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Antimicrobial resistance of *Campylobacter jejuni* and *Campylobacter coli* isolated from chickens in a diagnostic laboratory

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The aim of this study was to determine the resistance profile of 24 *Campylobacter jejuni* and 16 *Campylobacter coli* isolates from chickens in a diagnostic laboratory in Nigeria. Susceptibility testing was done by a broth microdilution MIC method with MICRONAUT – S anaerob test plates (Merlin Diagnostika, GmbH, Germany). MIC assay was performed according to CLSI (formally NCCLS) methods. Resistance to ciprofloxacin (57.5%) was the highest, followed by nalidixic acid (47%), tetracycline (35%) and trimethoprim/sulphamethoxazole (22%). *Campylobacter jejuni* were more resistant than *Campylobacter coli* to nalidixic acid, tetracycline and trimethoprim/sulphamethoxazole while *Campylobacter coli* were more resistant than *Campylobacter jejuni* to erythromycin and streptomycin. 47.5% of the isolates were multi - drug resistant with nalidixic acid and ciprofloxacin as the most frequently occurring antimicrobial agent in the pattern. This work has shown that majority of the *Campylobacter* isolates were resistant to most of the antimicrobial agents used with multi - drug resistance, thus the need for surveillance and rational use of antimicrobial agents in poultry production.

Key words: Antimicrobial resistance, campylobacter, chickens, diagnostic laboratory, MIC, multi-drug resistance.

INTRODUCTION

Campylobacter species are among the leading cause of bacterial enteritis in humans throughout the world (Friedman et al., 2000). Campylobacteriosis is a zoonotic disease with domestic animals as well as wild animals acting as reservoirs for *Campylobacter* species (Padungton and Kaneene, 2003). The emergence of antimicrobial resistance species due to the use of antimicrobial agents in husbandry is a matter of concern (Luber et al., 2003). Several studies have linked the use of antimicrobial agents in Veterinary Medicine to the emergence and spread of resistance among *Campylobacter* with potentially serious effects on food safety in both veterinary and human health (Endtz et al., 1991; Deckert et al., 2010; Economou et al., 2015).

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Table 1. Antimicrobial resistance of *Campylobacter* isolates from chickens.

Antimicrobial agent	Break point of resistance (µg/ml)	N (%) resistance		
		C.j (n=24)	C.c (n=16)	T (n=40)
CP	>4	19 (79.2)	4 (25.0)	23 (57.5)
NAL	>32	15 (62.5)	4 (25.0)	19 (47.5)
AZM	>4	3 (12.5)	2 (12.5)	5 (12.5)
ERY	>8	0 (0)	2 (12.5)	2 (5.0)
CLIN	>8	0 (0)	0 (0)	0 (0)
CMP	>32	0 (0)	0 (0)	0 (0)
TET	>16	9 (37.5)	5 (31.3)	14 (35.0)
TLS	>4/76	7 (29.2)	2 (12.5)	9 (22.5)
GEN	>16	1 (4.2)	0 (0)	1 (2.5)
STREP	>16	2 (8.3)	5 (31.3)	7 (17.5)

CP = ciprofloxacin; NAL = nalidixic acid; AZM = azithromycin; ERY = erythromycin; CLIN = clindamycin; CMP = chloramphenicol; TET = tetracycline; TLS = trimethoprim/sulphamethoxazole; GEN = gentamicin; STREP = streptomycin; n = number; % = percentage; C. j = *Campylobacter jejuni*; C.c = *Campylobacter coli*; T = total.

Campylobacter resistance to antimicrobial agents have been reported in both developed and developing countries (Cardinale et al., 2003; Ge et al., 2013; Nobile et al., 2013). The situation seems to be more in developing countries where there is wide spread and uncontrolled use of antimicrobials in both veterinary and human health (Cardinale et al., 2003; Pollett et al., 2012). Recent studies in developed countries have also showed increased resistance to flouroquinolones and macrolides (Gu et al., 2009; Marinou et al., 2012). There is limited information on the prevalence of antimicrobial resistance in poultry in most developing countries, including Nigeria. This study was designed to determine the antimicrobial resistance of thermophilic *Campylobacter* species isolated from chickens in Nigeria.

