Int.J.Curr.Microbiol.App.Sci (2017) *6*(4): 324-333

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 6 Number 4 (2017) pp. 324-333**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2017.604.035

Isolation and Antibiotic Susceptibility of *Campylobacter* Species from Cattle Offals in Gwagwalada Abattoir, Abuja-FCT Nigeria

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Keywords

Isolation, Antibiotic Susceptibility, Campylobacter species, Cattle offals, Gwagwalada abattoir

Article Info

Accepted:
02 March 2017
Available Online:
10 April 2017

A B S T R A C T

This study was conducted to establish the occurrence and antibiotic susceptibility testing on isolates of *Campylobacter* obtained in cattle offals slaughtered within Gwagwalada abattoir. A total of 75 samples were collected over a period of five weeks using sterile swab sticks for cultures on blood free selective *Campylobacter* agar (modified CCDA-Preston) enriched with selective supplement and incubated at 42°C for 48 hours microaerobically. The colonies were subjected to biochemical reactions of oxidase, catalase, citrate, indole reaction, hydrogen sulphide production and motility test. Antibiotic sensitivity test was also performed using an antibiotic impregnated multi-disk (Optudisc, UK) Gentamycin (10µg), Streptomycin (30µg), Rifampicin (20µg), Erythromycin (30µg), Ampiclox (20µg), Amoxicillin (20µg), Chloramphenicol (30µg), Levofloxacin (20µg) and Norfloxacin (10µg). Cultural and Gram staining characteristics showed 68% were positive for *Campylobacter spp* as gram negative curved rods. Biochemical reaction further revealed isolates were motile, oxidase, catalase and citrate utilization positive, as well as indole and hydrogen sulphide negative. Antibiotic sensitivity testing revealed that isolates were sensitive to Gentamycin and Amoxil and resistant to Norfloxacin, Rifampicin, Chloramphenicol, Streptomycin and Ampiclox but showed some effect to Ciprofloxacin, Levofloxacin and Erythromycin. These *Campylobacter* isolates within offals has a potential ability to contaminate meat obtained from the abattoir which may increase the risk of human infection. This finding indicates the presence of *Campylobacter* isolates in cattle offals showing resistance to commonly used antibiotics. Awareness campaign amongst both butchers and the general public on the occurrence and possible contamination of beef with *Campylobacter* is recommended with emphasis on safe and wholesome meat preparation and good hygienic slaughtering practices.

Introduction

Campylobacteriosis is a significant emerging bacterial foodborne zoonosis caused by the bacterial genus of *Campylobacter*, primarily associated with consumption of undercooked poultry, other meat products (Mazick *et al.*, 2006) contaminated with faeces (Friedman *et al.*, 2000) especially in several industrialized countries (Altekruse *et al.*, 1999) and characterized by *Campylobacter* gastro-enteritis (Kapperud *et al.*, 2003). The genus *Campylobacter* comprises of about 16 species and 4 sub - species (Vandamme, 2002) which are Gram negative, micro-aerophilic, curved or spiral rods, with a single polar flagellum

and a rather unique corkscrew motility (Quinn *et al.,* 1994).

Campylobacter have been incriminated in a variety of animal diseases including abortion in sheep and goats (Andersen *et al.,* 1983), infertility and abortion in cattle, diarrhea in sheep and cattle (Al-Mashat and Taylor, 1980), intestinal adenomitis in swine and gastroenteritis and abortion in dogs (Adak *et al.,* 2005). Genital Campylobacteriosis in animals have occurred during coitus and artificial insemination (AI) in cows (Skirrow, 1977). However, Chicken and cattle are the principal sources of *C. jejuni* pathogenic to humans, whereas wild animal and environmental sources have been associated with about 3% of the disease (Wilson *et al.,* 2008).

The routes of transmission of *Campylobacter* between food animals and humans are numerous and complex (Andersen *et al.,* 2006). Foodborne transmission is the mode by which majority of the cases occur. Raw poultry meat has often been implicated as the major source of human Campylobacteriosis (Wingstrand *et al.,* 2006).

