limit  $T_0$  580/g) no data in the Philippines is available for comparison. Nonetheless, in Thailand they reported 13.3 % from positive samples having non countable *Campylobacter* in broiler skin from slaughterhouses (Chokboonmongkol et al., 2013). Moreover, the study results of Chokboonmongkol et al. (2013) suggests cross-contamination during the slaughter process increases *Campylobacter* prevalence on broiler skin compared with the prevalence in broiler ceca. In addition, other studies in Thailand suggested the likelihood of transmission of *Campylobacter* spp. from the environment particularly of non-poultry sources to poultry, mainly at markets (Meeyam et al., 2004, Padungtod and Kaneene, 2005). Such prevalence of *Campylobacter* spp. contamination in chicken meat at retail determined in this study might also due to these reasons. Hence, we suggests the implementation of good and efficient

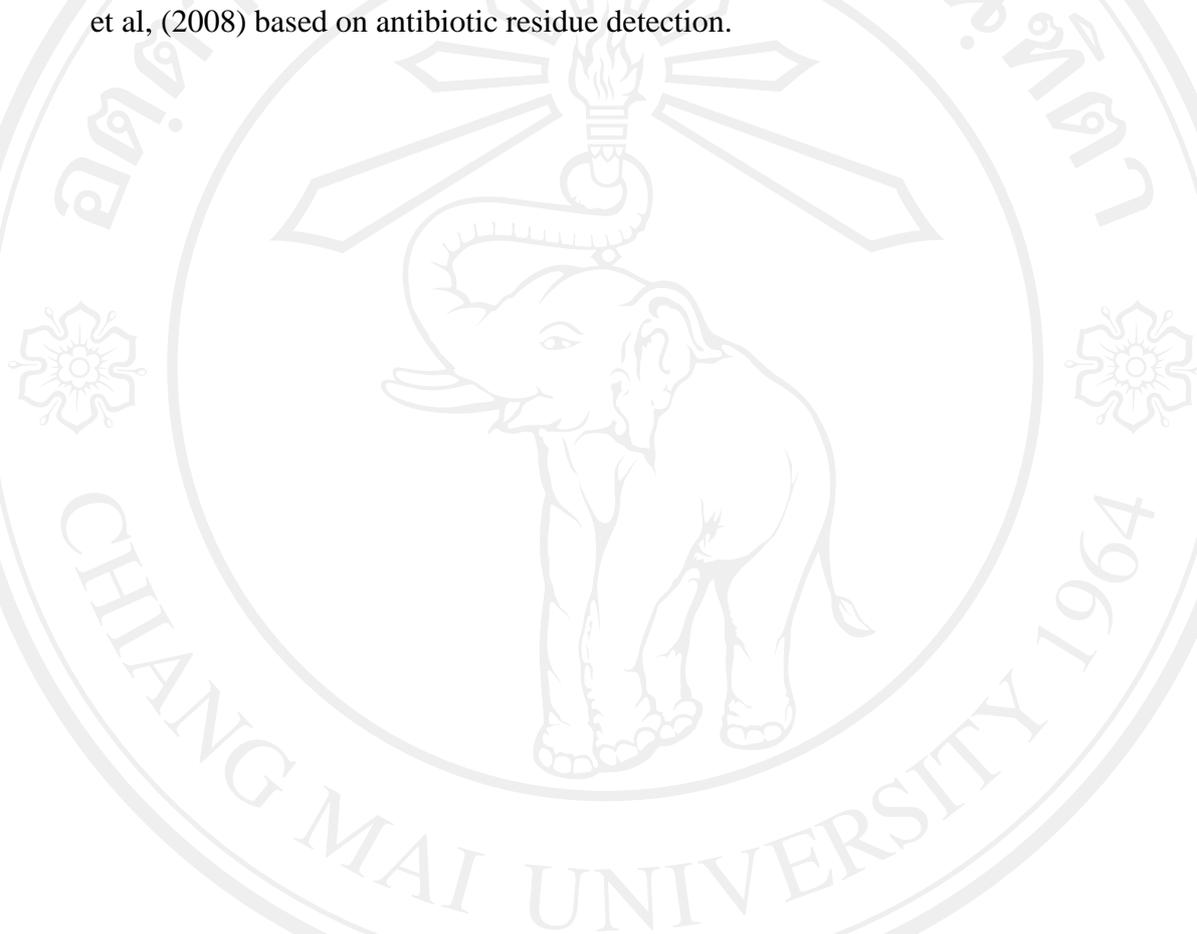
intervention measures at slaughterhouses to minimize fecal contamination of broiler skin and reduce cross-contamination. Furthermore, studies should be conducted to assess the risk factors of *Campylobacter* spp. contamination in the broiler production chain focusing more on local conditions. Awareness should be raised for the sanitary handling of poultry meat due to high *Campylobacter* number on breast skin of fresh chicken meat.

### 5.5. Antimicrobial resistance pattern

On the specific percentages of *Campylobacter* isolates resistance to the 5 antimicrobial agents (Figure 7), high percentage was observed to ampicillin (77.27%) followed by ciprofloxacin (70.45%) and tetracycline (54.55%) and low percentage to erythromycin and gentamicin was observed with only 20.25% and 11.36%, respectively. This was comparable to a study conducted in the Philippines except for gentamicin where they determined 91.7% (Baldrias and Raymundo, 2009). Similarly, Ibrahim et al, (2004) reported a constant increase in antimicrobial resistance to quinolones, ampicillin and tetracycline. However, in Thailand, comparably similar result was observed for susceptibility to gentamicin: 89.64 % in this study and 100% for Chokboonmongkol et al, (2013). Consistent high resistance of ampicillin, ciprofloxacin and tetracycline in this study was supported by Baldrias et al, (2008) where they determined, by Four Plate Test, that penicillin, florquinolones and tetracycline were the most commonly detected antibiotic residues from chickens. The same with the low resistance to erythromycin by *Campylobacter* isolates which was observed by Baldrias et al, (2008) as the least commonly detected among the antibiotic residues. Likewise, for the isolates tested, most common combination of multidrug resistance was to ampicillin, tetracycline, and ciprofloxacin (38.64%). This is similar to a study in Thailand where they also tested the same 5 antimicrobial drugs and get the combination of multidrug resistance to ampicillin, tetracycline, and ciprofloxacin being the highest (Chokboonmongkol et al., 2013).

Indeed, a dramatic increase in the number of resistant *Campylobacter* to quinolone, ampicillin, and tetracycline was reported from various areas of the world.

Multidrug-resistant *Campylobacter* isolates also were widespread as observed in the Philippines by Baldrias and Raymundo (2009) and other investigators from other countries (Chokboonmongkol et al., 2013, Ibrahim et al., 2004, Gaudreau and Giblert, 2003, Selaha, 2002). Moreover, resistance of *Campylobacter* isolates were probably related to antimicrobial usage in poultry production which was observed by Baldrias et al, (2008) based on antibiotic residue detection.



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