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Prevalence and antibiotic resistance of thermophilic *campylobacter* spp. isolates from raw beef, mutton and camel meat in Sokoto, Nigeria

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Abstract

Campylobacter is one of the common causes of human gastroenteritis worldwide. The organism is transmitted mostly via foods of animal origin. The study was conducted to investigate the prevalence of contamination of raw beef, mutton and camel meat in Sokoto, Nigeria, with thermophilic *Campylobacter* spp. and determined antibiotic susceptibilities of thermophilic *Campylobacter* spp. isolated from these carcasses. From March 2008 to February 2009, a total of 531 raw meat samples from beef (n=242), mutton (n=181) and camel (n=108) were collected randomly from meat processing facilities and retail stalls in Sokoto, and were evaluated for the presence of thermophilic *Campylobacter* spp. Thermophilic *Campylobacter* spp. were isolated from139 (26.33%) of the tested samples and the individual prevalence are 22.08%, 37.22% and 17.49% for beef, mutton and camel meat respectively. The most prevalent thermophilic *Campylobacter* spp. isolates from the raw meat samples was *Campylobacter jejuni* (74.10%). The antibiotic susceptibility of the isolates were determined for 10 antibiotic, revealed that resistance to tetracycline was the most common (71.4%) resistance observed, followed by ciprofloxacin (42.9%) and nalidixic acid (37.1%). All the isolates tested were susceptible to chloramphenicol and gentamycin. The results of our study have demonstrated that high proportion of meat samples are contaminated by thermophilic *Campylobacter* spp. which may have serious effects on public health. Most of the isolates are antimicrobial resistant strains. Campylobacteriosis is transmitted primarily through food of animal origin, the presence of antimicrobial-resistant strains in meat is of serious concern to food safety and public health.

Keywords: Thermophilic *Campylobacter*, Beef, Mutton, Camel meat, Antimicrobial Resistance, Sokoto

Introduction

Campylobacter is a leading cause of human gastroenteritis worldwide. The most important *campylobacter* spp associated with human illness are *Campylobacter jejuni* and *Campylobacter coli* (Wenley et al., 2000). Food animals may harbour this organism in their intestine asymptomatically and animal food products can become contaminated by this pathogen during slaughter and carcass dressing (Jorgensen et al., 2002; Whyte et al., 2004). Campylobacter infections occur as a result of consumption of raw or insufficiently cooked meat especially poultry meat (Evans et al., 1998; Friedman et al., 2000; Andersen et al., 2006). Foods of animal origin are considered as important sources of infection (Mead et al., 1999; Salihu et al., 2009; Salihu et al., 2010). Cross-contamination of ready-to-eat foods during food preparation with *Campylobacter* spp as well as direct contact with animals infected with *Campylobacter* has been reported (Hussain et al., 2007).

Antibiotics are extensively used worldwide in human and veterinary medicine for treatment and prevention of microbial diseases and as a feed additive for growth promotion (Philips et al., 2004). This has contributed to the development of antimicrobial resistance in many enteric bacteria and encouraged the persistence and transfer of antimicrobial resistance determinant in microbial genomes (Houndt and Ochman, 2000; Serum and L'Abee-lund, 2002). Antibiotic resistance is an increasing problem in some pathogens and the capability of multidrug resistance is of serious concern. In zoonotic bacteria such as Campylobacters, use of antimicrobial agents especially antibiotic in animals can enhance a drug-resistant bacteria population that may pose a potential threat to the consumer (Kurincic, et al., 2005).

There is paucity of information regarding the prevalence and antimicrobial susceptibility patterns of

Campylobacter in raw meat in Nigeria. This study was designed to determine the prevalence and antibiotic resistance of campylobacter species isolated from beef, mutton and camel meat in Sokoto metropolis.

Materials and Methods

A total of 531 raw meat samples were randomly collected from meat processing facilities and retail stalls in Sokoto. This was carried out from March 2008 to February 2009. A total of 242 beef, 181 mutton and 108 camel samples collected. Each collected sample was placed in separate sterile plastic bags to prevent spilling and cross contamination and immediately transported to the laboratory in ice pack cooler.

