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Full Length Research Paper

Prevalence of *Campylobacter* in poultry meat in Sokoto, Northwestern Nigeria

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The study was conducted in Sokoto, Nigeria from November 2007 to October 2008 to investigate the presence of *Campylobacter* spp. in poultry meat, by standard culture techniques. *Campylobacter* was detected in 558 (81.9%) of the 681 meat samples, the isolates were characterized by conventional phenotypic tests. *Campylobacter jejuni* was the most commonly identified species accounting for 340 (60.9%) of the positive samples and 413 (62.3%) of the total isolates, while *C. coli* and *C. lari* had 156 (28.0%) and 39 (7.0%) of the positive samples respectively. Thermophilic strains not identified as *C. jejuni*, *C. coli*, or *C. lari* were grouped as "other thermophilic species" and these account for 23 (4.1%) of the positive samples and 32 (4.8%) of the total isolates. Among these, 19 (59.4%) of the 32 strains were also identified as *C. upsaliensis* while 5 (15.6%) were identified as *C. hyointestinalis.* The most frequently identified biotype of *C. jejuni, C. coli* and *C. lari* was biotype I which accounts for 58.8, 68.0 and 59.2% respectively of the total individual isolates. The presence of *Campylobacter* in poultry meat may play a role as food borne pathogen of more importance than hitherto reported.

Key words: Prevalence, *Campylobacter,* meat, poultry, biotypes, Sokoto.

INTRODUCTION

Campylobacter spp. is recognized worldwide as important food pathogen; it is the leading cause of zoonotic enteric human infections in most developed and developing countries (WHO, 2001; Anonymous, 2004). The main source of human *Campylobacter* infection is believed to be poultry meat based on the frequent occur-rence of *Campylobacter* in this food type (Anonymous, 2004; European Commission, 2004; Rosenquist et al., 2006) and the fact that case-control studies conducted worldwide repeatedly have identified handling of raw poultry and eating poultry products as important risk factors for sporadic campylobacteriosis (Schorr et al., 1994; Adak et al., 1995; Neal and Slack, 1997; Studahl and Andersson, 2000; Effler et al., 2001; Kapperud et al., 2003; Friedman et al., 2004; Rosenquist et al., 2006; Vincenza et al., 2007).

The presence of the organisms on poultry meat derives from symptomless intestinal carriage in the live bird which is rather frequent (Pezzotti et al., 2003; Anonymous, 2004; Workman et al., 2005). During slaughter the intestinal content will inevitably contaminate the carcasses (Oosterom et al., 1983; Berrang and Dickens, 2000; Stern and Robach, 2003) and since no process operations eliminate the organism, poultry meat is often contaminated with thermotolerant *Campylobacter* (Anonymous, 2004; European Commission, 2004). Consumer exposure to *Campylobacter* is unavoidable if chicken meat is not handled hygienically, that is by cross-contamination from raw poultry meat to ready-to-eat food and/or if the meat is not properly cooked before consumption (Humprey et al., 2001; Kusumaningrum et al., 2003; Rosenquist et al., 2006).

Surface contamination of poultry meat may occur during

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Table 1. Thermophilic *Campylobacter* isolates from raw poultry samples.

Campylobacter spp.	Number of isolates	Percentage isolate
C. jejuni	340	49.9
C. coli	156	22.9
C. lari	39	5.7
Other thermophilic	23	3.4
Total	558	81.9

slaughter, but is more common in the scalding, defeathering and evisceration phases, which facilitate the transmission of microorganisms from one carcass to another (Izat et al., 1988; Oosterom et al., 1983; Vincenza et al., 2007). The formation of contaminated aerosols during the defeathering phase is the source of contamination of not only the carcass (Hinton et al., 1996) but increase the risk of slaughter house workers contracting infection (Oosterom et al., 1983; European Food Safety Authority, 2005). Poultry meat has been incriminated as being responsible for 20 - 40% of all sporadic cases of infection in several countries (Nadeau et al., 2002). Microbiology surveys carried out in the United States (1999 - 2000) and in the United Kingdom (1998 - 2000) on raw poultry meat taken from retail stores demonstrated *Campylobacter* spp. contamination of 70.7 and 83.0% respectively (European Food Safety Authority, 2005; Zhao et al., 2001). Thermotolerant *Campylobacters* especially *Campylobacter jejuni* is responsible for extraintestinal forms (meningitis, peritonitis, pancreatitis, unrinary infections, neonatal sepsis and abortion) and some chronic immunomediated diseases (endocarditis nodal fever, reactive arthritis). Guillain-Barre syndrome (GBS), a neurological, post-infective form, is also associated with *C. jejuni* infection (Ang et al., 2004). Levels of *Campylobacter* contamination reported in the literature show a wide range of values which although generally low, are not comparable as they are affected by different sampling methods (Food Standard Agency, 2001) and by different poultry samples analyzed (Lake et al., 2003). The goal of this study was to determine the prevalence of thermotolerant *Campylobacter* in poultry meat in Sokoto-Northwestern, Nigeria.