Contamination of cattle carcasses during processing either directly or indirectly have also been reported (Sharon *et al.,* 2013). However, person–to-person spread of infection was reported but is uncommon (Blaser *et al.,*1981) Human foodborne illness have been reported post consumption of *Campylobacter* contaminated bovine products like unpasteurized milk (Sato *et al.,* 2004) and meat (Osano and Arimi, 1999) with serious public health consequences (Besser *et al.,* 2005, Friedman *et al.,* 2004). Contaminated surface water run-off from bovine reservoirs and cattle pastures and or direct cattle contact (Friedman *et al.,* 2000) were also documented during disposal of abattoir effluents and slurries which contaminates water for human consumption (Tauxe, 1992).

Epidemiological studies have identified a significant association between *Campylobacter* infection in humans and consumption as well as handling of poultry (Wingstrand *et al.,* 2006). However, other studies reported similar association with cattle (Garcia *et al.,* 1985). This direct contact exposure to bovine faeces and consumption of unpasteurized cow milk are the leading causes of acute bacterial Campylobacteriosis outbreaks in cattle (Sato *et al.,* 2004) and humans globally (Nachamkin, 1995) with enteric Campylobacteriosis been prevalent amongst HIV-infected patients (Sorvillo *et al.,* 1991) and found to be resistant to antimicrobial therapy especially C. *jejuni* (Altekruse *et al.,* 1999) alongside other observed complications of Neuropathies such as Guillian-Barre syndrome (GBS) (Godschalk *et al.,* 2006), myocarditis (Cunningham and Lee, 2003).

The increasing concern based on previous epidemiological studies on the potential role of non-poultry sources for human clinical infections has been underestimated (Ngulukun, 2009, 2011). The relative direct and indirect contributions of cattle and sheep to human infections are still poorly understood (Frost, 2001). This premised the study to investigate the occurrence and antibiotic susceptibility testing of *Campylobacter* isolates in slaughtered cattle offals from Gwagwalada abattoir for the purpose of designing a disease control plan.

Materials and Methods

Study area

Gwagwalada is one of the six Area Councils of the Federal Capital Territory of Nigeria, alongside Abaji, Kuje, Bwari, Kwali and Abuja municipal area council. University of Abuja is located in Gwagwalada, which has an area of 1,043 km^2 and population of 157,770 during the 2006 census. Gwagwalada

is on geographical coordinates of 8° 56' 29" North, 7° 5' 31" East as shown on satellite images (3D Google Earth) with an extremely hot and daily temperature of 31°C. The abattoir is located mid-way between the popular 'kasuwandere' and the Federal Radio Coporation of Nigeria (FRCN) along old Kutunku road. The abattoir has been the main source of wholesome meat for the culturally diverse inhabitants of Gwagwalada metropolis and its environs (Olabode, *et al.*, 2011).

Study design and sampling method

The study was conducted between July and August 2016 in Gwagwalada metropolis abattoir, Gwagwalada Area Council of the Federal Capital Territory (FCT) Abuja. There were five (5) visits to the abattoir (forth nightly) during which samples were collected randomly from intestinal (offals) lumen of cattle immediately post slaughter. Fifteen (15) samples were collected weekly and stored 4^{o}C and transported in cold boxes to the laboratory for analysis over a period of five weeks.

Sample collection and processing

A total of seventy five (75) samples were collected during the study period and location. Fifteen samples were collected on each visit to the abattoir over the five weeks period using sterile swab sticks. These intestinal swabs were appropriately labeled and designated as abattoir cattle using the abbreviation ''AC'': AC1, AC2 -------- AC75 and transported in cold boxes to the Microbiology Laboratory of the Faculty of Veterinary Medicine, University of Abuja, for analysis.

