CHAPTER IV

RESULTS

4.1 *Campylobacter* spp. prevalence in Nueva Ecija, Philippines

Using both standard culture method (ISO, 2010) and multiplex-polymerase chain reaction, 57 out of the 120 samples tested were confirmed to be positive for *Campylobacter* spp. The estimated prevalence was 47.5 % (95 % CI: 38.66-56.72). Out of the 57 positive samples, 54.39% (n=31) were identified to be *C. coli* and 45.61% (n=26) to be *C. jejuni* (Table 6).

Table 6 Distribution of *Campylobacter* spp. in positive chicken breast skin at retail

No. of positive samples	No. of *C. jejuni* isolates (%)	No. of *C. coli* isolates (%)
57	26 (45.61%)	31 (54.39%)

Among the four local wet markets, the highest prevalence was recorded in Cabanatuan with 66.7%, followed by Gapan 50.0%, San Jose 47.7% and Guimba 26.7%, respectively (Table 7).

**Table 7** Prevalence of *Campylobacter* spp. in chicken breast skin collected at four wet markets in Nueva Ecija

Location	N	Positive	Prev %	95% CI	
Cabanatuan	30	20	66.7	47.18	82.71
Gapan	30	15	50.0	31.29	68.70
San Jose	30	14	46.7	28.34	65.67
Guimba	30	8	26.7	12.27	45.88
TOTAL	120	57	47.5	38.31	56.81

Among the four local wet markets, the highest ratio was determined in Cabanatuan with 35.09 %, followed by Gapan 26.32%, San Jose 24.56% and Guimba 14.04%, respectively (Table 8).

Table 8 Ratio of *Campylobacter* spp. isolated in four local wet market

Wet market location	*Campylobacter* (%)	95% CI	
Cabanatuan	31.5%	22.91	48.86
Gapan	26.3%	15.54	39.66
San Jose	24.1%	14.13	37.76
Guimba	14.0%	6.26	25.79

Figure 5 is a gel documentation of multiplex PCR assay products from isolated *Campylobacter* spp. from breast skin of fresh chicken meat at retail market in Nueva Ecija, Philippines. Isolates were identified and differentiated into *Campylobacter jejuni* and *Campylobacter coli* by showing the expected amplification products.
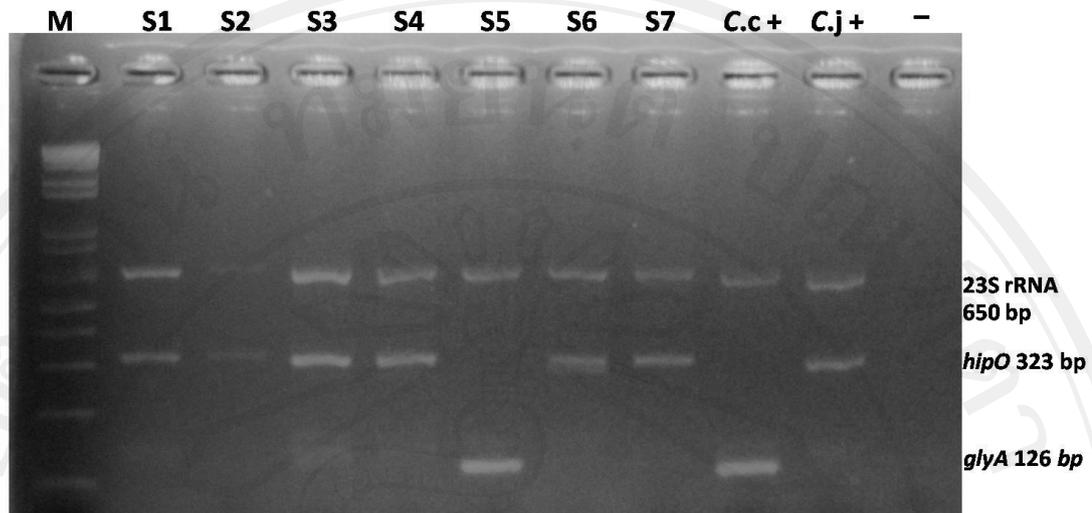
(M)=1kb marker; (S1-S7)=Samples; (C.*c*+)=*Campylobacter coli* positive control; (C.*j*.+) *Campylobacter jejuni* positive control; *(hipO* 323 bp)=323 bp fragment of *hipO* gene; (*glyA* 126 bp)=126 bp fragment of *glyA* gene(23s rRNA)= 650-bp fragment of 23S rRNA (which occurred in all *Campylobacter* spp.*Arcobacter* and *Helicobacter*.

Figure 5 Gel documentation of multiplex PCR assay products from set of isolates.

