Original article

Prevalence and antimicrobial susceptibility profiles of thermotolerant *Campylobacter* strains in retail raw meat products in Ethiopia

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Abstract

Background: *Campylobacter jejuni/coli* are frequent causes of diarrhea in humans worldwide originating in foods of animal origin mainly from poultry.

Objectives: The aim of this study was to determine prevalence and antimicrobial susceptibility profiles of thermotolerant *Campylobacter* strains in retail raw meat products.

Methods: During a 4-month period from November 2006 to April 2007, a total of 540 raw meat samples were collected from beef (n=227), sheep (n=114), goat (n=92), chicken (n=60) and pork (n=47) and analyzed for *Campylobacter* spp.

Results: *Campylobacter* spp. were isolated from 50 (9.3%) out of 540 meat samples. The highest prevalence (21.7%) was recorded in chicken meat, followed by sheep meat (10.5%), pork meat (8.5%), goat meat (7.6%) and beef (6.2%). Among the isolates, 39 (78%) were identified to be *C. jejuni*, 9 (18%) were *C. coli* and 2 (4%) were *C. lari*. Lower resistance rates (2-6%) were observed for amoxicillin, chloramphenicol and erythromycin than (10-20%) for ampicillin, gentamicin, kanamycin, streptomycin and tetracycline. Multidrug resistance to two or more drugs was detected in 20% of strains.

Conclusion: Raw meat from food animals could serve as potential source of campylobacter, indicating possible risks of infection to people through the consumption of raw/under-cooked meat. Low percentages of resistance to most antimicrobial agents tested in this study may be the indirect result of low/no usage of these agents as a growth promoter or treatment in food animals in the Ethiopian animal farm setting. [*Ethiop.J.Health Dev.* 2008;22(2):195-200]

Introduction

Campylobacters are small gram-negative, non-spore-forming, helical bacteria with a distinctive 'darting' motility, and are catalase and oxidase positive. *Campylobacter* spp. can be found in the reproductive organs, intestinal tracts, and oral cavity of animals and humans. They are the leading cause of bacterial diarrheal disease worldwide, resulting mainly from the contamination of poultry or other meats, raw milk, other milk products and surface water (1). Food animals, mainly poultry, cattle, sheep and pigs, may act as asymptomatic intestinal carriers of *Campylobacter* and animal food products can become contaminated by this pathogen during slaughter and carcass dressing (2).Cross contamination of ready to eat foods during preparation by food handlers as well as direct contact with animals have also been identified (2). Treatment with antibiotics for uncomplicated campylobacter infection is rarely indicated. However, antimicrobial resistance to clinically important drugs used for treatment (especially macrolides and fluoroquinolones) is increasingly reported for campylobacters (3). There is evidence that patients infected with antibiotic-resistant strains suffer worse outcomes (invasive illness or death) than those infected with sensitive strains (4). There is growing scientific evidence that the use of antibiotics in food animals, particularly in developed countries, leads to the development of resistant pathogenic bacteria that can reach humans though the food chain (5, 6, 7). This underlines the need to limit the use of antimicrobials in veterinary practice to limit the occurrence of resistance. Few study reports of *Campylobacter* spp. as human enteric pathogens in Ethiopia showed isolation rates ranging from 13.6% to 13.8% (8, 9) and 39.6% from apparently healthy food animals (10). The susceptibility pattern of isolates from humans (11, 12) and food animals (13) has been reported. No documented reports exist yet on the occurrence and susceptibilities of thermotolerant campylobacter strains in foods of animal origin in Ethiopia where raw meat is widely consumed. To readdress this situation, we investigated the occurrence and antimicrobial susceptibility profile of thermophilic *Campylobacter* strains from retail raw meat collected from abattoirs, butcher shops and supermarkets in Ethiopia.

Methods

Study area: The study was conducted in Addis Ababa, the capital city of the Federal Democratic Republic of Ethiopia and Debre Zeit which is located 45 km south east of Addis Ababa during the period between November 2006 and April 2007.

