# CHAPTER 4

# RESULTS

## 4.1 Recovered *Campylobacter* isolates

A total number of 415 were isolated from 849 collected samples and inoculated onto Brucella agar supplemented with 5% sheep blood. Only unrecovered isolates from the first attempt were resuscitated in Bolton broth before inoculating onto the same medium. After second passage, these cultures were confirmed with colony morphology and gram stain. Finally, 271 of the 415 (65.03%) of samples were recovered.

## 4.2 Optimization of the assay

The reference strain of *Campylobacter jejuni* 33560 was used for optimization of the assay. Two methods of DNA template preparations were compared: whole cell procedure and phenol-chloroform extraction. Both methods produced the amplified products, however, the whole cell procedure gave a better result in the initial trial.

Magnesium chloride concentration used varied from 10, 15, 20, 25, 30, 35 mM. However, 20 mM Magnesium chloride was used because this concentration gave the best amplification efficiency. The concentration of DNA templates was also optimized. The dilution of 1:1, 1:10, 1:50, 1:100, 1:500, 1:1,000 of 0.5 McFarland whole cell suspensions were compared. The most optimal dilution was 1:1.

**4.3 The prevalence of *Campylobacter spp.***

Of the 271 samples, the prevalence of *Campylobacter spp.* in Northern Thailand was found to be *C.coli* (46.49%), *C.jejuni* (35.79%) and other *Campylobacter* (8.86%). Some sample (8.86%), which were confirmed as positive with microbiological method, did not give the 23s rRNA amplified products.

The prevalence of *Campylobacter* at the farm showed that *C.jejuni* was the major species (42.53%) compared to *C.coli,* which was found at 39.08% and some other *Campylobacter spp.* (8.05%). In contrast, the prevalence at the slaughterhouse was found to be *C.coli* predominantly (72.41%), which was much higher than *C.jejuni* (17.24%) and other *Campylobacter* (3.45%). Similar to the market, the most prevalent species was *C.coli* (54.41%) while *C.jejuni* prevalence was 26.47% and other *Campylobacter* 13.24%.(Figure 4.1)

Moreover, the prevalence of *Campylobacter spp.* from farm worker isolates were found to be *C.coli* predominantly (75%), while *C.jejuni* was found only for one isolate (25%).

**4.4 Antimicrobial resistance of *Campylobacter spp.***

Ten antimicobial agents were tested with *Campylobacters* samples. The results indicated the 4 top antimicrobial agents that showed resistance were: ceeftiofur, trimethoprim-sulfamethoxazole, nalidixic acid, and cephalothin. *Campylobacter jejuni* was resistance to all 10 antimicrobial agents tested; the highest rate of resistance was cephalothin 96.67%,which is the natural characteristic of most *Campylobacter*. Alike resistance ceftiofur was also as high as 92.85%, while trimethoprim-sulfamethoxazole and nalidixic acid were 76.67 and 71.91%, respectively. The other antimicrobial agents that showed resistance were tetracycline 44.32%, streptomycin 6.67%, florfenicol 4.45%, ampicillin 7.69%, clindamycin 4.5% and erythromycin 6.8%. All *C.coli* isolates were resistance to cephalothin (100%) which was higher than ceftiofur 83.78%, trimethoprim-sulfamethoxazole 86.67%, and nalidixic acid 86.99%. Other *Campylobacter* were found to be resistance to 6 antimicrobial agents. The highest rates of resistance observed were to trimethoprim-sulfamethoxazole (83.33%) and cephalothin (83.33%). While ceftiofur resistance was found to be 75% and nalidixic acid 69.33%. Other *Campylobacter* were also resistant to tetracycline (34.62%) and erythromycin(10%).

At the farm level *C.coli* were resistant to more agents than *C.jejuni* (P=0.138), *C.coli* were resistant to 3 (48.98%) and 2 (34.69%) antimicobial agents, while 31.82% and 43.18% of *C.jejuni* were resistant to 3 and 2 antimicrobial agents, respectively(Figure 3.3). Besides, 33.33% and 22.22% of other *Campylobacter* were

resistant to 2 and 3 antimicrobial. There were a small number of *Campylobacter* from farm-level that showed had a multiresistance to 1 to 6 antimicrobial agents.

At the slaughterhouse, only resistance to 1 to 3 agents were found. More *C.jejuni* were resistant to more agents than *C.coli* (p=0.196) Most of *C.coli* (70.59%) were resistant to 2 antimicrobial. Whereas *C.jejuni* had a high resistance with 3 (55.56%) and 2 (44.44%). Sixty percentage of other *Campylobacter* were resistance to 2 and 40% to 3 antimicrobial agents. At the market, most *Campylobacter* were found to be resistant to 2 anitmicrobial agents (p=0.019); *C.coli* 76.19% and *C.jejuni* 60%. All of other *Campylobacter* were found resistant to 1 antimicrobial agent (100%).

All of human samples were resistance to cephalothin (100%). trimethoprim-sulfamethoxazole was also showed a high rate of resistance (66.67%). Other antimicrobial agents to which *Campylobacter* isolates from human showed resistance were ampicillin (33.33%) and erythromycin (33.33%).

