Occurrence of multidrug resistant (MDR) *Campylobacter* species isolated from retail chicken meats in Selangor, Malaysia and their associated risk factors

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Received 6 September 2017; Received in revised form 20 December 2017; Accepted 15 February 2018

ABSTRACT

**Aims:** *Campylobacter* infection is one of the leading bacterial food-borne illness and most frequently reported in humans in developed countries. This study was designed to determine the prevalence of multidrug resistant (MDR) *Campylobacter* and the risk factors associated with their occurrence in broiler chicken meat retailed in markets.

**Methodology and results:** A total of 210 samples consisting of 140 chicken meat and 70 swabs from weighing scales and cutting boards were collected. Isolates were cultured by passive filtration method, identified by biochemical tests and confirmed using PCR assay. Thirty-two (32/210) 15.2% were positive for *Campylobacter* of which (25/210) 11.9%, (6/210) 2.9% and (1/210) 0.5% were *Campylobacter jejuni*, *C. coli* and *C. upsaliensis* respectively. The isolates showed high resistance to ampicillin (62.5%), enrofloxacin (56.3%) and nalidixic acid (50.0%), while only 3.1% were resistant to streptomycin. Multidrug resistant isolates (resistance to at least one antibiotics in three classes or more) was high at 71.9%. The risk factors significantly (p<0.05) associated with *Campylobacter* contamination on chicken’s meat included poor workers hygiene (OR: 5.250 (95% CI: 0.988-27.895)), wearing improper work attire (OR: 2.700 (95% CI: 1.144-6.374)), poor protective equipment (OR 38.50 (95% CI: 2.915-508.463)), poor environment/stall hygiene (OR 44.00 (95% CI: 2.193-882.66)), and using tiled counter top surface (OR 6.667 (95% CI: 0.597-74.506)).

**Conclusion, significance and impact of study:** The finding of this study affirmed that lack or poor work hygiene, unclean environmental stall and protective equipment are associated with high occurrence of multidrug resistant *Campylobacter* species isolated from chicken meat

**Keywords:** *Campylobacter*, chicken, multidrug resistance, risk factors

INTRODUCTION

*Campylobacter* is one of the leading causes of foodborne diarrhea illness in the developed countries and a significant public health concern worldwide (Goni et al., 2017). In humans, infections caused by *Campylobacter jejuni* and *C. coli* are well known and transmission is generally through water, milk, and food animals (Huang et al., 2009). Retail meat products, particularly poultry meat, have been frequently implicated as sources of infection, and are considered a major risk factor for Campylobacteriosis in humans (Meldrum and Wilson, 2007). The pathogenicity and survivability of *Campylobacter* strains is enhanced by the expression of the antibiotic resistance characteristics and distinct virulence determinants (Young et al., 2007; Luo et al., 2005).

In humans, poultry meat is considered the principal source of *Campylobacter* infection, with infection acquired through fecal-oral route. In the United Kingdom, Food Standards Agency (FSA) (EFSA, 2009) reported a 62.5% prevalence of *C.* in poultry meat. Similarly, several studies
have reported the occurrence of C. jejuni in poultry and chickens (Hussain et al., 2007; Stoyanchev et al., 2007; Little et al., 2008). Berrang et al. (2004), illustrated that during processing, Campylobacter from the intestinal tracts could contaminate the surface of poultry carcasses. Cross contamination can also occur during preparation in the home kitchen from the raw meat products which can contaminate other foods and kitchen utensils. Cross- contamination to other ready-to-eat products and handling of contaminated raw meats are some of the possible risk factors associated with human Campylobacter and Salmonella infection (Smerdon et al., 2001). Consumption of under-cooked poultry meat can lead to sporadic human incidence, while outbreaks are usually related with raw milk (Denis et al., 2011). Foods prepared in restaurants have been associated in nearly half of the total sporadic Campylobacter illnesses in the United States (Friedman et al., 2004).

