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Prevalence and Antimicrobial Resistance of Thermophilic *Campylobacter* Isolates from Commercial Broiler Flocks in Sokoto, Nigeria

M.D. Salihu, A.U. Junaidu, A.A. Magaji and Y. Yakubu
Department of Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto, Nigeria

Corresponding Author: M.D. Salihu, Department of Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto, Nigeria Tel: +2348035950593

ABSTRACT

The study was carried out to determine the prevalence and the antimicrobial resistance profile of thermophilic *Campylobacter* sp. from broiler chickens. A total of 270 cloacal swab samples from randomly selected commercial broiler farms in the state capital were collected and examined for the presence of *Campylobacter* sp. The prevalence of *Campylobacter* was 139 (51.5%). *Campylobacter jejuni* constitute 87 (62.6%) of the positive isolates while *C. coli* and *C. lari* accounts for 30 (21.6%) and 22 (15.8%), respectively of the positive isolates. The results of antimicrobial susceptibility testing shows that isolates were resistant to the tested agent except Chloramphenicol. The resistance rates ranges from 11.6 to 32.1%. A total of 92 (82.1%) of the tested isolates showed resistance to one or more antibiotics. Twenty one (18.7%) of the isolates were resistant to single antibiotic, 30 (26.8%) were resistant to two antibiotics while 41 (36.6%) were resistant to more than three antibiotics. Resistance to ampicillin was more accounting for about 32.1% and lowest resistance was observed with erythromycin accounting for 11.6% . All the isolates were susceptible to chloramphenicol. The isolation of antibiotic-resistant strains from broiler chickens is of serious concern to food safety and public health.

Key words: Broilers, thermophilic campylobacter, antimicrobial resistance, prevalence, sokoto

INTRODUCTION

Thermophihc *Campylobacter* sp., primarily are *Campylobacter jejuni* and *C. coli* are among the most common bacteria causing acute human gastroenteritis throughout the world (Snelling *et al.*, 2005; Trachoo and Brooks, 2005; Humphrey *et al.*, 2007; Englen *et al.*, 2007; WHO, 2002; Dipineto *et al.*, 2008). Both species colonize the intestinal mucosa of most warm-blooded animals, including food-producing species and humans (Newell and Fearnley, 2003; Fallon *et al.*, 2003). Many avian species are considered the main reservoirs of *Campylobacter* sp. (Sahin *et al.*, 2002; Newell and Fearnley, 2003; Saleha, 2004; Lee and Newell, 2006). Poultry meat has been established as an important source of human infection with both *C. jejuni* and *C. coli* (Skirrow, 1991; Pearson *et al.*, 1996; Ramabu *et al.*, 2004; Baserisalehi *et al.*, 2006; Salihu *et al.*, 2009a; Frederick and Huda, 2011. A significant association exists between *Campylobacter* infection in humans and consumption of contaminated poultry products as reported by Altekruse *et al.*

(1999), Stern *et al.* (2001) and Friedman *et al.* (2004). Epidemiological studies have examined risk factors for the infection with *Campylobacter* such as the presence of other animals on the farm, contamination of previous flocks as well as vertical transmission (Gregory *et al.*, 1997; Newell and Fearnley, 2003).

Several studies have revealed high level of antimicrobial resistance in thermophilic *Campylobacter* sp. in poultry and humans. In animals and humans, the use of antibiotics may cause an increase in the resistance of their enteric flora. Resistant bacteria from animals can reach the human population by direct contact and also via food products of animal origin (Fallon *et al.*, 2003; Van de Bogaard and Stobberingh, 2000). The consumption of poultry meat has been commonly associated with the development of *Campylobacter* enteritis (Deming *et al.*, 1987; Evans, 1992; Van de Giessen *et al.*, 1992). Resistance among *Campylobacter* isolates from broilers may have implications for the treatment of poultry-acquired *Campylobacter* infections (Fallon *et al.*, 2003). There is dearth of information on the prevalence and antimicrobial resistance profiles among thermophilic *Campylobacter* isolates of poultry origin in Nigeria.

The study was carried out to determine the prevalence of thermophilic *Campylobacter* species and the antimicrobial resistance profiles of the isolates from broiler flocks.

MATERIALS AND METHODS

The study area was Sokoto state which is the second largest livestock producer in Nigeria. There are various species of livestock in the state these includes various species of poultry. Commercial poultry production is gradually gaining acceptance in the state. However, there are more commercial poultry farming activities in Sokoto the capital city of the state than other parts of the state. Most households and major restaurants and fast food centers within Sokoto metropolis depends on the commercial broiler poultry farms in Sokoto for broiler poultry meat supply.

