Original Research Article

Multi-Drug Resistant~~tceto~~ *Campylobacter Species* of Poultry Origin in Ado-Ekiti

Abstract

The occurrence of Campylobacter jejuni~~enu~~ in the large intestine of domestic fowls in Ado-Ekiti was assessed using standard microbiological procedures. One hundred faecal swabs were inoculated into modified cefoperazone charcoal deoxycholate agar (mCCDA). Twenty-seven isolates of Campylobacter jejuni~~enu~~ were recovered from the birds. Biochemical identification of the isolates was carried out using oxidase and catalase tests. Antibiotic susceptibility test was carried out using standard disc diffusion method as specified by the Clinical Laboratory Standard Institute (CLSI), to the following antibiotics; amoxicillin, cefoperazone, ceftazidime, aztreonam, ceftriaxone, pefloxacin, ciprofloxacin, levofloxacin, enrofloxacin and norfloxacin. The pattern of resistance was as follows; Amoxicillin (66.7%), cefoperazone(48.1%), ceftazidime(66.7%), aztreonam(40.7%), ceftriaxone(74.15), pefloxacin(51.9%), ciprofloxacin(33.3%), levofloxacin(40.7%), enrofloxacin(22.25) and norfloxacin(59.3%). Twenty-three different multiple resistance pattern were observed among the isolates. The high level resistance observed in this study poses significant health risk to the general public, a synergistic collaboration is therefore suggested between public health policy-makers and researchers to curb this ugly trend.

Keywords: gene, virulence, *Campylobacter jejuni~~staphyloccus aureus~~*, invasiveness, genetic diversity

Introduction

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Campylobacter is a gram negative, curved spiral or rod shaped catalase positive and oxidase positive bacterium that is micro-aerophilic in nature (Corry *et al.,* 2003). *Campylobacter* are very important cause of food illness worldwide. *Campylobacter* are micro-aerophilic, motile, helical to ~~vibroid~~vibrioid gram negative rods 0.2-0.5 µm by 1.5-5 µm in size. Their appearance varies from curved to spiral or gull wing shaped. Gull wing shapes are formed when daughter cells do not separate (Leonard, 2002).They are motile by means of polar flagellate at one or both ends and more in straight lines with a cork-screw motion (Van der walt 2004).

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Campylobacteriosisis an infectious diseases caused by *Campylobacter jejuni.* It is also referred to as *Campylobacter enteritis.* Most cases of *Campylobacter jejuni*are sporadic. *Campylobacter jejuni*is a species of curved, helical shape forming gram negative, microphilic bacteria commonly found in animal feaces (Ryan and Ray, 2004).It is one of the most common causes of human gastroenteritis in the world. Food poisoning caused by *Campylobacter* species can be severally debilitating, but rarely life threatening. It has been linked with subsequent development of Guillain-Barre Syndrome (GBS), which usually develops two to three weeks after the initial illness (Fujimoto and Amakon, 1990)

Most cases of Campylobacteriosis do not require antimicrobial treatment since they are clinically mild and ~~self limiting~~self-limiting in nature, although antimicrobial therapy is required for serious enteritis and systemic infection. Macrolides and fluroquinolones are considered as drugs of choice for the treatment of enteric infections and intravenous aminoglycoside for those cases present with systemic manifestations (Gaudreau *et al.*, 2003). In many cases fluoroquinolone are preferred if the differential ~~diadnosis~~diagnosis include *Salmonella*, *Shigella* or other enteric bacterial pathogens. Antimicrobial resistance to various drugs is on the rise and has been reported from several countries during previous years (Michaud *et al.*, 2003).

Recently, a dramatic rise in the number of resistant *Campylobacter* to quinolone, ampicillin and erythromycin was reported from various centers of the developed world (Chuma*et al.*, 2004). Similarly, Ibrahim *et al* reported a consistent rise in antimicrobial resistance to quinolones, ampicillin (Ibrahim *et al.*, 2004).