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Sample collection: A total of 540 raw ready-to-eat meat samples including beef (n=227), sheep (n=114), goat (n=92), pork (n=47) and chicken (n=60) were collected from randomly selected abattoirs, butcher shops and supermarkets located at Debre Zeit and Addis Ababa, Ethiopia (Table 1). The samples purchased from the supermarkets were either deep frozen or refrigerated and wrapped in polyethylene plastic bags in order to avoid contamination and were kept for a maximum of 1-2 days while samples purchased from abattoirs and butchers were ready-to-eat fresh. Samples were transported to the Microbiology Laboratory of the Faculty of Veterinary Medicine, Addis Ababa University on the day of purchase/collection in plastic bags containing ice packs and analyzed within 24 hours. An estimated (expected) 50% of prevalence of campylobacter in foods, 95% confidence interval and a precision of 5% was used for study sample size determination.

Table 1: **Retail meat samples investigated for thermophilic *Campylobacter* spp. (November 2006 to April 2007)**

Types of raw meat	Sources No. (%)		
	Abattoirs	Butehers shops	Supermarkets
Beef (n=227)	138 (60.7)	69 (30.4)	20 (8.8)
Sheep meat (n=114)	93 (81.6)	10 (8.8)	11 (9.6)
Goat meat (n=92)	67 (72.8)	11 (12.0)	14 (15.2)
Pork (n=47)	30 (63.8)		17 (36.2)
Chicken (n=60)	30 (50.0)		30 (50.0)
Total=540	**358 (66.3)**	**90 (16.7)**	**92 (17.0)**

Sample preparation and selective enrichment: Approximately 25 grams of raw meat samples were aseptically removed using sterile forceps and scissors and placed in 90 ml of Preston Campylobacter selective enrichment broth (Oxoid Ltd, Basingstoke, UK) supplemented with polymyxin B, rifampicin, trimethoprim and cycloheximide (Oxoid) and 5% lysed horse blood in sterile plastic bags and homogenized for 1 minute in a laboratory stomacher (Lab Blender 400, Seward Medical, London, UK). The homogenized material was then transferred into a sterile bottle and additional broth was added to minimize headspace within the bottle as recommended by Robert *et al.* (14). Following processing, all the samples were incubated at 42°C for 48 hours in a microaerophilic atmosphere achieved in anaerobic jar (Oxoid) without catalyst and by using CampyGen® gas generating kits (5% O_2 and 10% CO_2).

Culture and identification of thermophilic Campylobacter species: All enriched meat samples were subsequently subcultured onto Preston Campylobacter selective agar (Oxoid). The same supplements were used as for Preston broth. The plates were incubated in a microaerophilic atmosphere at 42°C for 48 hours. Preliminary identification of *Campylobacter* spp. was performed based on the characteristic Gram-staining reactions, positive tests for oxidase, and catalase reactions. Species differentiation was based on hippurate hydrolysis, H_2S production and susceptibility to nalidixic acid (30 µg, Oxoid) and cephalothin (30µg, Oxoid). These parameters formed the basis for the identification of *C. jejuni*, *C. coli* or *C. lari*, as proposed by others (15). The type strains *C. jejuni* (NCTC 11351), *C. coli* (LMG 6440) and *C. lari* (NCTC 11352) were included as positive controls.

Antimicrobial susceptibility testing: Antimicrobial susceptibilities to all antibiotics were performed for 50 isolates of *Campylobacter* spp. using the disk diffusion method as recommended by the National Committee for Clinical Laboratory Standards (NCCLS) (16). The antimicrobials for disc diffusion testing were obtained from Oxoid at the following concentrations: amoxicillin (AML) (10µg), ampicillin (AMP) (10 µg), chloramphenicol (C) (30 µg), erythromycin (E) (15 µg), gentamicin (CN) (10 µg), kanamycin (K) (30 µg), norfloxacin (NOR) (10 µg), streptomycin (S) (10 µg) and tetracycline (TE) (30 µg). Briefly, fresh bacterial colonies were inoculated in 0.85% NaCl suspension to turbidity equivalent to 0.5 McFarland standards. With sterile cotton swab, the culture was swabbed onto a Mueller-Hinton agar (Oxoid) supplemented with 5% sheep blood; antibiotic discs were applied after drying the plates for 3–5 min. The plates were incubated in microaerophilic atmosphere at 42°C for 48 hours. A standard reference strain of *E. coli* (ATCC 25922), sensitive to all antimicrobial drugs being tested was used as a control strain. Diameters of the zone of inhibition around the disc were measured to the nearest millimeter using a metal caliper, and the isolates were classified as sensitive, intermediate, and resistant using the breakpoints of the NCCLS (16).