Sample collection: A total of 270 cloacal swab samples from randomly selected commercial broiler farms in the state capital were collected. The samples were collected in August, 2009 and cover a period of 20 days. The collected samples were individually packed and transported on ice to the Veterinary Public Health laboratory of Usmanu Danfodiyo University, Sokoto within 3 hours of collection.

Isolation and identification: The *Campylobacter* species were isolated from cloacal swab samples using a direct plating method. The swabs were cultured on modified Charcoal Cefoperazone deoxycholate Agar, mCCDA (Oxoid) supplemented with selective supplement (SR155, Oxoid). The plates were incubated under microaerophilic conditions generated using Campygen® (oxoid), for 48 h at 42°C. Isolates that were small, curved, catalase and oxidase positive, Gram negative bacilli were presumed *Campylobacter* sp. The presumed *Campylobacter* isolates were identified to species level based on hydrolysis of sodium hippurate and indoxylacetate, production of H_2S in triple sugar iron agar and susceptibility to cephalotin and nalidixic acid as described by On and Holmes (1992) and ISO (2006). Among the thermophilic strains those that hydrolysed hippurate were identified as *C. jejuni*, while strains that are hippurate negative and showing a positive indoxyl acetate hydrolysis were identified as *C. coli*. *Campylobacter* strains that showed negative indoxyl acetate hydrolysis are identified as *C. lari* (ISO, 2006).

Antimicrobial susceptibility testing: A total of 112 (80%) of the *Campylobacter* isolates comprising *C. jejuni* (70), *C. coli* (24) and *C. lari* (18) were tested for resistance to streptomycin (10 µg), erythromycin (15 µg), Ciprofloxacin (5 µg), ampicillin (10 µg), tetracycline (30 µg) and chloramphenicol (30 µg) by the agar disc diffusion method. All the antimicrobial discs were sourced from Oxoid. A suspension of approximately 0.5 Mcfarland standard prepared in Mueller Hinton agar (Oxoid) supplemented with 5% (V/V) defibrinated sheep blood and incubated at 37°C for 48 h microaerobically. The inhibition zones were recorded and interpreted according to the guidelines of National Committee of Clinical Laboratory Standard (NCCL, 1999) and methods of Khosravi *et al.* (2009) and Jafari *et al.* (2009).

RESULTS

Prevalence of thermophilic campylobacter: Out of the 270 cloacal swabs processed, 139 (51.5%) were positive for thermophilic *Campylobacter* sp. *Campylobacter jejuni* constitute 87 (62.6%) of the positive isolates while *C. coli* and *C. lari* accounts for 30 (21.6) and 22(15.8%), respectively of the positive isolates (Table 1). The results of antimicrobial susceptibility testing shows that isolates were resistant to the tested agents except Chloramphenicol. The resistance rates ranges from 11.6 to 32.1%. The resistance pattern of the isolates to the antimicrobial agents is as shown in Table 2. A total of 92 (82.1%) of the tested isolates showed resistance to one or more antibiotics. Twenty one (18.7%) of isolates were resistant to single antibiotic, 30 (26.8%) were resistant to two antibiotics while 41 (36.6%) were resistant to more than three antibiotics. Resistance to ampicillin was more accounting for about 32.1% and lowest resistance was observed with erythromycin accounting for 11.6% (Table 2). All the isolates were susceptible to chloramphenicol.

DISCUSSION

The prevalence of *Campylobacter* sp. in this study is 51.5% (139/270) and this is in line with findings of similar studies carried out in other countries (Jozwiak *et al.*, 2006; Parisi *et al.*, 2007;

Table 1: Prevalence of *Campylobacter* sp. isolates from broiler poultry

Campylobacter sp.	Positive isolates (n = 139)	Positive isolates (%)
Campylobacter jejuni	87	62.6
Campylobacter coli	30	21.6
Campylobacter lari	22	15.8
Total	139	100

Table 2: Antimicrobial resistant *Campylobacter* strains isolated from broiler poultry

Antimicrobials	*C. jejuni* (n = 70)	*C. coli* (n = 24)	*C. lari* (n = 18)	Total (n = 112)
Streptomycin	6 (8.6)	6 (25.0)	2 (11.1)	14 (12.5)
Ciprofloxacin	15 (21.4)	4 (16.7)	3 (16.7)	22 (19.6)
Erythromycin	9 (12.9)	2 (8.3)	2 (11.1)	13 (11.6)
Ampicillin	27 (38.6)	5 (20.8)	4 (22.2)	36 (32.1)
Tetracycline	13 (18.6)	7 (29.7)	7 (38.9)	27 (24.1)
Chloramphenicol	-	-	-	-
Resistance one antibiotic	10 (14.3)	4 (16.7)	7 (38.9)	21 (18.7)
Resistance to two antibiotics	17 (24.3)	9 (37.5)	4 (22.2)	30 (26.8)
Resistance to more two antibiotics	28 (40.0)	7 (29.2)	8 (44.4)	41 (36.6)