It has been reported that the use of antibiotic in animals intended for food has led to the development of antibiotic resistance in E. coli, Salmonella, Enterococcus and Campylobacter in animals, which can be transferred to humans through the food chain (Akinbowlale et al., 2006). Campylobacteriosis is an important foodborne zoonotic disease. In the US, Campylobacter isolates resistant to fluoroquinolones (FQ) in human are consistently increasing due to the widespread use of the drug in animals and poultry production (Price et al., 2005; Oliver et al., 2011). Multidrug resistance (MDR) is frequently observed in food borne pathogens. MDR in Campylobacter have been reported in a number of studies; for instance, Zoran et al. (2010) in Serbia reported that 10% of C. jejuni and 18.3% of C. coli were resistant to three or at least one in three classes of antibiotics. Other studies, reported that multidrug resistance was common in C. coli isolates compared to C. jejuni (Ubboi et al., 2012; Tambur et al., 2010; Englen et al., 2007). Thus, this study was designed to determine the occurrence of multidrug resistant (MDR) Campylobacter and the possible risk factors associated with their occurrence in broiler chicken meat retailed in markets in Selangor.

**MATERIALS AND METHODS**

**Samples collection**

A total of 210 samples consisting of 140 chicken meat parts and 70 weighing scales and cutting boards that were swabbed randomly in seven different selected markets in Selangor. Each meat sample was placed in a sterile plastic bag and each swab sample of weighing scale and cutting board was placed in a sterile bottle containing 2 mL of Bolton Selective Enrichment Broth (Oxoid) supplemented with Bolton Antibiotic Supplements (Oxoid SR0208E) and 5% defibrinated lysed horse blood. The samples were kept in a box with ice packs and transported to the laboratory and processed within 2-4 h post sampling.

**Isolation and identification of Campylobacter**

The isolation of Campylobacter in broiler chickens was carried out using the Cape Town Protocol developed by Le Roux and Lastovica (1998). Twenty-five grams of each meat sample was taken, homogenized, enriched in a broth and incubated at 42 °C for 48 h under microaerophilic condition generated using CampyGen gas pack (Oxoid CN 0025A). A volume of 200 µL each of enriched mixture were dropped onto a membrane filter of 0.65 µm pore-size cellulose nitrate and of 47 mm diameter (Millipore, Sartorius Stedim, Biotech, Goettingen Germany) which was placed onto Colombia Blood Agar (CBA) (Oxoid) plate. Passive filtration was set for 45 min before incubation under microaerophilic condition at 42 °C for 48 h. Presumptive Campylobacter colonies were selected and identified on the basis of colony morphology, motility using wet mounts, cellular morphology from Gram staining and biochemical tests which included: hippurate hydrolysis, indoxyl-acetate hydrolysis, urease, and oxidase and catalase tests.

**PCR confirmation of Campylobacter species**

Bacterial DNA was extracted by a crude boiling method. A bacterial suspension of fresh overnight culture was prepared in a 1.5 mL Eppendorf tube (Eppendorf, Australia) containing 1000 µL of sterile distilled water. The suspension was incubated for 10 min in dry bath at 94 °C, and allowed to cool at room temperature (25 °C). The bacterial suspension was centrifuged for 3 min at 13,000 rpm and the supernatant was used DNA template.

Multiplex PCR (mPCR) was performed using specific primers, appropriate cycling conditions and standardized positive controls (C. jejuni (ATCC 29428), C. coli (ATCC 33559) and C. upsaliensis (CCLG 14913T) as described by Yamazaki-Matsune et al. (2007) (Table 1). The reaction mixture was performed in a 50 µL reaction volume containing 25 µL of TopTaq Master Mix (QIAGEN), 1 µL (10 mM) of each forward and reverse primer (Table 1), 5 µL of Coral load (QIAGEN), 4 µL of DNA templates and 14 µL of RNase-free water (QIAGEN). The reaction mixtures were amplified in a thermal cycler (Eppendorf) with the following cycling parameters: pre-denaturation at of 95 °C for 2 min, followed by 25 cycles of denaturation at 95 °C for 30 sec, annealing at 58 °C for 2 min and elongation at 72 °C for 1 min, ending with a final elongation at 72 °C for 7 min.

Amplified PCR products were resolved in a 3% agarose gel (w/v) (Promega, USA) prepared in 1X TBE buffer contains Tris-Borate-EDTA (TBE) solution and (89 mM Tris Base, 89 mM Boric acid, 2 mM EDTA, pH 8.3) at 100V for 90 min. The gel was visualized under transilluminator UV with the aid of Alpha Imager (Bio-Rad) after staining in ethidium bromide for 2 min and de-stained in distilled water for 30 min (Figure 1).
Table 1: Primers used for the amplification of Campylobacter genes.