Antimicrobials have been used in animal feed (farm animals and poultry), most commonly used drugs are either identical to or are related to those administered to humans, including cephalosporin and fluoroquinolone. These antimicrobial agents are given to animals as therapy for an infection, or in the absence of illness for sub therapeutic purposes with the goals of growth promotion and enhanced feed efficiency. Food producing animals, especially poultry, are

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considered as one of the most important sources of *Campylobacter* infection among human being. Many studies have shown that poultry meat available at supermarket have been contaminated by *Campylobacter jejuni*(Luber*et al.,* 2003).

Moreover, various studies suggest that the incidence of antimicrobial resistant strains have increased with the introduction of the sub-therapeutic and therapeutic use of these drugs in animals (Engberg *et al.*, 2001). The use of antimicrobials in animal feed selects resistant strain and enhances their persistence in the environment. Drug resistance in *Campylobacter* and other organism can increase the frequency and severity of infections (Molbak*et al.,* 2000).

Campylobacteriosis is an infectious diseases caused by *Campylobacter* species. This infection can be treated with the use of antibiotics but may have one or multiple resistance to some antibiotics. Fluoroquinolone and cephalosporin for the treatment of *Campylobacter*infections have been doubtful following the emergence of multi-drug resistance in *Campylobacter*. This study was therefore carried out to isolate and characterize *Campylobacter* from some domestic animals and to show the number of multiple resistance as *Campylobacter* is currently a zoonotic disease of considerable magnitude.

Due to the emergence, dissemination and public health risks that may be posed by various mechanisms contributing to βeta lactamase resistance in *Campylobacter* isolates from poultry.

Methodology

Study Area

This study was carried in the Microbiology lab of Ekiti State University, Ado-Ekiti, southwest, Nigeria. Ado Ekiti, lies between latitude $7^{o}35`$ and $7^{o}38`$ North of the equator and Longitude $5^{o}10`$ and $5^{o}15`$ East of the Greenwich Meridian. It has a population of 308, 626. (Oriye, 2008).

Sample Collection

One hundred poultry faecal samples were collected from apparently healthy chickens within the study area- Ado-Ekiti. The samples were collected with the aid of sterile swab stick which is carefully dipped into the rectum of the chicken and rotated gently. One swab stick was used for each chicken; samples were taken to the laboratory and processed within 1hr of collection.

Sample Processing

Thermophilic *Campylobacter* spp. was isolated by direct inoculation on selective /enrichment medium. Ten millilitre of faecal suspension was streaked into mCCDA and incubated micro aerobically (6%, O_2, 7%CO_2, 7%H_2, 80%N_2) at 42^0C for 24 to 48hrs after which the swab stick streaked into mCCDA plates and incubated micro aerobically as above at 42^0C for 2 to 3days.

Faecal sample were inoculated directly into sterile plates of mCCDA (modified charcoal cefoperazone deoxycholate agar). Streaked to obtain discrete colonies and incubate at 42^0C within 24-48hrs (Lund *et al.*, 2003). Characterisation of the isolates was done through catalase and oxidase tests.

Antibiotic Sensitivity Test (AST) was carried out using standard disc diffusion procedure specified by the clinical lab standard institute (CLSI). Muller hinton agar plates were prepared according to manufacturer's specification; the organism was incubated into the tryptone soy broth and then standardized using 0.5 MacFarland turbidity standard dried at 42^0C for 3hrs. After incubation, sterile swab sticks were used to inoculate the Muller Hinton agar plates. The plates were left on the bench for few minutes and allowed to dry. The antibiotic discs (OXOID) were firmly placed on the ~~innoculated~~inoculated plates with sterile forceps. The antibiotics from the disc were allowed to diffuse into the medium for 30minutes at room temperature and the plates were incubated micro aerobically at 42^0C for 48hrs. The antibiotics discs for gram negative bacteria

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used contained: amoxycillin (Amx) 25µg, cefoperazone (Cfp) 75µg, ceftazidime (Caz) 30µg, aztreonam (Atm) 30µg, ceftriaxone (Cro) 30µg, Pefloxacin (Pef) 5µg, ciprofloxacin (Cip) 5µg, Levofloxacin (Lev) 1µg, Enrofloxacin (Enr) 5µg and Norfloxacin (Nor) 10 µg. After incubation, the plates were examined for zone of inhibition around the paper disc. The zones of inhibition were interpreted by comparing with the standard antibiotic sensitivity chart to determine the resistance pattern.