Media

The media used for this study include *Campylobacter* blood-free selective agar (Modified CCDA- Preston) (Oxoid, Hampshire, England) and CCDA Selective supplement (SR155E) for Isolation, SIM [Sulphide-Indole-Motility] (Merck, Germany), Simmon Citrate Agar (Hi-Media, India), Kovacs reagent (Hi-Media, India) for biochemical reactions and Muller Hinton Agar (Hi Media, India) for Antibiotic susceptibility testing. All these media were prepared in accordance with manufacturer's instructions and sterilized using an autoclave at 121°C for 15 minutes.

Sample plating and inoculation

Samples were innoculated unto solid agar plates by streaking out technique on modified CCDA-preston using swab sticks. Inoculated agar plates were then transferred into anaerobic gas jar with a control plate (not inoculated), and the lid closed, (this is to create a microaerobic environment for normal growth and metabolism of *Campylobacter*). The jar was then transferred into the incubator for a period of 48 hours at 42°C.

Culture and identification

Post incubation, the cultural growth were visually and macroscopically identified as described by Teufel, (2002) for Flat, smooth, glossy and grayish colonies with no confluent growth. The colonies were later Gram stained as described by Bergey *et al.*, (1994) for Gram negative, curved or spiral rods, with single polar flagellum post microscopic examination.

Biochemical reaction

Suspicious colonies of *Campylobacter* species were used for biochemical characterization post sub culturing on *Campylobacter* blood-free selective agar (Modified CCDA- Preston) (Oxoid, Hampshire, England) and CCDA Selective supplement (SR155E). The isolates were subjected to biochemical tests (Catalase,

Oxidase, Motility, Indole, Hydrogen Sulphide test, and Citrate utilization) in accordance with standard methods.

Antibiotic susceptibly testing

The isolates were subjected to antibiotic susceptibility test using disc diffusion method as described by Taradon, *et al.,* (2007). Antibiotics impregnated disk (OPTUDISC, UK) used include; Ciprofloxacin (10µg), Norfloxacin (10µg), Gentamycin (10µg), Amoxicillin (20µg), Streptomycin (30µg), Erythromycin (30µg), Rifampicin (20µg), Chloramphenicol (30µg), Levofloxacin (20µg), Ampiclox (20µg). The isolates were uniformly and aseptically inoculated unto a set of dried sterile Mueller-Hinton agar plates and kept for 3-5 minutes post streaking to allow for drying off excess surface. Then, the antibiotic Multi-discs were aseptically placed on the agar using sterile forcep and incubated at 37°C for 24 h. The clear zones of inhibition were measured to the nearest millimeter using a transparent Millimeter ruler. The results were expressed as susceptible, intermediate, and resistant as indicated by the Clinical and Laboratory Standards Institute guidelines (CLSI, 2006).

Statistical analysis

The data generated from the research work was analyzed using descriptive statistics such as frequency, percentages and chart.

Results and Discussion

Out of the seventy five (75) samples collected from the intestinal tracts of sampled cattle in Gwagwalada abattoir during the study period. Fifty-one (51) [68%] samples were positive with typical morphological characteristics (gram negative curved rods)for *Campylobacter* as indicated in table 1 and figure 1.

For week one 8 (53%) were positive for *Campylobacter*, week two had 10 (67%) samples positive for *Campylobacter*, week three had 13 (86%) samples positive for *Campylobacter*, week four had 12 (80%) samples positive for *Campylobacter*, week five had 8 (53%) samples positive for *Campylobacter* as indicated in table 2.

Biochemical characterization showed that the isolates were motile, oxidase positive, indole negative, catalase positive, citrate utilization positive, and Hydrogen sulphide negative as indicated in table 3.

Antibiotic sensitivity testing further revealed that the isolates were sensitive to Ciprofloxacin, Gentamycin, Amoxil, Erythromycin and Levofloxacin and were resistant to Norfloxacin, Rifampicin, Chloramphenicol, Streptomycin and Ampiclox as shown in table 4.