MATERIALS AND METHODS

Study area

The study was carried out in Sokoto the capital city of Sokoto state of Nigeria. The state is located in the Northwestern part of the country, bordering the Republic of Benin to the West and Niger Republic to the North. The state falls within two vegetational zones; the Sudan savanna and Northern guinea savanna. The climate is semi-arid and characterized by alternating wet and dry seasons with a short cool and dry period 'harmattan'- which starts in late October and ends in late February. The mean monthly temperature is generally high, 20 - 38°C, with the highest temperature occurring in April. Relative humidity ranges from 12 to 71% with the highest occurring in August (Sokoto State, 2000). The state is ranked second in livestock production. Averages of 250 poultry of different species are slaughtered and processed daily in Sokoto.

Sampling and sample collection

The study was conducted between November, 2007 and October, 2008. A total of 681 raw poultry samples were collected from the three main poultry process plants within the metropolis during the study. The samples were transported in a cool pack within 2 - 3 h of collection to the laboratory for analysis. Twenty five grams of each of the collected materials was placed in a plastic bag containing 225 ml of broth and homogenized in stomacher blender. The selective enrichment both (Preston broth) with the following formulation was used: nutrient both (Oxoid, CM067B), Laked horsed blood (5%), growth supplement (Oxoid SR084E) and Preston *Campylobacter* selective supplement (Oxoid, SR117E). The homogenates were incubated in jars at 42°C for 48 h in micro-aerophilic atmosphere obtained using commercial gas generating kits (CAMPYgen) (Oxoid, CN35). Following the incubation, one loopful of culture was streaked onto plates of *Campylobacter* blood-free selective medium (modified CCDA-Preston) (Oxoid, CN739, SR155E) and incubated in modified atmosphere at 42°C for 48 h.

Campylobacter suspect colonies were subcultured, one or more times until monocultures were obtained. Curved or spiral Gram-negative rods, oxidase positive were presumptively identified as *Campylobacter.* Further biochemical investigation were performed for identification and biotyping of the strains (Baron et al., 1994); indoxyl-acetate hydrolysis (indoxyl-acetate reagent disks RMEL 21087), resistance to nalidixic acid and cephelotin. Rapid H_2S test (Lior, 1984) was carried out in a semisolid agar base medium supplemented with FBP (containing ferrous sulphate, sodium metabisulphite and sodium pyruvate) that corresponds to *Campylobacter* growth supplement (Oxoid, SR044E). Hippurrate hydrolysis test and DNase test (DNase agar, Oxoid, CM321) were also performed according to the scheme described by Lior (1984). Nitrate reduction and hydrogen sulphide production in triple sugar iron medium were used as additional tests for identification of the *Campylobacter* strains not identified as *C. jejuni* or *C. coli.*

RESULTS

Thermotolerant *Campylobacter* was isolated in 558 samples (81.9%). The results of identification showed that *C. jejuni* was isolated in 340 (49.9%) of the 681 raw poultry samples, *C. coli* in 156 (22.9%) of raw poultry samples, *C. lari* in 39 (5.7%) and other species were identified in 23 (3.4%) of the raw poultry samples (Table 1). In all 663 strains of *Campylobacter* were isolated as follows: *C. jejuni* 413 (62.3%), *C. coli* 169(25.5%), *C. lari* 49 (7.4%) and 32 (4.8%) as other *Campylobacter* spp (Table

Table 2. Identified strains of *Campylobacter* species from positive raw poultry samples.

Campylobacter species	Number of strains (%)	Number of positive samples (%)
Campylobacter jejuni	413(62.3)	340(60.9)
Campylobacter coli	169(25.5)	156(28.0)
Campylobacter lari	49(7.4)	39(7.0)
Other thermophilic spp.	32(4.8)	23(4.1)
Total	663	558

Table 3. Biotypes of *Campylobacter* species isolates from raw poultry samples.

