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

Muhammad Jalo Ibrahim [1,5], Saleha Abdul-Aziz[1*], Asinamai Athliamai Bitrus[1,4], Dauda Goni Mohammed[1,3,5,] Jalila Abu[2], Siti Khairani Bejo[1], Mohamed Abdelrahman Mohamed[1], Mohamed Yousif Ibrahim Mohamed [1]

[1]Department of Pathology and Microbiology, Faculty of Veterinary Medicine, University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
[2]Department of Veterinary Clinical Studies, Faculty of Veterinary Medicine, University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
[3]Unit of Biostatistics and Research Methodology, School of Medical Sciences, Health Campus, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia.
[4]Research Unit in Microbial food safety and Antimicrobial resistance, Department of Veterinary Public Health, Faculty of Veterinary Sciences, Chulalongkorn University, 10330 Pathumwan, Bangkok, Thailand.
[5]Veterinary Service Department, Ministry of Agriculture and Environment, Damaturu Yobe State Nigeria.
Email: aasaleha@yahoo.com

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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

***Corresponding author**

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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 (Akinbowale *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 16.3% 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* (Uaboi *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 (Milipore, 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* (CCUG 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).

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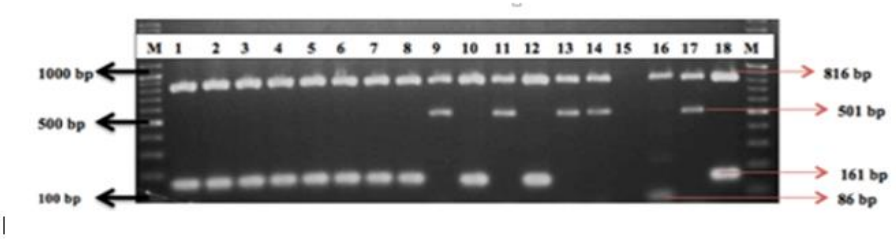
Table 1: Primers used for the amplification of *Campylobacter* genes.

Species	Size (bp)	Target gene	Oligonucleotide sequence (5'-3')	References
Genus *Campylobacter*	816	16S rRNA	C412F 5'-GGA TGA CAC TTT TCG GAG C-3′ C1228R 5′-CAT TGT AGC ACG TGT GTC-3′	Linton *et al*. (1996)
C. coli	501	*ceu*E gene	F -5′-ATG AAA AAA TAT TTA GTT TTT GCA-3′ R-5′-ATT TTA TTA TTT GTA GCA GCG-3′	Gonzalez *et al*. (1997)
C. upsaliensis	86	*lpx*A	CU61F -5′-CGA TGA TGT GCA AAT TGA AGC-3′ CU146R- 5′-TTC TAG CCC CTT GCT TGA TG-3′	Klena *et al*. (2004)
C. Jejuni	323	*hip*	CJF-5'-ACT TCT TTA TTG CTT GCT GC-3' CJR-5'-GCC ACA ACA AGT AAA GAA GC-3'	Wang *et al*. (2002)



Lane M: 100 bp molecular DNA marker, Lane 1-14 *Campylobacter* isolates, Lane 15: Negative control, Lane 16: *C. upsaliensis* CCUG 14913, Lane 17: *C. coli* ATCC 33559, Lane 18: *C. jejuni* ATCC 29428

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 (cip), 5 µg; ampicillin (Amp), 10 µg; tetracycline (Te), 30 µg; erythromycin (E), 15 µg; gentamicin (Cn), 10 µg; cefotaxime (ctx), 30 µg; penicillin G (P), 10 µg; streptomycin (S), 10 µg; nalidixic acid (Na), 30 µg; enrofloxacin (Enr), 5 µg; amoxicillin-clavalunic acid (Amc), 10µg and trimetophrim-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 ($\chi 2$) 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 ($\chi 2$) 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.

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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.

