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

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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 (62.8%) 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
Thermophilic 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; Dipinoto 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; Basemisalehi 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 Boegaard 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., 2006). 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 Cefoperezone 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, estalase 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 H₂S 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 (NCCLS, 1999) and methods of Khrarvaf 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;

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<tr>
<th>Table 1: Prevalence of Campylobacter sp. isolates from broiler poultry</th>
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<tr>
<td><strong>Campylobacter sp.</strong></td>
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<tr>
<td><em>Campylobacter jejuni</em></td>
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<tr>
<td><em>Campylobacter coli</em></td>
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<td><em>Campylobacter lari</em></td>
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<td><strong>Total</strong></td>
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<th>Table 2: Antimicrobial resistant Campylobacter strains isolated from broiler poultry</th>
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<tr>
<td><strong>Antimicrobials</strong></td>
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<td>---------------------</td>
</tr>
<tr>
<td>Streptomycin</td>
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<tr>
<td>Ciprofloxacin</td>
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<td>Erythromycin</td>
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<td>Ampicillin</td>
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<td>Tetracycline</td>
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<td>Chloramphenicol</td>
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<td>Resistance to two antibiotics</td>
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<td>Resistance to more two antibiotics</td>
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Values in bracket indicate percentage
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 Lucey 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 (Triebel 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 (Triebel and Taylor, 2000). Resistance to tetracycline ampicillin was second in this study. In a similar study in Irish, Lucey 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 livestock and humans (Triebel 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 Baseresalehi 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.

**REFERENCES**