Values in bracket indicate percentage

Baserisalehi *et al*., 2007; Cokal *et al*., 2009). *Campylobacter jejuni*, *C. coli* and *C. lari* were the species isolated in this study. The isolation of these *Campylobacter* sp. from broiler is of public health importance, as these species of *Campylobacter* are known to cause infection in humans. These species have been reported in other species of animal including cattle (Salihu *et al*., 2009a), goats (Salihu *et al*., 2009b), Sheep (Salihu *et al*., 2009c) free range chickens (Salihu *et al*., 2009d), dogs and cats (Salihu *et al*., 2010). Litter materials from broiler poultry farms in the study area are usually collected for use as manure in the farms; this may contaminate the soil and water body around the farms.

Although, *Campylobacter* infections are usually self-limiting and antibiotics are not required, severe cases often require treatment. Antibiotic-resistant *Campylobacter* species from animals can colonise or infect the human population via occupational exposure or through the food chain (Van de Bogaard and Stobberingh, 2000; Fallon *et al*., 2003).

In this study, the campylobacter isolates were resistant to all the antimicrobial agents used except Chloramphenicol. The level of resistance of the isolates to ampicillin in this study is 32.1% which is also the highest resistance level observed. This observation correlate with the work of Narvarro *et al*. (1993) who reported that ampicillin and other β-lactams are usually not recommended for the treatment of *Campylobacter* species infection due to high incidence of resistant to the drug family by the organisms. The resistance pattern of *C. jejuni* observed in this study is in line with the report of Fallon *et al*. (2003), but in contrast to the report of Cokal *et al*. (2009) who reported that *C. jejuni* strains from broilers were susceptible to ampicillin. Erythromycin was considered as the drug of choice for the treatment of *C. jejuni* infections and in this study, the level of resistance of the isolates to erythromycin is generally low and similar to the findings of Luccy *et al*. (2000), who reported 9.7% resistance to erythromycin by poultry *campylobacter* isolates. However, 9 (12.9) and 2 (8.3%) of *C. jejuni* and C. *coli*, respectively were showed resistance to erythromycin. This observation is contrary to the observations of Cabrita *et al*. (1992) and Aarestrup *et al*. (1997) who reported higher levels of resistance to erythromycin by *C. coli* isolates from food animals. Higher levels of resistance to erythromycin by *Campylobacter* from clinical isolates have been reported (Rautelin *et al*., 1991; Jozwiak *et al*., 2006). Aminoglycoside resistance has been reported less frequently in *C. jejuni* and *C. coli* (Trieber and Taylor, 2000). The resistance levels of *Campylobacter* isolates to streptomycin in this study is higher than the report of Cabrita *et al*. (1992) and Fallon *et al*. (2003) who reported lower resistance between 0 and 6% for *C. jejuni* and *C. coli* from broilers. Tetracyclines have been listed as an alternative drug for treatment of *Campylobacter* gastroenteritis in the past and are widely used both therapeutically and subtherapeutically as feed additives for livestock and poultry (Trieber and Taylor, 2000). Resistance to tetracycline ampicillin was second in this study. In a similar study in Irish, Luccy *et al*. (2000) reported 19.4% tetracycline resistance for poultry isolates. Fallon *et al*. (2003) also reported 20.5% for *C. jejuni* and 18.2% for *C. coli*. However, in Taiwan, tetracycline resistance level is high with 83% of *C. jejuni* and 90% of *C. coli* isolates from chicken products (Li *et al*., 1993). All *campylobacter* isolates tested in this study were susceptible to chloramphenicol. Some studies have reported rare resistance to chloramphenicol by *campylobacter* isolates from livestocks and humans (Trieber and Taylor, 2000; Moore *et al*., 2001; Aquino *et al*., 2002; Fallon *et al*., 2003; De Vega *et al*., 2005). The zero resistance of *Campylobacter* to chloramphenicol observed in this study may be due to the banned on the use of the drug for both human and livestock. However, a study by Baserisalehi *et al*. (2007) recorded resistance to chloramphenicol by *Campylobacter* isolates from India and Iran.

It is obvious that the irrational usage of antimicrobials in animal production can lead to development of resistance in zoonotic bacteria; products from such animals can be source of transmission of resistance pathogens to humans. About 36.6% of the isolates were resistance to multiple (more than two antibiotics) antibiotic agent and most of the multiple drug resistant isolates were *C. lari* and *C. jejuni* (44.4 and 40.0%), respectively.

The results of this study have shown that *Campylobacter* isolates from broiler poultry are resistant to antimicrobial agent. The isolation of antibiotic-resistant strains from broiler is of serious concern to food safety and public health.

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