Statistical analysis: EPI-INFO Version 6, software (CDC, Atlanta GA) was used for statistical analysis. Comparisons were made using Chi-square test with Yates' correction or Fisher's exact tests. A p-value of <0.05 was considered indicative of a statistically significant difference.

Ethical Considerations: The research project has been approved by the Academic Commission of the Faculty of Veterinary Medicine, Addis Ababa University.

Results

Prevalence and species distribution of thermophilic Campylobcater spp.: The numbers and percentages of strains isolated from each meat sample were - beef: 14/227=6.2%; sheep: 12/114=10.5%; goat: 7/92=7.6%; pork: 4/47=8.5%; chicken: 13/60 (21.7%) (Table 2). Chicken meat was found to be more highly contaminated than other raw meats examined ($p < 0.05$). The numbers of *C. jejuni, C. coli* and *C. lari* per meat sources were, respectively: beef 12/2/0; sheep 10/2/0; goat 5/2/0; pork 1/2/1 and chicken 11/1/1 (Table 3). *C. lari* was isolated only from pork and chicken meat. There was no statistically significant difference in the isolation frequency of different thermophilic *Campylobacter* spp. in various meats ($p > 0.05$).

Table 2: **Prevalence of thermophilic *Camplylobacter* species in various raw meat samples (November 2006 to April 2007)**

Meat type investigated	Prevalence rates (%) Sources			
	Abattoir	Butcher	Supermarket	Total
Beef	9/138 (6.5)[a]	4/69(5.8)	1/20(5.0)	14/227(6.2)
Sheep meat	11/93(11.8)	1/10(10.0)	0/11(0)	12/114 (10.5)
Goat meat	6/67(9.0)	1/11(9.0)	0/14 (0)	7/92(7.6)
Pork	3/30(10.0)	-	1/17(5.9)	4/47(8.5)
Chicken	8/30(26.7)	-	5/30(16.7)	13/60(21.7)
Total	**37/358(10.3)**	**6/90(6.7)**	**7/92(7.6)**	**50/540(9.3)**

Results expressed as the number of thermophilic *Campylobacter*-positive samples/total number of meat samples analyzed. [a] Percentage of positive samples

Table 3: **Distribution of thermophilic *Campylobacter* species in various meat samples (November 2006 to April 2007)**

Meat source	No. (%)		
	C. jejuni	*C. coli*	*C. lari*
Beef (n=14)	12(85.7)	2(14.3)	-
Sheep meat (n=12)	10(83.3)	2(16.7)	-
Goat meat (n=7)	5(71.4)	2(28.6)	
Pork (n=4)	1(25.0)	2(50.0)	1(25.0)
Chicken (n=13)	11(84.0)	1(8.0)	1(8.0)
Total (50)	**39(78.0)**	**9(18.0)**	**2(4.0)**