Location of the wet markets	Number of positive samples (%)	
	*Chicken meat n (%)	**Cutting board and weighing scale (%)
Semenyih wet market	1/20 (5.0%)	0/10 (0%)
Sentul wet market	0/20 (20.0%)	0/10 (0%)
Pasar Raya Awam Serdang	4/20 (20.0%)	3/10 (30.0%)
Pasar Borong Selangor	5/20 (25.0%)	0/10 (0%)
Chow-Kit wet market	4/20 (20.0%)	1/10 (10.0%)
Pasar Awam Bangi	8/20 (40.0%)	0/10 (0%)
Pasar Awam Banting	6/20 (30.0%)	0/10 (0%)
Overall total	28/140 (20.0%)	4/70 (5.7%)

* 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.

Variables	Description scores and categorization	
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 Clean gloves =3
3. Using protective equipment	Good (All 3 scores) Fair (2 scores) Poor (1 or 0 score) Others	Hand gloves = 1 Boot = 2 Apron = 3
4. Stall environment hygiene	Good (All 3 scores) Fair (2 scores) Poor (1 or 0 score)	Clean counter surface =1 Clean floor =2 No much flies =3
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…	

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

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

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

Additionally, high resistance towards amoxicillin-clauvalunic, 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 sulphamethoxazole-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 (TeAmcEnrAmp) and 5 (EPTeCtxAmp) classes of antibiotics at 15.6% each (Figure 2).

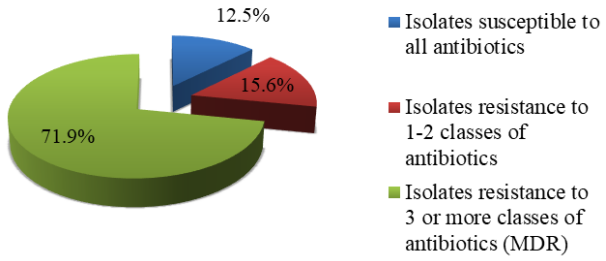

Figure 2: Percentage of MDR *Campylobacter* on chicken meat, weighing scales and cutting boards.

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 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.

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

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Table 5: Univariate logistic regression for risk factors associated with *Campylobacter* isolates in markets.

Risk Factors	Category	Prevalence (%)	*P*-value	Odd ratio	95% Confidence interval	
					Lower	Upper
Workers hygiene	Good	19.0	REF	NA	NA	NA
	Fair	23.8	0.266	2.917	0.442	19.234
	Poor	57.1	0.052	5.250	0.988	27.895
Wearing working attire	Yes	23.8	REF	NA	NA	NA
	No	76.2	0.033*	2.700	1.144	6.374
Wearing protective equipment	Good	4.8	REF	NA	NA	NA
	Fair	42.9	0.036*	12.60	1.186	133.899
	Poor	52.4	0.006*	38.50	2.915	508.463
Stall hygiene	Good	4.8	REF	NA	NA	NA
	Fair	42.9	0.253	4.00	0.371	43.139
	Poor	52.4	0.013*	44.00	2.193	882.66
Type of counter surface	Stainless steel	19.0	REF	NA	NA	NA
	Tiles	28.6	0.123	6.667	0.597	74.506
	Wood	52.4	0.029*	6.111	1.198	31.164
Source of water	Tap	14.3	REF	NA	NA	NA
	Container	85.7	0.259	1.167	0.980	1.389

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

clothing while working in the markets. These results corroborated the findings of Mensah *et al*. (2000) and Caballero *et al*. (1988) who identified personnel as mechanical vehicles of *Salmonella* and other zoonotic bacteria causing cross-contamination from raw chicken, vegetables, or environment via utensils or other tools or equipments (Jacobs-Reistma, 1997).

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 the meat and equipment 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 (Sneed *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 Hahne´, 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 stall were not included in the univariate risk factor analysis because there was no difference in the variables. To decrease carcass contamination, decontamination procedures can be instituted which could be physical or use of chemical to decrease the microbial load. Dipping can reduce contamination of carcasses with *Campylobacter* or spraying of carcasses using chlorinated water, acidified sodium chlorite (ASC) or acetic or lactic acids. Trisodium phosphate (TSP) has also been widely used, but due to processing and environmental problems its use is now minimal. It is widely assumed that harvesting or gradual depopulation is a significant risk factor for flock colonization.

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

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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 *Campylobacte*r, 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 antibiotics (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 enrofloxacins. One *C. coli* isolate was resistant to four classes of antibiotics including fluoroquinolones and macrolides. Most of *Campylobacter* species (62.5%) 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 Miflin *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.

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