MATERIALS AND METHODS

A total of 40 *Campylobacter* isolates (*C. jejuni* = 24; *C. coli* = 16) from a collection of 68 strains isolated from the caecal samples of chickens submitted for routine diagnostic tests to a central Diagnostic laboratory located in Plateau state, north central Nigeria, from different parts of the country between 2008 – 2009 was used for this study. The remaining 28 isolates could not be recovered on culture. The caecal contents were cultured on modified charcoal, cefoperazone deoxycholate agar (MCCDA), (Oxoid, Basingstoke, UK) and incubated at 42^{o}C under microaerobic condition generated by CampyGen^{R} (Oxoid) for 48 hr. The identification of *Campylobacter* species was based on colony and microscopic morphology, motility, oxidase, catalase, hippurate hydrolysis and indoxyl acetate tests. The isolates were confirmed using multiplex PCR as described by Wang et al. (2002). Susceptibility testing was performed by a broth micro dilution method with MICRONAUT – S anaerob test plates (Merlin Diagnostika, GmbH, Germany) following the Clinical and Laboratory Standards Institute (CLSI, formally NCCLS) guidelines (CLSI, 2007) as described by the manufacturer. We tested the following ten (10) antimicrobial agents at the indicated concentration ranges: ciprofloxacin and azithromycin 0.0625 – 8 µg/ml; clindamycin, tetracycline and gentamicin 0.125 – 16 µg/ml; erythromycin, streptomycin and chloramphenicol 0.5 – 64 µg/ml; nalidixic acid 1 – 128 µg/ml and trimethoprim/sulphamethoxazole 0.0625/1.1875 – 8/152 µg/ml. Isolates were grown on Mueller – Hinton agar plates (Oxoid) with 5% horse blood and were incubated for 48 h at 42°C in a microaerobic atmosphere (10 % CO_2, 5 % O_2 and 85 % N_2). Several colonies of *Campylobacter* were transferred into 2 ml Wilkins Chalgren broth (Sigma - Aldrich) until the turbidity matches a McFarland of 0.5. Then 200 µl of the bacteria suspension was pipetted into 11 ml Mueller – Hinton II broth supplemented with 2.5 % laked horse blood and homogenized well. One hundred microliter (100 µl) of the suspension was inoculated into each well of the 96 well MICRONAUT – S plate using a multichannel pipette. After the inoculation the plates were covered with the perforated plate sealer and incubated at 42°C for 24 – 48 h under microaerobic conditions (10 % CO_2, 5 % O_2 and 85 % N_2). After incubation, the plates were removed and read visually under a black background. The MICs were defined as the lowest concentration where no viability was observed in the wells of the microplates after incubation.

The MIC break points used for resistance to the antimicrobials were chosen on the basis of earlier publications (Luber et al., 2003; Hakanen et al., 2003; Ge et al., 2003). They were > 4 µg/ml for ciprofloxacin and azithromycin; > 8 µg/ml for erythromycin and clindamycin; > 16 µg/ml for tetracycline, gentamicin and streptomycin; > 32 µg/ml for nalidixic acid and chloramphenicol; > 4/76 µg/ml for trimetoprim/sulphamethoxazole. Multiresistance was defined as resistance to three or more antimicrobial agents.

Statistical analysis

The frequencies of resistance were tested between *Campylobacter* species using the Fisher's Exact Test for R x C contingency table (R = Rows and C = columns) using Microsoft Excel software for Windows.