The samples were processed immediately on arrival at the laboratory using aseptic techniques. For each of the meat samples, 25g was homogenized and added with 225ml of Preston broth (CM67 plus selective supplements SR117 and SR48; Oxoid, UK) incubated at 35^{o}C for 4hours followed by incubation at 42^{o}C for 48hours in microaerophilic condition (10% CO_2, 5% O_2, 85% N_2) generated by a gas generating pack (CampyGen, CN35, Oxoid). One loopful (10µl of broth was streaked onto plates containing modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA) (CM739 and SR155, Oxoid). The inoculated plates were incubated at 42^{o}C for 24-48hours under microaerophilic condition. Following this incubation the isolates were examined for characteristic colony formation (gray, moist and flat spreading on mCCDA). One to three typical colonies were randomly selected and subcultured and presumptive isolates were identified to the species level using standard microbiological and biochemical procedure including Grams staining, growth at 25^{o}C, production of catalase, and oxidase, hippurate hydrolysis, urease activity, indoxyl acetate hydrolysis, and susceptibility to cephalotin. Isolates that do not show growth at 25^{o}C were identified as thermophilic campylobacter species (TCS) (ISO, 2006). Among the thermophilic strains, those that hydrolysed hippurate were identified as *C. jejuni*, while strains that are hyppurate negative and showing a positive indoxyl acetate hydrolysis were identified as *C. coli*. *Campylobacter* strains those that showed negative indoxyl acetate hydrolysis was identified as *C. lari* (ISO, 2006).

The isolates were subjected to antibioticl susceptibility testing using the disc diffusion method described by CLSI, (2006). Mueller-Hinton agar plates (CM337, Oxoid) with 5% sheep blood was used. The following antibiotic impregnated discs were used for the susceptibility study; nalidixic acid (30µg), ciprofloxacin (15µg), tetracycline (15µg), erythromycin (15µg), streptomycin (30µg) gentamycin (10µg), ampicillin(10µg), amoxicillin (30µg), chloramphenicol (30µg) and enrofloxacin (10µg). Susceptibility of the isolates to the antibiotic agents was determined according to the CLSI, (2006) after incubation for 48hours at 42^{o}C microaerobically.

Results

One hundred and thirty nine (26.33%) of the 528 meat samples were positive for *Campylobacter* spp. isolates. The prevalence rates in the individual samples are beef (22.08%), mutton (37.22%) and camel meat (17.59%). The rate of *C. jejuni*, *C. coli* and *C. lari* isolations within the positive isolates were 69.81%, 22.64% and 1.49% respectively in beef carcasses; 91.05%, 7.46% and 1.49% respectively in lamb carcasses. The prevalence of *C. jejuni*, *C. coli* and *C. lari* in camel meat was 26.31%, 57.90% and 15.76% (Table 1).

Seventy (50%) of the campylobacter isolates were selected for the antibiotic susceptibility test. This comprised 52 (*C. jejuni)*, 14 (*C. coli*) and 4(*C. lari*) from beef, mutton and camel meat. The resistance pattern of the isolates to10 antibiotics tested in this study is as shown in Table 2. A total of 63 (90.0%) of the 70 campylobacter isolates (90.0%) showed resistance to one or more antibiotics. Nineteen (27.1%) of the isolates were resistant to single antibiotic, while 20 (28.6%) were resistant to two antibiotic agents. Campylobacter isolates showing multidrug resistance were 24 (34.3%). Resistance to tetracycline was the most common (71.4%), followed by resistance to ciprofloxacin (42.9%) and nalidixic acid (37.1%) (Table 2). All the isolates were susceptible to chloramphenicol and gentamycin. *Campylobacter lari* isolates were also susceptible to amoxicillin, erythromycin and nalidixic acid.

Table 1: Number and percentage isolate of *Campylobacter* spp from beef, mutton and camel meat

Species	Beef (n=240)	Mutton (n=180)	Camel (n=108)	Total (n=528)
Campylobacter Spp	53(22.08%)	67(37.22%)	19(17.49%)	139(26.33%)
C. jejuni	37(69.81%)	61(91.05%)	5(26.31%)	103(74.10%)
C. coli	12(22.64%)	05(07.46%)	11(57.90%)	28(20.14%)
C. lari	04(01.49%)	01(1.49%)	03(15.79%)	08(05.76%)

Table 2: Antimicrobial resistance of *Campylobacter* isolates from raw meat

Antibiotics	*Campylobacter* spp. (n=70)	*C. jejuni* (n=52)	*C. coli* (n=14)	*C. lari* (n=4)
Amoxicilin	3(04.30%)	2(03.90%)	1(07.1%)	0(00.0%)
Ampicilin	9(12.9%)	7(13.5%)	1(07.1%)	1(25.0%)
Chramphenicol	0(00.0%)	0(00.0%)	0(00.0%)	0(00.0%)
Ciproflaxacin	30(42.9%)	26(50.0%)	3(21.4%)	1(25.0%)
Enroflaxacin	19(27.1%)	15(28.8%)	2(14.3%)	1(25.0%)
Erythromycin	10(14.3%)	8(15.4%)	2(15.4%)	0(00.0%)
Gentamycin	0(00.0%)	0(00.0%)	0(00.0%)	0(00.0%)
Nalidixic acid	26(37.1%)	22(42.3%)	4(28.6%)	0(00.0%)
Streptomycin	7(10.0%)	5(09.6%)	1(07.1%)	1(25.0%)
Tetracycline	50(71.4%)	37(71.2%	10(71.4%)	3(75.0%)
Resistance to one antibiotic	19(27.1%)	11(57.9%)	06(31.6%)	2(10.5%)
Resistance to two antibiotics	20(28.6%)	14(70.0%)	5(25.0%)	1(05.0%)
Resistance to more than two antibiotic	24(34.3%)	19(79.1%)	3(12.5%)	2(8.30%)