Results

Tentative identification of *Campylobacter* spp was based on its morphological appearance of curved, spiral and distinct colonies with mucoid on mCCDA. Biochemically, all the isolates were positive for catalase, oxidase test. The overall susceptibility of the isolates revealed that 18(66.7%) were resistant to ceftazidime, 20(74.1%) to ~~ceftriazone~~ceftriaxone, 11(40.7%) to aztreonam, 18(66.7%) to amoxicillin, 13(48.1%) to cefoperazone, 14(51.9%) to pefloxacin, 9(33.3%) to ciprofloxacin, 6(22.2%) to enrofloxacin, 16(59.3%) to norfloxacin, 11(40.7%) to levofloxacin. Each of the isolates was resistant to all the antibiotics with resistance to ceftazidime and amoxicillin having the highest percentage and resistance to enrofloxacin being the least (TABLE 1). 14 different multiple resistant pattern were resistant to cephalosporin from the *Campylobacter* isolated from poultry birds in this study (TABLE 2&3). A total of 23 different multiple

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resistant patterns were observed in the *Campylobacter*spp isolated from the poultry birds in this study. The predominant pattern for cephalosporin and fluoroquinolone was CAZ-CRO-AMC-CFP-ATM-ENR-NOR-LEV-CIP-PEF. (TABLE 4).

TABLE 1: OVERALL RESISTANCE OF *CAMPYLOBACTER* SPP. ISOLATED FROM CHICKEN TO SINGLE ANTIBIOTICS

S/N	ANTIBIOTICS	RESISTANCE N=27
1	CAZ	18(66.7%)
2	CRO	20(74.1%)
3	ATM	11(40.7%)
4	AMC	18(66.7%)
5	CIP	9(33.3%)
6	NOR	16(59.3%)
7	LEV	11(40.7%)
8	ENR	6(22.2%)
9	CFP	13(48.1%)
10	PEF	14(51.9%)

LEGEND
CAZ-Ceftazidime, CFP-Cefoperzone, AMC-Amoxycillin, ATM-Aztreonam, CRO, Ceftriaxone, PEF-Pefloxacin, CIP-Ciprofloxacin, LEV-Levfloxacin, ENR-Enrofloxacin, NOR-Norfloxacin

TABLE 2: MULTIPLE ANTIBIOTIC RESISTANCE PATTERN TO FLUOROQUINOLONE AMONG ISOLATES.

ANTIBIOTICS	RESISTANCE	SUB TOTAL	
2	NOR-CIP	2	
	NOR-LEV	2	
	ENR-NOR	1	
	LEV-PEF	1	
	NOR-PEF	2	8
3	NOR-LEV-PEF	1	
	NOR-CIP-PEF	3	
	ENR-NOR-LEV	1	
	ENR-LEV-PEF	1	
	NOR-LEV-CIP	1	
	LEV-CIP-PEF	1	8
4	ENR-NOR-CIP-PEF	1	
	ENR-NOR-LEV-PEF	1	2
5	ENR-NOR-LEV-CIP-PEF	1	1

TABLE 3: MULTIPLE ANTIBIOTIC RESISTANCE PATTERN TO CEPHALOSPORIN AMONG ISOLATES.