<table>
<thead>
<tr>
<th>Species</th>
<th>Size (bp)</th>
<th>Target gene</th>
<th>Oligonucleotide sequence (5′-3′)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genus Campylobacter</td>
<td>816</td>
<td>16S rRNA</td>
<td>C412F 5′-GGA TGA CAC TTT TCG GAG C-3′</td>
<td>Linton et al. (1996)</td>
</tr>
<tr>
<td>C. coli</td>
<td>501</td>
<td>ceuE gene</td>
<td>F-5′-ATG AAA AAA TAT TTA GTT TTT GCA-3′</td>
<td>Gonzalez et al. (1997)</td>
</tr>
<tr>
<td>C. upsaliensis</td>
<td>86</td>
<td>lipA</td>
<td>CU61F-5′-CGA TGA TGT GCA ATT TGA GGC GGC-3′</td>
<td>Klena et al. (2004)</td>
</tr>
<tr>
<td>C. Jejuni</td>
<td>323</td>
<td>hip</td>
<td>CJF-5′-ACT TCT TTA TTG CTT GCT GC-3′</td>
<td>Wang et al. (2002)</td>
</tr>
</tbody>
</table>

Figure 1: Electropherogram showing PCR detection of Campylobacter isolates.

Antibiotic susceptibility test

The antibiotic susceptibility test was performed using disc diffusion method as described by the Clinical Laboratory Standard Institute (CLSI) (2014). Campylobacter isolates were tested against 12 antibiotics from different classes of veterinary critically important antibiotics (OIE, 2015) which included ciprofloxacin (cipro), 5 µg; ampicillin (Amp), 10 µg; tetracycline (Tc), 30 µg; erythromycin (E), 15 µg; gentamicin (Cm), 10 µg; cefotaxime (Cfx), 30 µg; penicillin G (P), 10 µg; streptomycin (S), 10 µg; nalidixic acid (Na), 30 µg; enrofloxacin (Enr), 5 µg; amoxicillin-clavulanic acid (Amc), 10µg and trimethoprim-sulfamethoxazole (sxt) 25 µg. Isolates were classified as sensitive, intermediate and resistant using zone diameter breakpoints as in CLSI (2014).

Statistical analysis

Chi-square test (χ²) was used to determine the proportion of Campylobacter species resistant to each tested antibiotic using IBM SPSS version 21.

Risk factor analysis

Descriptive analysis, chi-square (χ²) and univariate logistic regression for risk factor analysis using IBM SPSS version 21 were performed. Two variables, how the meat was displayed and use of disinfectant were excluded in the univariate risk factor analysis because all the meat was displayed without putting ice and disinfectant was not using to clean the stall as such there was no variation and could not be compared. A conditional logistic model was used for univariate analysis, and exposures with P<0.05 were considered significant. For both univariate logistic regression and chi-square, continuous variables were dichotomized, and the median value was chosen as the breakpoint. Variables were constructed by combining multiple items from the questionnaire.

Questionnaire design

The questionnaire used in this study was designed to assess the factors that are associated with the occurrence of Multidrug resistant (MDR) Campylobacter at selected retails outlet within Selangor namely; Semenyih wet market, Sentul wet market, Pasar Raya awam Serdang, Pasar Borong Selangor, Chow-kit wet market, Pasar Awam Bangi and Pasar awam Banting. The targeted respondents were chicken meat sellers. The consent of each respondent was sort for, before they were given the questionnaire to fill with the aid of a Bahasa Melayu language translator. The questionnaire was designed to take about 15 min to complete Table 2.
RESULTS

A total of 32 (15.2%) Campylobacter were isolated, 28 (20%) were from chicken meat and 4 (5.7 %) from weighing scales and cutting boards (Table 3). Three (3) species of Campylobacter were identified, namely C. jejuni, C. coli and C. upsaliensis at 11.9%, 2.9% and 0.5% respectively. Campylobacter isolates showed high resistance to ampicillin, enrofloxacin and nalidixic acid at 62.5%, 56.3% and 50.0% respectively, while the least resistance was to streptomycin at 3.1%. At species level, C. jejuni showed exceptionally high resistance to all 12 antibiotics tested. Resistance towards streptomycin was observed in all the isolates (100%). Eighty-four (84.6%) of C. jejuni isolates were resistant to cefotaxime, while more than 60% of C. jejuni isolates were resistant to Erythromycin (76.9%), Penicillin G (73.3%), Nalidixic acid (76.5%), Ciprofloxacin (70%), Tetracycline and Trimethoprim-sulfamethoxazole (71.4%), Gentamicin (75%), Enrofloxacin (68.4%), Ampicillin (76.2%), and Amoxicillin (63.6%) respectively Table 4. Similarly, all C. coli isolates showed resistance towards all the antibiotic tested with the exception of streptomycin.