**Table 4.1** The number of recovered Campylobacter isolates that were tested.

| Source | Sample type | 2000 | 2001 | 2002 | Total |
|---|---|---|---|---|---|
| Farm | Cloacal swab | 42/97 | 76/126 | 38/41 | 156/264 |
| | Environment | -/- | -/1 | 14/14 | 14/15 |
| | Worker | -/- | 2/2 | 2/3 | 4/5 |
| Slaughterhouse | Cloacal and Carcass swab | 16/46 | 52/53 | -/- | 68/99 |
| Market | Meat | -/- | 29/32 | -/- | 29/32 |
| | Total | 58/143 (40.56%) | 159/214 (74.23%) | 54/58 (93.10%) | 271/415 (65.30%) |

**Table 4.2** The number of *Campylobacter spp.* from each source

| Source | N* | Total | CC | CJ | Other | OR (CC:CJ) | p-value |
|---|---|---|---|---|---|---|---|
| farm | 425 | 156 | 68 | 74 | 14 | 0.85 | 0.57 |
| market | 72 | 27 | 21 | 5 | 1 | 15.40 | <0.01 |
| slaughter | 352 | 64 | 37 | 18 | 9 | 3.50 | <0.01 |
| Grand Total | 849 | 247 | 126 | 97 | 24 | 1.93 | <0.01 |

N* = The number of total collected samples

**Table 4.3** Percentage of Antimicrobial resistance to *Campylobacter.spp.*

| Antimicrobilal agents | *C.jejuni* | *C.coli* | Others *Campylobaceter* | N* |
|---|---|---|---|---|
| streptomycin | 6.67 | 0 | 0 | 69 |
| florfenicol | 4.55 | 0 | 0 | 54 |
| ampicillin | 7.69 | 3.23 | 0 | 123 |
| clindamycin | 4.55 | 3.74 | 0 | 212 |
| erythromycin | 6.06 | 4.67 | 10 | 212 |
| tetracycline | 44.32 | 46.67 | 34.62 | 266 |
| ceftiofur | 92.45 | 83.78 | 75 | 123 |
| trimethoprim-sulfamethoxazole | 76.67 | 86.67 | 83.33 | 69 |
| nalidixic acid | 71.91 | 86.99 | 69.23 | 266 |
| cephalothin | 96.67 | 100 | 83.33 | 69 |