RESULTS

The antimicrobial resistance among *C. jejuni* and *C. coli* isolates is presented in Table 1. Overall, resistance to ciprofloxacin (57.5%) was the most common, followed by nalidixic acid (47%), tetracycline (35%), trimethoprim/

Table 2. Multi-resistance patterns among isolates of *Campylobacter* species

Resistance patterns	N (%) resistance		
	C.j (n=8)	C.c (n=5)	T (n=13)
CP,NAL,TET	4 (50)	2 (40)	6 (46.2)
CP,NAL,TLS	1 (12.5)	0 (0)	1 (7.7)
TET,TLS,STREP	1 (12.5)	0 (0)	1 (7.7)
CP,NAL,TET,TLS	1 (12.5)	0 (0)	1 (7.7)
NAL,AZM,ERY,STREP	0 (0)	1 (20)	1 (7.7)
CP,AZM,ERY,TET,STREP	0 (0)	1 (20)	1 (7.7)
CP,NAL,TET,TLS,STREP	0 (0)	1 (20)	1 (7.7)
CP,NAL,AZM,TET,GEN,STREP	1 (12.5)	0 (0)	1 (7.7)

Susceptible to all antimicrobials = 8 (20%); Resistant to 1 antimicrobial = 13 (32.5%); Resistant to 1 or more antimicrobials = 32 (80%); Multi-resistant = 13 (32.5%) (C.j 8; 33.3%; C.c 5; 31.3%). CP = ciprofloxacin; NAL = nalidixic acid; AZM = azithromycin; ERY = erythromycin; TET = tetracycline; TLS = trimethoprim/sulphamethoxazole; GEN = gentamicin; STREP = streptomycin; n = number; % = percentage; C. j = *Campylobacter jejuni*; C. c = *Campylobacter coli*; T = total.

sulphamethoxazole (22.5%) and streptomycin (17%). A significantly higher resistance to ciprofloxacin, nalidixic acid and tetracycline was recorded ($P < 0.05$). Considering the resistance by species, *C. jejuni* were significantly more resistant than *C. coli* to ciprofloxacin, nalidixic acid and trimethoprim/sulphamethoxazole ($P < 0.05$). On the other hand, *C. coli* were significantly more resistant than *C. jejuni* to erythromycin and streptomycin ($P < 0.05$). All the isolates were susceptible to clindamycin and chloramphenicol. The overall resistance patterns exhibited by the isolates are shown in Table 2. Of the 40 *Campylobacter* strains tested, 32 (80%) were resistant to one or more of the antimicrobial agents tested while 8 (20%) were susceptible to all the antimicrobial agents. Thirteen (32.5%) were multiresistant, being resistant to three or more antimicrobial agents. Eight (33.3%) of the *C. jejuni* and 4 (31.3%) of the *C. coli* strains were multiresistant. Overall, tetracycline was the most frequently occurring antimicrobial agent being found in 6 of the 8 multiresistant patterns in this study followed by ciprofloxacin, nalidixic acid and streptomycin (5 of the 8 patterns), trimethoprim/sulphamethoxazole (4 of the 8 patterns) and Azithromycin (3 of the 8 patterns). The least was gentamicin being found in only one of the eight patterns. The multiresistant profile also showed that resistance to 3 antimicrobial agents were 3 while resistance to 4, 5 and 6 antimicrobial agents were 2, 2 and 1 respectively. Six (46.2%) of the isolates (*C. jejuni*, 4; *C. coli,* 2) were resistant to ciprofloxacin/nalidixic acid/tetracycline.