Discussion

The contamination of raw meat with *Campylobacter* spp in this study was 26.33% (139 of 528). The study showed that camel meat had the lowest contamination level; this findings may be linked to low rate of daily slaughter of camel in the state, which give the processors at the camel unit ample time to ensure maximum hygiene, when compared to cattle unit where higher number of cattle are slaughter daily. The low contamination level in camel may also be associated with the fact that there are lower population of heterogenic bacteria in the rumen of the camel leading to the accumulation of hydrogen (H_2) which affects the survival of some bacteria such as *Campylobacter* spp. However, *Campylobacter* spp has been isolated from faeces of camel (Salihu et al., 2009b).

The prevalence of *Campylobacter* spp. in the beef samples in this study was 22.08%, which was higher than reported (Ono and Yamamoto, 1999; Whyte et al., 2004; Bostan et al., 2009; Rahimi et al., 2010). *Campylobacter jejuni* was the most common isolates accounting for 69.81% of the total isolates from beef meat. This is in contrast to the report of Bostan et al., (2009) who reported that *C. coli* was the most frequently isolated *Campylobacter* species from beef. The prevalence of *Campylobacter* in mutton showed 37.22%. This is higher than the report by Rahimi et al., (2010). The frequently isolated *Campylobacter* spp. from mutton was *C. jejuni* 91.05%. The higher prevalence of *Campylobacter* positive samples in the present study may be associated to cross-contamination during manual flaying, evisceration and processing in the slaughter house and during transportation.

Generally, *C. jejuni* was the most common *campylobacter* spp recovered from the meat samples in this study. Other studies identified *C. jejuni* as the most common *Campylobacter* spp. isolates from foods of animal origin, especially poultry meat (Zanetti et al., 1996; Ghafir et al., 2007; Hussain et al., 2007; Salihu et al., 2009a). The higher prevalence rates of *C. jejuni* in this study from raw meat are in agreement with reports from other countries (Whyte et al., 2004; Ghafir et al., 2007; Hussain et al., 2007). The variance observed in the level of isolation of campylobacter from raw meat samples reported in other studies may be due to different sampling techniques used, seasonal effects and laboratory method employed in various studies.

The results of antimicrobial susceptibility testing in this study indicate that the isolates were in general resistant to the tested antibiotics at rates ranging from 4.3% to 71.4%. The rates were comparable to those reported by other investigator (Yildirin et al., 2005; Taremi et al., 2006; Bostan et al., 2009; Rahimi et al., 2010). There was higher resistance of campylobacter isolates to tetracycline (71.4%), ciprofloxacin (42.9%) and nalidixic acid (37.1%) in this study. This observation is similar to that of Rahimi et al., (2010) in Iran. About 34.3% of the isolates were resistant to multiple (more than two antibiotics) antibiotic agents & the majority of the multidrug-resistant isolates were *C. jejuni* (76.1%). This findings is in line with reports of Yildirin et al., 2005; Taremi et al., 2006; Rahimi et al., 2010). The outcome of antibiotic resistance observed in this study correlates to antibiotics used in therapeutic treatment of infection in food animals in Nigeria.

Susceptibility to nalidixic acid is recommended for differentiation of *Campylobacter* strains at species level. However, increasing resistant to nalidixic acid has been reported (Yildrin et al., 2005; Kang et al., 2006; Sallam, 2007). In our study, 26 (37.1%) of the 70 isolates subjected to antibiotic resistance test were resistant to nalidixic acid. This result confirms the resistance development to nalidixic acid and indicates that the test of susceptibility to this antibiotic has lost its usefulness in identification.

Campylobacter is considered mainly a food-borne pathogen, with raw or undercooked poultry serving as

an important source of sporadic campylobacter infection (Blaser, 1997). The identification of antibiotic resistant *Campylobacter* organisms isolated in food-borne campylobacteriosis case may be compromised, because antibiotic resistant campylobacter strains cause more prolonged or severe illness (Traver and Barza, 2002).

The results of our study have demonstrated that high proportion of meat samples are contaminated with thermophilic *Campylobacter* spp. which may have serious effects on public health. Most of the isolates are antibiotic resistant strains. Campylobacteriosis is transmitted primarily through food of animal origin, the presence of antibiotic-resistant strains in meat is of serious concern to food safety and public health.

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