Antimicrobial susceptibility pattern: The results of antimicrobial susceptibility testing for *C. jejuni, C. coli* and *C. lari* isolated from raw meats against 9 chosen antimicrobial agents are presented in Table 4. Lower resistance rates were seen for amoxicillin (6%), chloramphenicol (4%) and erythromycin (2%) than for ampicillin, gentamicin, kanamycin, streptomycin and tetracycline with 10%, 14%, 12%, 20% and 10% respectively. Resistance was not observed for norfloxacin. Resistance to gentamicin and tetracycline (33.3%-55.6%) in *C. coli* was higher than in *C. jejuni* and *C. lari* (5.1% and 0%, respectively) ($p<0.05$). Regarding kanaymycin resistance, a higher prevalence of resistance (50.0%) was found in *C. lari* than *C. jejuni* and *C. coli* (10.3% and 11.1%, respectively) ($p> 0.05$). Resistance figures were not statistically different among *C. jejuni, C. coli* and *C. lari* ($p> 0.05$) except for gentamicin and tetracycline. Multidrug resistance (an isolate being resistant to two or more tested drugs) was detected in 20% of strains. Multidrug resistance was found in 4/39 (10.3%) of the *C. jejuni* strains, but was more common in 6/9 (66.7%) of *C. coli* ($p<0.05$).

Discussion

Most human campylobacter enteritis is caused by thermophilic campylobacters, namely: *C. jejuni, C. coli* and *C. lari* although *C. upsaliensis* is also important in the developing world. It is now accepted that campylobacteriosis is predominantly acquired through the consumption of contaminated foods (17).

In the present study, chicken meat was more highly contaminated (21.7%) than other meats examined (Table 2). It is a well known fact that poultry appeared to be a significant source of campylobacter and chicken were found to be heavy intestinal carriers of campylobacters when compared with other food animals (17). Wide variation (0–90%) in the prevalence of *Campylobacter* in fresh poultry meat had been reported in different countries (18, 19, 20). *Campylobacter* was recovered at lower prevalence in other meats obtained from beef (6.2%); sheep (10.5%), goat (7.6%) and pork (8.5%) than chicken meat in the present study. This finding is in close agreement with the reported prevalence of campylobacter bacteria in these meat samples ranging between 0% and

10.9% in different countries (19, 20, 21, 22, 23, 24). In this study, among the thermophilic campylobacters isolated from various meats, *C. jejuni* accounted for 78%, *C. coli* for 18% and *C. lari* for 4% (Table 3). Similar findings have been reported that prevalence of *C. jejuni* in meats of animal origin (except in pork meat), dairy products and vegetables were higher than *C. coli* ranging from 45 to 89% (20, 25, 26, 27).

Table 4: **Antimicrobial susceptibility of *C. jejuni*, *C. coli*, and *C. lari* isolated from raw meat samples in Ethiopia**

Campylobacter spp.	No. (%)								
	AMP	AML	C	E	CN	K	NOR		TE
C. jejuni (n=39)									
S[a]	29(74.4)	38 (97.4)	37(94.9)	38(97.4)	37 (94.9)	33(84.6)	31(79.5)	31 (79.5)	36.(92.3)
I[b]	5(12.8)	-	2(5.1)	-	-	2(5.1)	8 (20.5)	-	1 (2.6)
R[c]	5(12.8)	1(2.6)	-	1 (2.6)	2 (5.1)	4(10.3)	-	8 (20.5)	2 (5.1)
C.coli (n=9)									
S	9(100)	7 (77.8)	7(77.8)	9(100.0)	4 (44.4)	6 (66.7)	9(100.0)	7 (77.8)	-
I	-	-	-	-	-	2 (22.2)	-	-	6(66.7)
R	-	2(22.2)	2(22.2)	-	5 (55.6)	1 (11.1)	-	2 (22.2)	3(33.3)
C. lari (n=2)									
S	2 (100)	2 (100.0)	2(100.0)	2(100.0)	2(100)	1(50.0)	1 (50.0)	2 (100.0)	2(100.0)
I	-	-	-	-	-	-	1(50.0)	-	-
R	-	-	-	-	-	1(50.0)	-	-	-
Total (n=50)									
S	40 (80)	47 (94.0)	46(92.0)	49(98.0)	43 (86.0)	40(80.0)	41(82.0)	40 (80.0)	38(76.0)
I	5 (10)	-	2(4.0)	-	-	4(8.0)	9 (18.0)	-	7(14.0)
R	5 (10)	3(6.0)	2(4.0)	1(2.0)	7(14.0)	6(12.0)	-	10 (20.0)	5(10.0)