4.2 Multiple comparisons of *Campylobacter* spp. prevalences among the four local wet markets in Nueva Ecija, Philippines

Using Guimba as reference to compare the prevalence of *Campylobacter* spp. in the different wet markets, Table 9 shows that there was a significant difference in the prevalence between Guimba and Cabanatuan (OR=5.5; p=0.005).

Table 9 Likelihood-ratio test comparing the prevalence of *Campylobacter* spp. in chicken breast skin collected at four wet markets in Nueva Ecija

Location	Prev %	Odds Ratio	95% CI		P
Guimba*	26.7	1	-	-	-
Cabanatuan	66.7	5.5	1.813	16.681	0.005
Gapan	50.0	2.75	0.933	8.100	0.184
San Jose	46.7	2.41	0.816	7.095	0.319

*Used as reference for comparison

4.3 Semi-quantification of *Campylobacter* spp. in Nueva Ecija, Philippines

Almost 53% of the samples tested positive for *Campylobacter* spp. had a contamination of MPN = ∞ (LCL 580/g) (ISO, 2011). Semi-quantification results on *Campylobacter* spp. on chicken meat at retail is presented in the figure below (Figure 6).
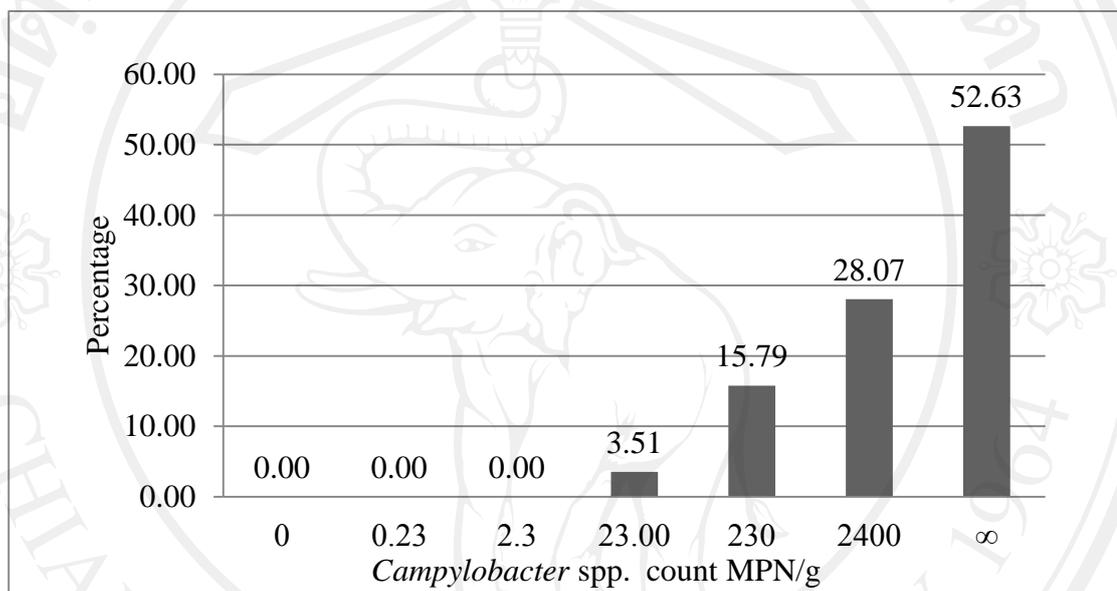

Figure 6 Percentage of *Campylobacter* spp. contamination on chicken meat at retail