Campylobacter species	No. of isolates (%)	Biotypes (%)			
		I	II	III	IV
Campylobacter jejuni	413(62.3)	243(58.8)	157(38.0)	9(2.2)	4(1.0)
Campylobacter coli	169(25.5)	115(68.0)	54(32.0)		
Campylobacter lari	49(7.4)	29(59.2)	20(40.8)		

N = number; (%) = percentage.

2). The results of biotyping of the thermotolerant *Campylobacter* isolates from poultry meats indicated that *C. jejuni* isolates showed a large prevalence of biotype I (58.8%). The *C. coli* and *C. lari* isolates also showed high prevalence of biotype I (68.0%) and (59.2%) respectively (Table 3). Thermophilic strains not identified as *C. jejuni, C. coli* or *C. lari* were grouped as "other thermophilic species". Among these, 19 (59.4%) out of the 32 strains were identified as *C. upsaliensis* and 5 (15.6%) were also identified as *C. hyointestinalis,* while doubtful biochemical results did not yield a complete identification of the remaining strains.

DISCUSSION

The result of the survey indicates a high prevalence of contamination with thermotolerant *Campylobacter* in poultry meat in Sokoto, Nigeria. The prevalence of *Campylobacter* (81.9%) observed in this study is in line with values reported by other authors (Hood et al., 1988; Jones et al., 1991a, b; Karib and Seeger, 1994; Lee et al., 1994; Cocchi et al., 2001; Lake et al., 2003; Pezzotti et al., 2003 Nannapaneni et al., 2005; Workman et al., 2005; Valdivieso-Garcia et al., 2007). These authors explained that the prevalence rate in poultry meat and product is influenced by a series of factors such as time of the year when sampling is performed, product storage type, sampling time, product batch. All these factors were not considered in our study. The contamination rate observed in this study may be attributed to that damage to intestinal tract during evisceration can lead to direct contamination of the carcasses. Contamination can also occur indirectly through the hands of the processors and material or instrument used in processing.

The common *Campylobacter* species found in poultry meat in this study was *C. jejuni* (49.9%) of the raw poultry samples and accounting for 62.3% of the isolated strains. The prevalence of *C. jejuni* in this study is however, lower than reports of other studies report 77.3% (Kramer et al., 2000), 74.0% (Food Standard Agency, 2001), 68% (Lake et al., 2003), 81.9% (Vincenza et al 2007). The differences observed could due to the type of samples, size of samples and method of isolation. Studies have shown that higher prevalence rate is observed in intestinal and liver samples. There are reports from other studies (Kramer et al., 2000; Nielsen and Nielsen, 1999) that more than one *Campylobacter* species may be found in the same sample. These reports support the isolation of more than one strain of *Campylobacter* from most of the positive raw poultry samples in this study. The rate of isolation of *C. jejuni* and *C. coli* from chicken carcasses in this study conformed with findings of Schoeni and Doyle (1992), Hernandez (1993), Chuma et al. (1997), Se (2003), Vincenza et al. (2007) who reported isolation of *C. jejuni* and *C. coli* from poultry meat and products. The isolation of "other" thermophilic species suggests that they may represent a predominant cluster and among these *C. upsaliensis* seemed to be particularly common. The isolation of *Campylobacter* species in this study is of serious public health concern, because *C. jejuni, C. coli* and *C. lari* have been implicated in causing human disease (Skirrow, 1998; Altekruse et al., 1999; Rautelin and Hanninen, 2000).

The identification of *C. jejuni* biotype I and *C. coli* biotype I as the predominant biotypes in this study is in agreement with the findings of Pezzotti et al. (2003). The implication of the isolations of these *C.* biotypes in this study, is that similar biotypes (*C. jejuni* and *C. coli* biotype I) prevailed in humans as reported by Lior (1984) and Varoli

et al. (1991), while biotype II was more common in animals. It is apparent that isolates of poultry meat in this study is closer to results obtained from humans by some authors (Pezzotti et al., 2003) thus, reinforcing the hypothesized role of these products as vehicles of infection to man.

In conclusion, the results allowed gathering of information on the distribution of *Campylobacter* in Northwestern Nigeria, a particularly important geographical zone for animal production and animal product processing. The presence of *Campylobacter* in poultry meat may play a role as food borne pathogen of more importance than hitherto reported. On the basis of the findings, formulation and implementation of specific control procedures is strongly recommended in order to improve the protection of the public against campylobacteriosis.

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