DISCUSSION

Results from recent susceptibility studies of *Campylobacter* species from poultry and poultry meat performed in different countries indicate substantial variation between countries. High resistance rates have been reported from Belgium (Habib et al., 2009), USA (Ge et al., 2003), Italy (Pezzotti et al., 2003; Nobile et al., 2013; Giacomelli et al., 2014) and Czech Republic (Bardon et al., 2008) while lower resistance rates have been reported from Australia (Miflin et al., 2007). Possible explanation for these differences has been due to different National and Regional policies in relation to the use of antimicrobial agents for food animals (Anderson et al., 2006). In this study, there was a high resistance to quinolones (ciprofloxacin and nalidixic acid) among the isolates. High resistance to flouroquinolones in poultry and poultry meat have been reported in many European countries, 72% in the Czech Republic (Bardon et al., 2008), 65% in Turkey (Cokal et al., 2008), and 82% in Spain (Prats et al., 2000). Similarly, high resistance to ciprofloxacin and nalidixic acid has been reported in other countries, 88 and 91% in Korea (Kang et al., 2006), 69.4 and 75% in Iran (Taremi et al., 2006). In contrast, Norstrom et al. (2007) reported no resistance to quinolones in *C. jejuni* isolated from broilers in Sweden. In this study, comparable resistance rates were observed for azithromycin, tetracycline, erythromycin, trimethoprim/sulphamethoxazole and streptomycin. Only 20% of the *Campylobacter* strains tested were susceptible to the antimicrobial agents tested, this finding provides evidence of potential role of chickens in the circulation of resistant *Campylobacter* strains in human and thus demands more careful attention on antimicrobial use in poultry production and veterinary medicine. Eighty percent of the strains were resistant to one or more of the antimicrobial agents with 32.5% being multiresistant (defined as resistance to 3 or more antimicrobial agents). This indicates that resistance to these antimicrobial agents in this study further confirm the global emergence of antimicrobial resistance of *Campylobacter* species.

The predominant multiresistant pattern of ciprofloxacin/nalidixic acid/tetracycline in this study is similar to other studies reported from other countries (Hakanen et al., 2003; Cokal et al., 2008; Nobile et al., 2013). It was observed that among isolates that were multi- resistant, ciprofloxacin, nalidixic acid, tetracycline and trimethoprim/sulphamethoxazole were prevalent in most patterns. This is in agreement with the findings of Rodrigo et al. (2007).

Resistance of *Campylobacter* strains in our study demonstrates a high resistance to ciprofloxacin, nalidixic acid, tetracycline and trimetoprim/sulphamethoxazole with higher resistance frequency in the *C. jejuni* than the *C. coli* strains. In a recent study by Lemos et al. (2015), a high resistance to nalidixic acid (100%), norfloxacin (100%), ciprofloxacin (95.8%), ampicillin (91.6%) and tetracycline (75%) was observed among *Campylobacter* species isolated from the liver of chickens. The high resistance profile reported in this study could be as a result of indiscriminate use of these drugs in poultry feed or treatment with flouroquinolones, macrolide and tetracycline in cases of gastroenteritis in chicks which is common in Nigeria. *Tet* (O) and mutation in the *gyrA* gene to *Thr* – 86 –Ile have been reported to be responsible for resistance to tetracycline and flouroquinolones repectively (Ge et al., 2003; Ekkapobyotin et al., 2008). In Nigeria, as in other developing countries, although regulations exist on the use of antimicrobial agents, their enforcement is always a problem and virtually non-existent. Though the sample size is relatively small, it gives a fair picture of the situation in the study area. Lower resistance was observed to azithromycin, erythromycin, gentamicin and streptomycin; this may be because these drugs are not commonly used to treat poultry diseases in Nigeria. All the strains were susceptible to clindamycin and chloramphenicol, thus, may be drugs of choice for the treatment of campylobacteriosis.

The occurrence of resistance to ciprofloxacin and multiresistant isolates in this study is of major concern since *Campylobacter* is a gram – negative organism which could transfer resistant genes to other gram – negative pathogens in the environment (Barlow et al., 2004). This could also have therapeutic implications in the treatment of human bacterial diseases originating from consuming contaminated poultry meat. This study demonstrates that Diagnostic laboratories could be used as surveillance points for antimicrobial resistance for *Campylobacter* species and other bacteria. Further investigation and surveillance is necessary, especially in developing countries to determine the extent of the resistant situation and proper control measures.

Conflict of interests

The author(s) did not declare any conflict of interest.

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