Table 3: Prevalence of Campylobacter in chicken meat and on cutting board and weighing scale in market.

<table>
<thead>
<tr>
<th>Location of the wet markets</th>
<th>Number of positive samples (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>*Chicken meat (%)</td>
</tr>
<tr>
<td>Sementiyh wet market</td>
<td>1/20 (5.0%)</td>
</tr>
<tr>
<td>Sentul wet market</td>
<td>0/20 (0.0%)</td>
</tr>
<tr>
<td>Pasar Raya Awam Serdang</td>
<td>4/20 (20.0%)</td>
</tr>
<tr>
<td>Pasar Borong Selangor</td>
<td>5/20 (25.0%)</td>
</tr>
<tr>
<td>Chow-Kit wet market</td>
<td>4/20 (20.0%)</td>
</tr>
<tr>
<td>Pasar Awam Bangi</td>
<td>8/20 (40.0%)</td>
</tr>
<tr>
<td>Pasar Awam</td>
<td>6/20 (30.0%)</td>
</tr>
<tr>
<td>Banting</td>
<td>Overall total</td>
</tr>
</tbody>
</table>

* Keel, wing, drumstick and breast meat included skin at 20 each / market
** Swabs of cutting board and weighing scale at 10 each

Table 2: Definition and description of exposure variables of meat handling practices associated with the occurrence of Campylobacter in stalls in selected market.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Description scores and categorization</th>
</tr>
</thead>
</table>
| 1. Wearing work-attire at the market | 1. Yes  
2. No |
| 2. Workers hygiene | Good (All 3 scores)  
Fair (2 scores)  
Poor (1 or 0 score)  
| Clean cloth =1  
Clean apron =2  
Apron = 3 |
| 3. Using protective equipment | Good (All 3 scores)  
Fair (2 scores)  
Poor (1 or 0 score)  
Others |
| 4. Stall environment hygiene | Good (All 3 scores)  
Fair (2 scores)  
Poor (1 or 0 score)  
Others |
| 5. Type of counter – top surface | Tiles  
Wooden table  
Stainless steel  
Others… |
| 6. Source of water for washing meat and counter surface | Collected in container  
Direct from the tap  
Others… |
| 7. Placing ice on the carcasses | Yes  
No |
| 8. Type of Cutting board | Wood  
Plastic  
Others… |
| 9. Use of disinfectants to clean stall premise | Yes  
No |
| 10. Mode of meat display on the counter | Open without ice pack  
Open with ice pack  
Others… |
Additionally, high resistance towards amoxicillin-clavulanic, enrofloxacin, ciprofloxacin and gentamicin at 27.3%, 26.3% and 25% was observed. This finding is in agreement with the report of Tang et al. (2009). In *C. upsaliensis* only 14.3% of isolates showed resistance towards the antibiotics sulphonamethoxazole-trimethoprim while none of the isolates were resistant cefotaxime, streptomycin and gentamycin respectively (Table 4). However, *C. upsaliensis* isolates were resistant Erythromycin (7.7%), Penicillin G (6.7%), Nalidixic acid (5.9%), Ciprofloxacin (10%), Tetracycline (7.1%), Enrofloxacin (5.3%), Ampicillin (4.8%), and Amoxicillin (9.1%) respectively (Table 4).

All isolates showed resistance to at least one antibiotic; the most was to 4 - 5 antibiotics at 15.6%. The MDR (resistant to at least one antibiotic, in three or more classes) was 71.9% (Figure 2). Twenty-three (23) different antibiotic resistance patterns were detected in nine (9) antibiogroups (that is groups showing the number and types of antibiotics to which each isolate was resistant). The most common multidrug resistance profile for *Campylobacter* isolates was to 4 (TeAmCnEnAmp) and 5 (EPcxt5Amp) classes of antibiotics at 15.6% each (Figure 2).