In this study the overall prevalence of *Campylobacter* isolates in cattle slaughtered in Gwagwalada abattoir was 68%. This prevalence is as high as the 66.7% (Ngulukun, *et al.,* 2011) reported in Plateau state. The increased rate of isolation in the study could be associated with the specific agar and enrichment medium employed. The increased occurrence could also be attributed to the management type (free ranged), and sources (markets/ herds) where the cattle were transited from, before slaughter in the study area.

The observed colonies of *Campylobacter* showed grey, butyrous, moist, flat and spreading topography, the isolates were gram negative curved rods in shaped as described (Quinn *et al.,* 1994). Biochemically, isolates were oxidase, catalase and citrate positive, isolates were motile, indole negative and did not produce Hydrogen Sulphide.

Table.1 Weekly distribution of *Campylobacter* isolates obtained from cattle offals in Gwagwalada abattoir

Weeks	Sources	Sample Collected	Number +ve	Number -ve
1	Cattle	15	8	7
2	Cattle	15	10	5
3	Cattle	15	13	2
4	Cattle	15	12	3
5	Cattle	15	8	7
Total		75	51(68%)	24

Keys: +ve: Positive -ve: Negative

Table.2 Occurrence of *Campylobacter* isolates in cattle offals slaughtered in Gwagwalada

Weeks	Sample Collected	Number +ve	Number -ve
1	15	8 (16%)	7
2	15	10 (20%)	5
3	15	13 (25%)	2
4	15	12 (24%)	3
5	15	8 (16%)	7
Total	75	51(68%)	24 (32%)

Keys: +ve: Positive -ve: Negative

Table.3 Biochemical characterization of *Campylobacter* isolates from intestinal content

No. of +ve	Motility	Oxidase	Indole	Catalase	Citrate	Gram staining	H_2S Production
AC 5	+	+	-	+	+	-	-
AC 6	+	+	-	+	+	-	-
AC 7	+	+	-	+	+	-	-
AC 8	+	+	-	+	+	-	-
AC 16	+	+	-	+	+	-	-
AC 19	+	+	-	+	+	-	-
AC 21	+	+	-	+	+	-	-
AC 24	+	+	-	+	+	-	-
AC 33	+	+	-	+	+	-	-
AC 36	+	+	-	+	+	-	-
AC 42	+	+	-	+	+	-	-
AC 47	+	+	-	+	+	-	-
AC 50	+	+	-	+	+	-	-
AC 54	+	+	-	+	+	-	-
AC 55	+	+	-	+	+	-	-
AC 60	+	+	-	+	+	-	-
AC 64	+	+	-	+	+	-	-
AC 67	+	+	-	+	+	-	-
AC 71	+	+	-	+	+	-	-

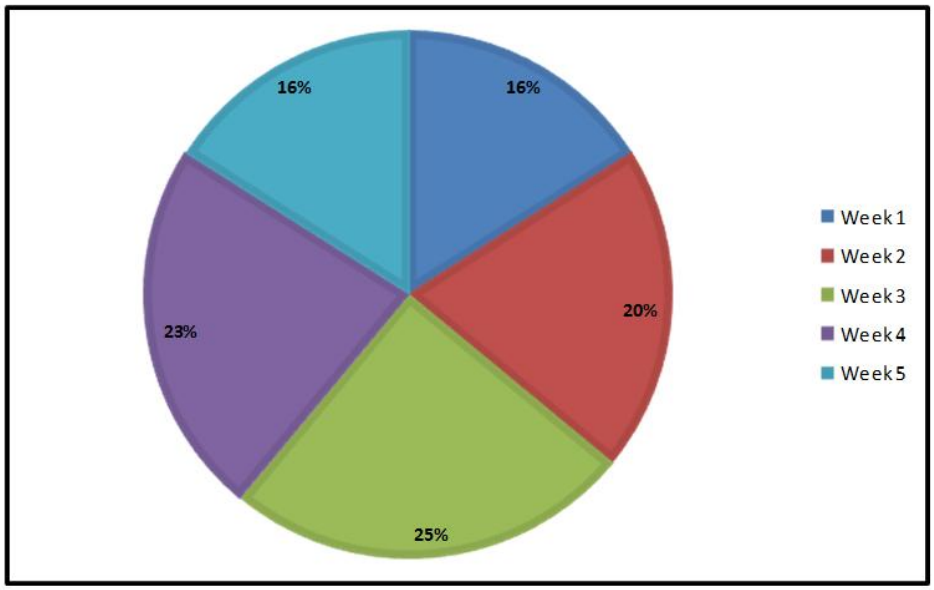
Keys: +ve Positive reaction, -ve Negative reaction AC: Abattoir cattle