4.4 Antimicrobial resistance pattern of *Campyobacter* spp.isolates

With regards to degree of resistance to five antibiotics, out of the 57 isolated *Campylobacter* spp., 44 isolates were tested. Of these 77.27% were resistant to ampicillin, followed by ciprofloxacin 70.45%, tetracycline 54.55%, erythromycin 20.25% and gentamicin 11.36%, respectively.
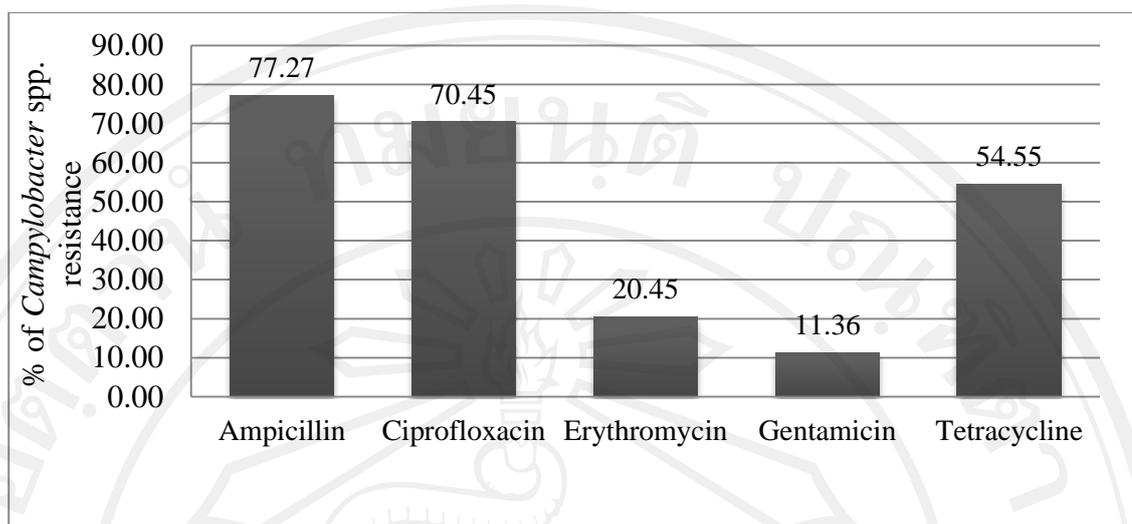
Figure 7 Percentage of *Campylobacter* spp. resistant to antimicrobial drugs

Moreover, 81.82% (n=36) of the isolates were resistant to atleast one antimicrobial agent. For two antimicrobial drugs, there were 13.64% (n=6), 38.64% (n=17) for three drugs, 13.64% for four drugs and 6.89% (n=3) were resistant to all five antimicrobial drugs. Overall, 8 different patterns were observed. Below is a table showing the pattern most frequently observed (Table 10).

Table 10 Most frequent multi-drug resistance pattern of *Campylobacter* spp. isolates

No. of Antimicrobial drug	Most frequent pattern	n
Two	Amp+Cip	6
Three	Amp+Cip+Tet	15
Four	Amp+Cip+Ery+Tet	4
Five	All Antimicrobial drugs	3

Note: Amp=Ampicillin; Cip=Ciprofloxacin; Tet=Tetracycline; Ery=Erythromycin.

The figure below shows the number of *Campylobacter* resistant to specific antimicrobial agent according to species. Most of the *C. jejuni and C. coli* isolates showed highest number resistant to ampicillin followed by ciprofloxacin and tetracycline. Low resistance to erythromycin and gentamicin by *C. jejuni* and *C. coli* isolates was also observed.
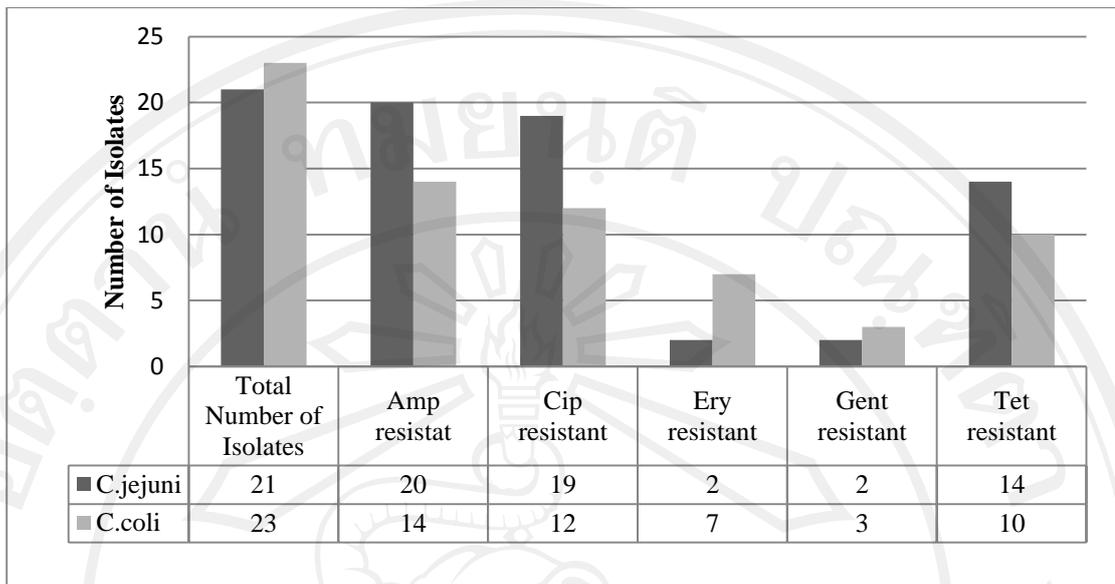
Amp=Ampicillin; Cip=Ciprofloxacin; Ery=Erythromycin; Tet=Tetracycline; Gent=Gentamicin.

Figure 8 Number of *Campylobacter* resistant to specific antimicrobial agent according to species