**Table 4:** Percentage of antibiotic resistance in *Campylobacter* isolates.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>E15</th>
<th>P10</th>
<th>Na30</th>
<th>cxt30</th>
<th>S10</th>
<th>cip5</th>
<th>Te30</th>
<th>sxt25</th>
<th>cn10</th>
<th>en5</th>
<th>Amp10</th>
<th>Am30</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. upsaliensis</em></td>
<td>7.7</td>
<td>6.7</td>
<td>5.9</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>7.1</td>
<td>14.3</td>
<td>0</td>
<td>5.3</td>
<td>4.8</td>
<td>9.1</td>
</tr>
<tr>
<td><em>C. coli</em></td>
<td>15.4</td>
<td>20</td>
<td>17.6</td>
<td>25</td>
<td>0</td>
<td>20</td>
<td>21.4</td>
<td>14.3</td>
<td>25</td>
<td>26.3</td>
<td>19</td>
<td>27.3</td>
</tr>
<tr>
<td><em>C. jejuni</em></td>
<td>76.9</td>
<td>73.3</td>
<td>76.5</td>
<td>84.6</td>
<td>100</td>
<td>70</td>
<td>71.4</td>
<td>71.4</td>
<td>75</td>
<td>68.4</td>
<td>76.2</td>
<td>63.6</td>
</tr>
<tr>
<td>Total</td>
<td>37.5</td>
<td>43.8</td>
<td>50</td>
<td>37.5</td>
<td>3.1</td>
<td>28.1</td>
<td>43.8</td>
<td>21.9</td>
<td>12.5</td>
<td>56.3</td>
<td>62.5</td>
<td>34.4</td>
</tr>
</tbody>
</table>

E: erythromycin; P: penicillin G; Na: Nalidixic acid; cxt: cefotaxime; S: streptomycin; Cip: Ciprofloxacin; Te: Tetracycline; sxt: trimethoprim-sulfamethoxazole; cn: gentamicin; en: enrofloxacin; Amp: ampicillin; Am: amoxicillin

DISCUSSION

Campylobacter contamination is found more frequently in chicken meat compared to other meat (Abu-Madi et al., 2016). Carcass contamination occurred mostly through cross contamination during slaughtering and dressing processes. According to Herman et al. (2003), it is difficult for any slaughterhouse to avoid carcass contamination with foodborne pathogens. Equipment and surfaces can be sources of direct contamination when they have not been effectively cleaned or remained wet between cleaning and subsequent usage (Evans et al., 2004). Cross-contamination of bacteria such as *Salmonella* and *Campylobacter* during food preparation in the kitchens or any processing plants is considered a risk factor for human exposure to these foodborne pathogens. An important route of cross-contamination being transfer of bacteria from contaminated chicken carcasses via cutting boards or other unwashed surfaces to ready-to-eat foods (Kusumaningrum et al., 2004). In this study however, there was an association (*p*=0.05) between poor hygiene and presence of *Campylobacter* on the chicken meat in the market. Poor waste disposal and unsatisfactory sanitation were five times more likely to cause poultry meat to be contaminated with *Campylobacter* than those with good hygiene. The condition of stall hygiene can predict the occurrence of cross contamination with *Campylobacter*. Poor stall hygiene was 44 times likely to cause contamination of the meat compared to good stall hygiene and this association was statistically significant at *p*=0.013. Furthermore, it was observed that the risk of contamination was high when the workers wore dirty working attire (OR 2.7, CI 1.144-6.374, *p*=0.033), using poor protective equipment (OR 38.50, CI 2.915-508.463, *p*=0.006), poor stall hygiene (OR 44.00, CI 2.193-882.66, *p*=0.013) and titled counter top surface (OR 6.1, CI 1.198-31.164, *p*=0.029) as shown in (Table 5). This means that the likely hood of contamination with *Campylobacter* as a result of the lack of using protective clothing OR= 38.5 and poor stall hygiene OR=44.0 is higher than the likely hood of contamination as a result of using proper working attire OR= 2.7 and titled counter top surface OR= 6.1.