AMP: ampicillin; AML: amoxycillin; C: chloramphenicol; E: erythromycin; CN: gentamicin; K: kanamycin: NOR: norfloxacin: S: streptomycin; Te: tetracycline [a] Sensitive [b]Intermediate [c]Resistant

In the present investigation, isolates from meat samples showed lower resistance rates to most antimicrobial agents tested (Table 4). Similar findings have been observed in a previous study conducted in Ethiopia where 80-100% of isolates from food animals were sensitive to these antimicrobial agents (13). However, there are reports from different parts of the world that antimicrobial resistance in food, food animals and human isolates is increasing. High levels of resistance to fluroquinolones (ciprofloxacin or other fluroquinolones) were observed from foods of animal origin e.g. in Spain (74.7%) (28), Estonia (66%) (29), Ireland (31.8%) (30), Korea (95.9%) (31), Italy (78.6%) (23), Iran (69.4%) (32), Japan (94.9%) (15) and Austria (40.7%) (19). Similar pattern of resistance was observed in strains isolated from various food animals (14-98.7%) and humans (10-90%) (33). Thus, there is convincing evidence today that quinolone resistance emerged and increased among food animals as a consequence of the use of fluroquinolones in animal production and then spread to via food chain and caused infection in man (3, 34). Resistance to macrolides (erythromycin or other macrolides) has been reported in 12 to 60% isolates from foods (23, 28, 29, 31) and 0-83% from food animals, particularly from pigs and 0.3-90% from humans (33). This observation indicated significant association between macrolide use such as tylosin, erythromycin and avopracin as a growth promoter in pork production (3, 33). Low and high level of resistance to chloramphenicol (0–60%), gentamicin (0–11.9%), streptomycin/ kanamycin (0–48%) and tetracycline (0–96%) has been reported among *Campylobacter spp.* isolated from foods, food animals and humans of different parts of the world (34) Several studies have been reported resistance to beta lactam antibiotics is in general high in food animals (6, 35, 36). Similar findings of resistance to these drugs in human and food animal isolates have been observed in Ethiopia (11, 12, 13), Spain (28) and Denmark (37). A high percentage of resistance (11.1-48.5%) to ampicillin/amoxicillin was also observed in food isolates from different parts of the world (29, 30, 32). Ten (20%) of the *Campylobacter* isolates were resistant to two or more antimicrobial agents. Multidrug resistance was found in 4/39 (10.3%) of the *C. jejuni* strains, but was more common in 6/9 (66.7%) of *C. coli* ($p<0.05$). Multidrug resistant isolates always remained susceptible to chloramphenicol, norfloxacin and erythromycin. The frequency of multidrug resistant strains (20%) in this study is higher than the previous finding in Ethiopia (14.5%) (13), but is much lower than what was reported in Belgium (60%) (6), Estonia (60%) (29), Iran (75%) (32) and Korea (93.4%) (31).

In conclusion, this study revealed that various raw meats from foods of animal origin are often contaminated with thermophilic *Campylobacter* spp., suggesting possible risks of infection to people through consumption of raw/undercooked meat. The study also showed that antimicrobial resistance is found only at relatively low frequencies for most antimicrobial agents tested. The low percentages of resistance to most antimicrobial agents tested in this study may be the result of low/no usage of these agents as growth promoters or treatment in food animals in the Ethiopian farm setting. To our knowledge, oxytetracycline and/or a combination of penicillin with streptomycin are the most frequently used antibiotics for treatment of infection in different animal farm settings in Ethiopia (personal communication). The detection of

multidrug resistant isolates may pose a threat to humans and further limits therapeutic options.

We recommend that coordinated actions are needed to reduce or eliminate the risks posed by this organism at a number of stages in the food chain. These include Good Agricultural Practice (GAP), Good Manufacturing Practice (GMP), and Hazard Analysis of Critical Control Points (HACCP) at every stages of the meat supply chain, from the farm, through the abattoir, to the retailer, and those involved with the handling and processing of such raw meat products in the home environment.

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