ANTIBIOTICS	RESISTANCE PATTERN	SUB TOTAL	
2	CRO-AMC	3	
	CAZ-CFP	1	
	CAZ-CRO	1	
	CAZ-AMC	1	6
3	CRO-CFP-ATM	2	
	CAZ-CRO-AMC	3	
	CAZ-CRO-CFP	1	6
4	CAZ-CRO-AMC-ATM	2	
	CRO-AMC-CFP-ATM	1	
	CAZ-CRO-AMC-CFP	3	
	CAZ-AMC-CFP-ATM	1	
	CAZ-CRO-CFP-ATM	1	8
5	CAZ-CRO-AMC-CFP-ATM	3	3

TABLE 4: OVERALL MULTIPLE RESISTANCE PATTERN TO FLUOROQUINOLONE AND CEPHALOSPORIN AMONG ISOLATES.

NO OF ANTIBIOTICS	RESISTANCE PATTERN	SUB TOTAL	TOTAL
2(2)	CAZ-AMC	1	
	NOR-PEF	1	2
3(3)	CAZ-CRO-CRP	1	
	CAZ-CRO-NOR	1	
	LEV-CIP-PEF	1	3
4(5)	AMC-NOR-LEV-PEF	1	
	CAZ-CRO-AMC-ATM	2	
	CAZ-ENR-NOR-LEV	1	
	CRO-AMC-ENR-NOR	1	5
5(7)	CAZ-CRO- AMC-CFP-PEF	2	
	CRO-CFP-ATM-NOR-CIP	1	
	CAZ-CRO-AMC-NOR-LEV	1	
	CAZ-CRO-AMC-CFP-ATM	1	
	CAZ-CFP-ENR-LEV-PEF	1	
	CRO-AMC-LEV-CIP-PEF	1	7
6(3)	CAZ-AMC-CFP-ATM-NOR-CIP	1	
	CAZ-CRO-AMC-NOR-LEV-CIP	1	
	CRO-AMC-CFP-ATM-LEV-PEF	1	3

7(1)	CAZ-CRO-AMC-CFP-NOR-CIP-PEF	1	1
8(2)	CAZ-CROAMC-CFP-ATM-NOR-LEV-PEF CAZ-CROAMC-ATM-ENR-NOR-LEV-PEF	1 1	2
9(2)	CAZ-CRO-CFP-ATM-ENR-NOR-LEV-CIP-PEF CAZ-CRO-AMC-CFP-ATM-ENR-NOR-CIP-PEF	1 1	2

Discussion

The results indicate that *Campylobacter's* are present in the large intestine of chicken. *Campylobacter* generally is considered to be a commensal that normally inhabits the gut of healthy birds, cattle and chicken including humans. Isolation of *Campylobacter* species by direct swabbing on *Campylobacter* selective agar (mCCDA) has been reported by other workers including (Yildrim*et al.,* 2005 and Savasan*et al.,* 2005). Recently, a diameter rise in the number of resistant Campylobacter to fluoroquinolone and Cephalosporin was reported from various centers of the developed world (Chuma*et al.,* 2005). In this study, high resistance of isolates to antibiotics was observed. The data showed high prevalence to most drugs tested among Campylobacter isolates. Infection with *Campylobacter's* is established zoonoses and the organism can be transmitted to human body via food (meat and milk), water and through contact with farm animals.

A number of potential risk factor associated with *Campylobacter* infection include inadequate cooked chicken, domestic pets, untreated water, food poor hygiene and handling practices (Mcmahon and Malmood, 1993). *Campylobacter* after entering into the environment use its particular characteristics, unique metabolism along with complete citric acid cycle, complex and highly branched respiratory chain and great regulatory infections enable them to colonize a number of environments in addition to mammalian gut (Kelly, 2001). Poultry are some of the most important sources of *Campylobacter* infection in humans and the water supply has shown to be a prominent factor in colonization of *Campylobacter* in chickens (Kapperud*et al.*, 1993), in addition distribution of *Campylobacter* species in chicken was similar to that seen in humans, suggesting that both of these food sources play a significant role in human infection.