This study identified five (5) risk factors for *Campylobacter* contamination on chicken meat in the market that, if taken together, might account for most contamination and although relevant but not statistically significant was poor work hygiene (OR 5.250, CI 0.988-27.895, *p*=0.05). This is because, there is 5 times likelihood of chicken meat contamination with *Campylobacter* as a result of poor hygiene. Those factors that were significant were without using proper cutting boards.
The findings of Mensa et al. (2007) and Lues and Van Tonder (2007) highlighted the importance of personal hygiene and good hand hygiene can prevent the spread of pathogenic bacteria causing cross-contamination from raw chicken, vegetables, or environment via utensils or other tools or equipments (Jacobs et al., 1988). Dipping can reduce the bacterial load. Another significant association (p=0.033) observed, was between those without proper work attire, which were three times likely to contaminate the meat and equipment than those with good work attire. Those with fair and poor protective equipment were 13 and 38 times more likely respectively to be exposed or contaminated with Campylobacter than those with a good protective equipment and this association was significant at p=0.036 and p=0.006 respectively. It is generally accepted that the hands of food handlers are an important vehicle of cross contamination on food and that improved personal hygiene and good hand hygiene can prevent the spread of pathogenic bacteria (Sneath et al., 2004; Lues and Van Tonder, 2007). This finding highlights the important role of good hygienic measures in the prevention of food contamination. Hence there is the need for regular cleaning with water in order to minimize the unavoidable environmental contamination. The protective effects of using stainless steel equipment was six times less likely to contaminate the chicken meat and equipment in comparison with wood counter top surface. This finding suggested the use of stainless steel is vital to reduce carcass contamination which is agreeable with a study by Kusumaningrum et al. (2002) who inoculated stainless steel surfaces with a test suspension of C. jejuni and noticed a three-log reduction in the first 30 min. After 4 h of incubation, no Campylobacter could be recovered. Cogan et al. (2002) computed cross-contamination in a study in which the volunteers were asked to cut a naturally Campylobacter-contaminated whole raw chicken carcass into pieces; the study found 85% of hands and 80% of cutting boards made up of wood were contaminated with Campylobacter. A qualitative cross-contamination study from the Netherlands indicated that C. jejuni were conveyed from raw chicken products to cutting boards, plates, and especially to hands (De Boer and Hahné, 1990). It is considered that cross-contamination and not undercooking is the dominant route of exposure to humans (Nauta et al., 2009). Two other risk factors which could also potentially accounted for Campylobacter contamination in meat and equipment included: how the meat is displayed and use of disinfectant to clean the stainless steel equipment which could also potentially accounted for Campylobacter contamination in meat and equipment.

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>Category</th>
<th>Prevalence (%)</th>
<th>P-value</th>
<th>Odd ratio</th>
<th>95% Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower</td>
</tr>
<tr>
<td>Workers hygiene</td>
<td>Good</td>
<td>19.0</td>
<td>REF</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Fair</td>
<td>23.8</td>
<td>0.266</td>
<td>2.917</td>
<td>0.442</td>
</tr>
<tr>
<td></td>
<td>Poor</td>
<td>57.1</td>
<td>0.052</td>
<td>5.250</td>
<td>0.988</td>
</tr>
<tr>
<td>Wearing working attire</td>
<td>Yes</td>
<td>23.8</td>
<td>REF</td>
<td>NA</td>
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</tr>
<tr>
<td></td>
<td>No</td>
<td>76.2</td>
<td>0.033*</td>
<td>2.700</td>
<td>1.144</td>
</tr>
<tr>
<td>Wearing protective equipment</td>
<td>Good</td>
<td>4.8</td>
<td>REF</td>
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<tr>
<td></td>
<td>Fair</td>
<td>42.9</td>
<td>0.036*</td>
<td>12.60</td>
<td>1.186</td>
</tr>
<tr>
<td></td>
<td>Poor</td>
<td>52.4</td>
<td>0.006*</td>
<td>38.50</td>
<td>2.915</td>
</tr>
<tr>
<td>Stall hygiene</td>
<td>Good</td>
<td>4.8</td>
<td>REF</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Fair</td>
<td>42.9</td>
<td>0.253</td>
<td>4.00</td>
<td>0.371</td>
</tr>
<tr>
<td></td>
<td>Poor</td>
<td>52.4</td>
<td>0.013*</td>
<td>44.00</td>
<td>2.193</td>
</tr>
<tr>
<td>Type of counter surface</td>
<td>Stainless steel</td>
<td>19.0</td>
<td>REF</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Tiles</td>
<td>28.6</td>
<td>0.123</td>
<td>6.687</td>
<td>0.597</td>
</tr>
<tr>
<td></td>
<td>Wood</td>
<td>52.4</td>
<td>0.029*</td>
<td>6.111</td>
<td>1.198</td>
</tr>
<tr>
<td>Source of water</td>
<td>Tap</td>
<td>14.3</td>
<td>REF</td>
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<td>NA</td>
</tr>
<tr>
<td></td>
<td>Container</td>
<td>85.7</td>
<td>0.259</td>
<td>1.167</td>
<td>0.980</td>
</tr>
</tbody>
</table>

*Significant (p < 0.05), NA: Not applicable, REF: Reference category.

