CHAPTER 5

DISCUSSION

A total of 271 (65.3%) samples were recovered from 415 stock isolates. There are three factors that may affect the recovery rates of *Campylobacter spp.* from stock isolates. 1) The storage duration of the isolates in the freezer may affect recovery. We noted, for in stance, that the recovery rates of 2002, 2001, and 2000 were 93.10%, 74.30%, and 40.56% respectively. These result suggest that long storage of *Campylobacter* freezer decreases the recovery rate of *Campylobacter spp.* 2) The proportion of glycerol in Bolton broth may affect the preservation of the organism cells, inappropriate amount of glycerol may reduce the number of *Campylobacter* that can survive. 3) There are many technical errors in laboratories such as technicians, temperature that could have happened.

Either template preparation or $MgCl_2$ concentration were similar to the reference protocol. The 23S rRNA amplicons were found in 91.14% of the samples. However, 8.86% from these samples did not give the other species-specific primers of *C.jejuni, C.coli, C.lari, C.upsaliensis*, and *C.fetus* subsp.*fetus*, which may indicate that these might be other *Campylobacter spp.*, *Arcobacter spp.* or *Helicobacter spp.* These 8.86% were not successfully amplified with 23S rRNA primers, which have positive results with bacteriological methods. The reason for this discrepency may be

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due to contamination during the isolation or the recovering stage. Moreover, the number of *Campylobacter* in the templates might have been a problem because the detectability level of this assay were reported to be 10^8 to 10^{13} for *C.jejuni*, 10^6 to 10^{13} for *C.coli* and *C.upsaliensis*, 10^7 to 10^{10} for *C.lari*, and 10^2 to 10^{13} for *C.fetus* subsp.*fetus*. Although the concentration of the template was estimated by colorimeter, the amount of bacteria may still not be exactly known.

Many studies described comparing molecular methods such as PCR with conventional method (biochemical tests). All of them found the higher sensitivity of PCR assay such as 10^3 cfu/g of human faeces (Misawa, et al.,2002), 1-5 cfu (Fermer and Engvall,1999), 2-3 cfu/ml or 0.01 pg/PCR in environmental sample (Bang, et al.,2002) and 10^2 cells of crude lysate, 10^3 cells in seeded faeces and 120 cells/ml of washing 1cm^2 of chicken skin (Korolik, et al.,2001).

To differentiate thermophilic *Campylobacter*, multiplex PCR assay were widely used for its convenience and efficiency (Denis, et al.,1999) , (Cloak and Reatamico,2002) , (Korolik, et al.,2001). Additionally, some techniques combined the PCR assay with another technique in order to improve their sensitivity and specificity. Examples of these include the filtration-enrichment culture (Misawa, et al.,2002), RFLP (Steinhauserova, et al.,2001) and (Fermer and Engvall,1999) or Nested PCR (Bang, et al.,2002). Moreover, PFGE typing was reported to be useful for identify epidemiology association in several small outbreaks (Hanninen, et al.,1998).

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The prevalence from this study showed that overall *C.coli* was the majority species of *Campylobacter* (46.49%) from every source. At the farm, the most common species was *C.jejuni* (42.53%), *C.jejuni* were identified 42.53% from chicken, which was the majority species similar to other studies that found *C.jejuni* 14.5% (Chattopadhyay, et al.,2001), 81%(Saenz, et al.,2000).

The contamination of chicken at the farm caused by environment sources and the hygiene measures of farmers can reduce the transmission from farm environment (Giessen, et al.,1998). Risk factors associated with chicken contamination with *Campylobacter spp.* were described. These included: the seasonal effect, the house with static air distribution, two or more people taking care of the flock, in poultry farm with three or more houses, and if the acidified drinking water for chicken (Refregier-Petton, et al.,2001).

Unlike the farm prevalence, the slaughterhouse and the market were mainly contaminated with *C.coli* (72.41% and 54.41%, respectively). From these results, the transmission of *Campylobacter*-contaminated chicken meat to the consumer may not all be associated with the chicken at farms. Many evidence mentioned the contamination of chicken during the processing from the environment and cross contamination between batches at the slaughterhouse. (Newell, et al.,2001) and (Rivoal, et al.,1999)

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Antimicrobial resistance profiles of *C.jejuni*, *C.coli* and other *Campylobacter* were similar for 4 antimicrobials that showed the highest level of resistance (ceftiofur, trimethoprim-sulfamethoxazole, nalidixic acid, and cephalothin).

The highest level of resistance in *Campylobacter jejuni* was to cephalothin 96.67%. However, resistance levels were high to the other three: ceftiofur was also as high as 92.85% and trimethoprim-sulfamethoxazole and nalidixic acid were 76.67% and 71.91 %, respectively. Other studies found *C.jejuni* were resistant to ciprofloxacin (25%) amipicillin (25%), and gentamicin (50%)(Cloak and Reatamico,2002). All *C.coli* were resistance to cephalothin (100%), which was higher than ceftiofur 83.78%, trimethoprim-sulfamethoxazole 86.67% and nalidixic acid 86.99%. These results were differ from reported by others where erythromycin was the highest rate resistant to *C.coli* (81.1%), followed by ampicillin (65.7%), gentamicin (22.2%) and amikacin (21.6%) (Saenz, et al.,2000). Additionally, a high frequency of quinoione-resistance was found in both *C.jejuni* and *C.coli* in our study which is in contrast to the work reported by (Chuma, et al.,2001) where high level of erythromycin resistance was found in only *C.coli* .

Moreover, the result of resistant to beta-lactam antbiotics showed the low prevalence of ampicillin resistance *Campylobacter*, which was found 7.69% of *C.jejuni* and 3.23% of *C.coli*. While the other antibiotic in the same group, cephalothin, was much higher rate resistant to both *C.jejuni* (96.67%) and *C.coli* (100%). There is the evidence that identified *CmeABC* of *Campylobacter*, which their function is as a tripartite multidrug efflux pump, that contributes to the intrinsic

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resistance of *C.jejuni* to a broad range of structurally unrelated antimicrobial. Consequently, the MICs of two beta-lactams were decreased 256-fold for cefotaxime and 32-fold for ampicillin (Lin, et al.,2002). From these finding, further research should investigate the relation of these results with the efflux pump mechanism of *Cme* ABC function.

From our study, the top 4 antimicrobial agents to which *C.jejuni* and *C.coli* showed resistance are similar. These finding are similar to those reported in a study by Isenbarger et al.(2001), *C.jejuni* and *C.coli* from humans had the same highest antimicrobial resistance.

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Conclusions

1. *C.coli* and *C.jejuni* are prevalent at farm, slaughterhouse, and market, but *C.coli* are more prevalence than *C.jejuni.*
2. *C.coli* are also found to be more prevalent than *C.jejuni* in human samples.
3. Antimicrobial resistance was demonstrated to all 10 antimicrobial agents in both *C.coli* and *C.jejuni*, which would suggest a widespread problem
4. The highest prevalence of resistance was observed with cephalothin, nalidixic acid, ceftiofur, and trimethoprim-sulfamethoxazole.

Area for further research

1. The reason why *C.coli* is more prevalence than *C.jejuni* in poultry in Thailand needs further investigation, since this is in contrast to what has been reported previously.
2. Direct comparisons of antimicrobial resistance patterns in *Campylobacter spp.* isolated from animals and humans working with animals or animal products need further investigation.

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