Based on forgoing evidence, domestic animal and poultry could be considered as a link between natural habitat of *Campylobacter* and human being. ~~Therefore~~Therefore, to determine possibility of dissemination of *Campylobacter* and estimate their frequency of occurrence in domestic animal and poultry, the present study was conducted to isolate *Campylobacter* spp. from fecal sample of poultry. The result obtained from the present study indicated that all survey was contaminated with different level of *Campylobacter*. According to our

observations, the major vehicle of *Campylobacter* in the area was relatively poultry. Several studies parallel to our finding have shown that the poultry is a major source of *Campylobacter* and chicken meat is predominantly associated with *Campylobacter* infection in man (Humphery *et al.,* 1993).

The *Campylobacter* species isolated in this study showed (22.2%) Enrofloxacin, (59.3%) Norfloxacin, which is contrary to the (50%) Enrofloxacin and (60%) Norfloxacin. But the result is in concordance with the result of (Moore *et al.*, 2006). The prevalence of antibiotic resistance of *Campylobacter* spp. from humans to fluoroquinolone resistance of these organism in poultry (Gallay*et al.*, 2007). This study showed that the resistance rate of *Campylobacter* to Ceftriazone was quite high in chicken (74.1%). This is consistent with the findings of (Ibrahim *et al.*, 2004). In this study it was observed that the resistance rate of Enrofloxacin was very low. This could be that the drug is not used for animals and or in humans in the study area, Enrofloxacin is a ~~first generation~~first-generation fluoroquinolone, which makes drugs the most effective and could be used as the last line of drug for treatment of antimicrobial resistant *Campylobacter* infections among chickens in the study area. It is also a ~~broad—spectrum~~broad-spectrum antibiotic, the original drugs in the fluoroquinolone family have been shown to exhibit excellent antibacterial activity. The high sensitivity of all the isolates to Enrofloxacin could be as a

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result of its restricted use for treatment of clinical infections in humans (Ibrahim *et al.*, 2004).

Multiple resistance in *Campylobacter* spp. was observed in this study among all the isolates. High findings have been reported by (Engberg *et al.*, 2001). Twenty three different multiple antibiotic resistance patterns were obtained and the most prominent antibiotics resistance patterns exhibited by some isolates recovered from the chicken were CAZ-CRO-AMC-CFP-PEF (which is the most dominant). The emergence of multiple antibiotic resistance *Campylobacter* in chicken implies that very few options of antibiotics may be available for therapeutic and sub therapeutic used in these animals in case of infection of *Campylobacter*. Similarly, these multiple resistant strains could be disseminated into the environment and even transmitted to humans either through contact or consumption (Luber*et al.*, 2003).

The successful outcome of antimicrobial therapy with antibiotics depends on several factors such as host mechanism, the location of infection and properties of antibiotics (Pankey*et al.*, 2004). However, the presence of antibiotic resistance *Campylobacter* could raise important questions concerning the acquisition or colonization of the gut of chickens, which are often raised domestically. They are neither fed with feed supplement containing antibiotics. However, the most likely route through which antibiotic resistant

Campylobacter could colonize the gut of local chicken could be feeding. Humans in the community who are carriers of antibiotics resistant bacteria could defecate and shed the bacteria into the environment (Molback*et al.*, 1999). During feeding, they may pick up the feces or other fecally contaminated food materials directly from the environment. The resistant bacteria may also be acquired from contaminated water sources while drinking.

Moreover, local chickens scavenge pit and waste dumps for food where they may possibly pick up residual antibiotics that may have been disposed.

Conclusion and Recommendation

As a result of the relatively high prevalence of antibiotic resistance *Campylobacter* in chicken, as discovered by the findings in the research work, it is absolutely necessary to prevent the emergence of resistant bacteria and possibly outbreaks of disease they may cause.

In conjunction with pharmaceutical companies, researchers can initiate continuous surveillance programme on a regular basis to monitor the prevalence in animals and possible transmission to antibiotic resistance bacteria to humans in the environment. Scientific and political efforts need to collaborate to eradicate the problem of antibiotic resistance. Control of antibiotic resistance is also needed to conserve the usefulness of the remaining drugs that are sensitive.

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