N*= number of tested samples

**Figure 4.1** Proportion of *Campylobacter spp* at each source

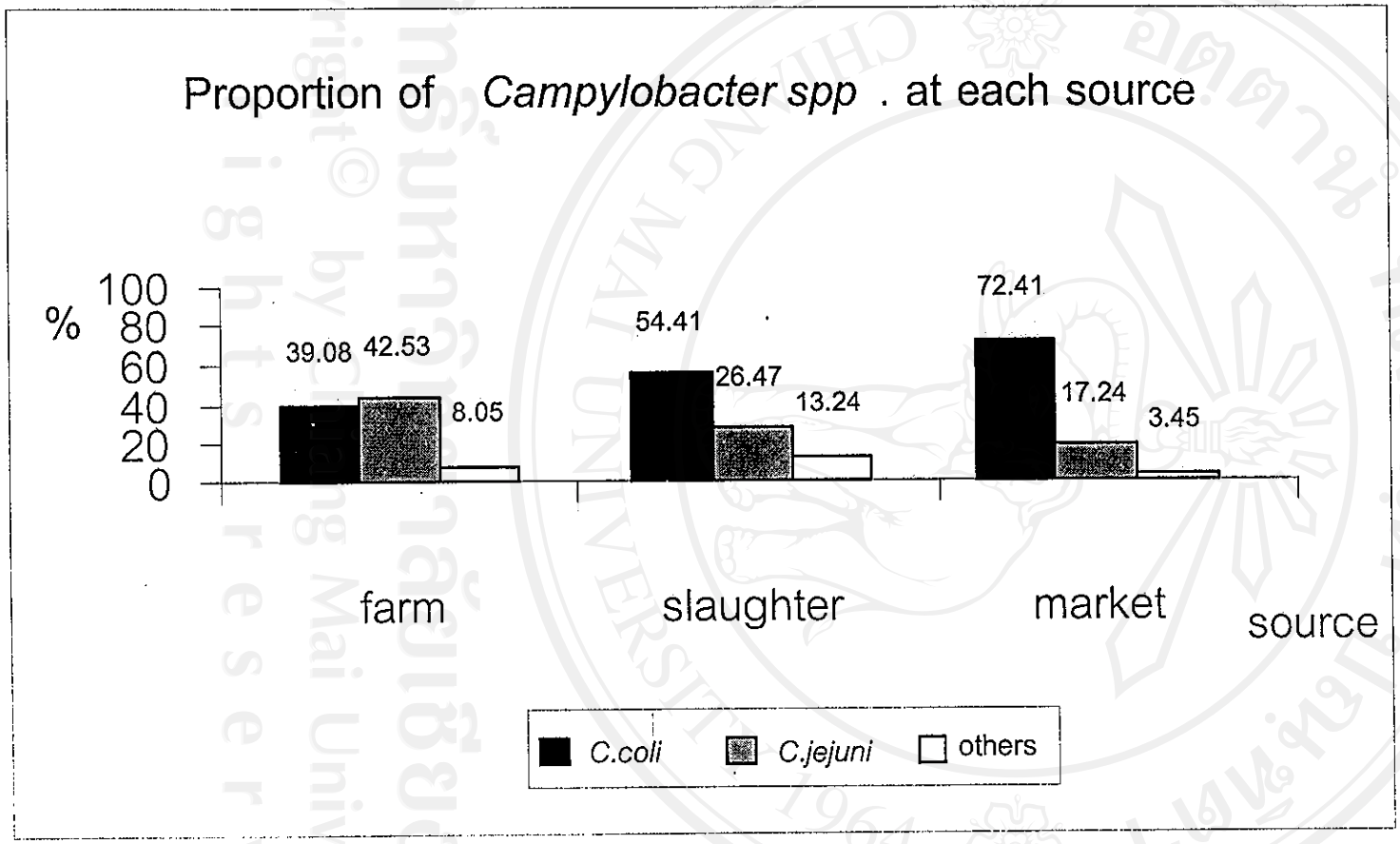
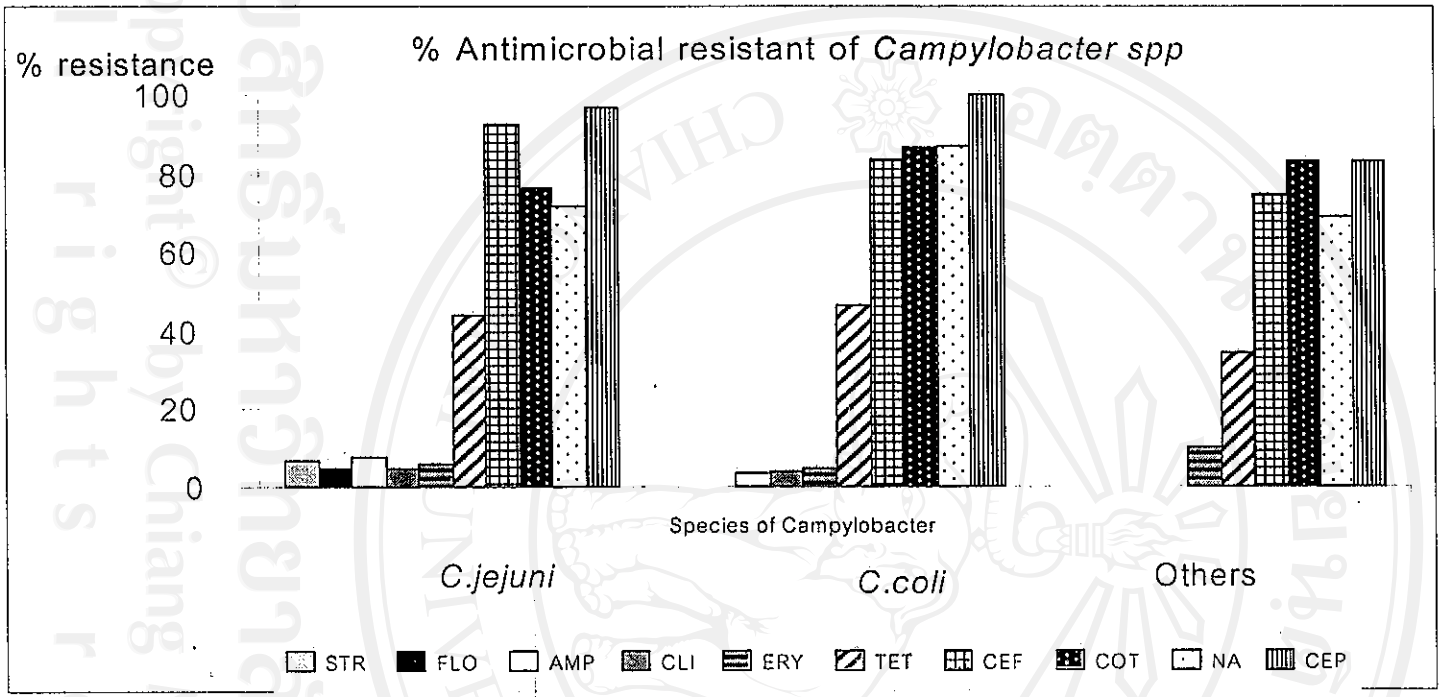


**Figure 4.2** Percentage of *Campylobacter spp*. resistance to antimicrobial agents




STR =streptomycin, FLO = florfenicol, AMP = ampicillin, CLI = clindanycin, ERY=erythromycin, TET = tetracycline, CEF = ceftiofur, COT= trimethoprim-sulfamethoxazole, NA = nalidixic acid, CEP = Cephalothin

**Figure4.3** Percentage of *Campylobacter* isolates resistant to more than one antimicrobial agent at each source