The high prevalence of Campylobacter in raw poultry meat found in this study was similar with other studies (Moore et al., 2002; Denis et al., 2001). Due to the high concentration of Campylobacter in the intestines, in

Table 5: Univariate logistic regression for risk factors associated with Campylobacter isolates in markets.
particular the caeca, chicken carcasses may become contaminated at the surface during processing as a result of contamination. Carcasses from Campylobacter negative broilers can become contaminated through contaminated equipment when they are processed after a positive Campylobacter flock (Frediani-Wolf and Stephan, 2003). However, this contamination usually results in a lower concentration of bacteria at the surface compared to carcasses from colonized chickens and reported to have a negligible impact on the risk for humans compared to products from Campylobacter positive flocks (Rosenquist et al., 2003). The high occurrence of Campylobacter during processing may lead to contaminated poultry carcasses in the retail market. If the initial carcasses are contaminated with Campylobacter, the contact surfaces in the processing environment become contaminated and contribute to cross-contamination to poultry meat. Therefore, starting with good microbiological quality poultry carcasses should reduce the contamination level of processed poultry products.

There is a limited option in the use of antibiotics for the treatment of Campylobacter infection, and resistance towards those antibiotics increase the need of an alternative antibiotic (Hong et al., 2007). The cross-resistance among Campylobacter strains to enrofloxacin, ciprofloxacin and other fluoroquinolones might also explain the increasing number of resistance toward fluoroquinolones (Jacobs-Reitsma et al., 1994; Hong et al., 2007). Low-level resistance to aminoglycosides (streptomycin) generally can be attributed to the intermittent usage of this group of antibiotics in the poultry either at prophylactic or therapeutic level due to its intramuscular route of administration, which may be impracticable for large-scale application (Rodrigo et al., 2007). Since erythromycin is the drug of choice for the treatment of Campylobacter infections, the resistance to this antibiotic, especially among strains isolated from food, should be a cause for special concern. In this study, majority of Campylobacter strains (70.0%) were resistant to one or more antibiotics. Furthermore, most of the isolates (50.0%) showed resistance to two or more different classes of antibiotics and this percentage was higher than that reported by other authors (Andersen et al., 2006; Sallam 2007; Rozynek et al., 2008). The findings above is in agreement with the documentation of Health Action International Asia Pacific (HAIAP) (2013) on the use of antibiotics in animals in Malaysia. The report showed that the mostly commonly used antibiotics in poultry farms in Malaysia are Erythromycin, Ampicillin, Amoxicillin, gentamycin and enrofloxacin. One C. coli isolate was resistant to four classes of antibiotics including fluoroquinolones and macrolides. Most of Campylobacter species (82.5%) isolated in this study were resistant to ampicillin. Seventy-six-point two percent (76.2%) of C. jejuni, 19.0 % of C. coli and 4.8 % C. upsaliensis isolated from chicken meat were found resistant to ampicillin. The result of this study was similar to the study by Sáenz et al. (2000) who reported 47.4% of C. jejuni and 90% of C. coli were resistant to ampicillin. A lower resistance was reported by Milfin et al. (2007) who found 17.6% of C. jejuni and 14.8% of C. coli were resistant to ampicillin. The high antibiotic resistance in this study may possibly be due to inappropriate use of antibiotics at sub-therapeutic doses in broilers for prophylaxis and growth promotion (Saleha, 2002; Olah et al., 2006).

CONCLUSION

Five risk factors for high antibiotic resistant Campylobacter contamination of poultry carcasses were identified. Most of the risk factors were associated with hygienic practices which called for good disinfection and hygienic practices by the workers. To enhance the hygienic level of retail outlets, retailers, managers and staff must be informed and sensitized about the risk of this bacteria especially with regards to food safety and also proper washing of contaminated hands, knives and chopping boards before and after meat handling is vital. The presence of high MDR Campylobacter species could compromise human health. Therefore, there is a need to further educate and emphasize among farmers the need to observe good husbandry practices and prudent use of antibiotics to reduce the menace of antibiotic resistance and among chicken meat stall owners to observe good hygienic practices.

ACKNOWLEDGEMENT

The authors wish to acknowledge the staff of Veterinary Public Health Laboratory, and Department of Pathology and Microbiology, Faculty of Veterinary Medicine for their assistance.

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