Table.4 Antibiotic sensitivity pattern of Bovine *Campylobacter* isolates

Antibiotics	Samples tested	Sensitive	Resistant	Zone of inhibition (mm)
Ciprofloxacin	10	I	-	18
Norfloxacin	10	-	R	Nil
Gentamycin	10	S	-	21
Amoxil	10	S	-	22
Streptomycin	10	-	R	Nil
Rifampicin	10	-	R	Nil
Erythromycin	10	I	-	19
Chloramphenicol	10	-	R	Nil
Ampiclox	10	-	R	Nil
Levofloxacin	10	I	-	17

Keys: S- Susceptible, I- Intermediate, R- Resistant
S +++: 20-30mm Zone of Inhibition
I ++: 10-20mm Zone of Inhibition
R: 0 < 10mm Zone of Inhibition

Fig.1 Pie chart showing the weekly distribution of *Campylobacter* isolates in Gwagwalada abattoir



However, hippurate hydrolysis that has capacity to differentiate *Campylobacter jejuni* from *Campylobacter coli* was not conducted as *C. coli* usually indicates a negative reaction to hippurate test and *C. jejuni* have been associated more with pathogenic infection

(Salihu *et al.,* 2009). Although, previous reports of *Campylobacter* species isolation have been documented (Ngulukun *et al.,* 2009) in apparently healthy cattle.

The *Campylobacter* species isolates tested were sensitive to gentamycin and amoxicillin in this study. Amoxicillin susceptibility contrast previous report Tajada *et al.,* (1996) that organisms are resistant to a large number of betalactams particularly ampicillin and amoxicillin. The isolates were moderately susceptible to erythromycin, ciprofloxacin, and levofloxacin. The erythromycin zone of inhibition is similar to previous findings (Gaudreau *et al.,* 2007; Okunlade, *et al.,* 2015) and ciprofloxacin susceptibility is in line with Okunlade, *et al.,* (2015) but contrast Gaudreau *et al.,* (2007) this in consistency indicates increasing resistance of *Campylobacter* to antibiotics particularly macrolides and fluoroquinolones as reported (Asrestrup and Enberg, 2001). The high resistance to most of the antimicrobial agents tested in this study may be the consequence of indiscriminate use and abuse of these drugs in livestock herds and farms.

The high occurrence of *Campylobacter* spp in offals of slaughtered cattle suggests the possible contamination of commercially obtained meat and butchers handling meat and offals during slaughter operations as well as environment especially the surface water during disposal of abattoir effluent and animal slurry to land (Inglis *et al.,* 2004).

The observed post mortem and sanitary operating standards during this study is poor, characterized by weak veterinary supervision. Intestinal gut contents are dump either in the drainages (gutter) constructed beside the slaughter slabs or spilled on the floor where carcasses are kept before transportation to the market. Thus, there exists the possibility of contamination and hence the occurrence of *Campylobacter* spp in the study area.

Therefore, this study provides a preliminary report on the existence of *Campylobacter* species in Gwagwalada as a potential zoonotic problem associated with the supply of unwholesome meat and offals from the abattoir for human consumption especially amongst vulnerable groups. In addition, *Campylobacter* species isolates were susceptible to Gentamycin and showed increased resistance to fluoroquinolones and macrolides antibiotics most commonly used antibiotic for the treatment of human diarrhea. Hence, the needs to further conduct molecular biotyping studies to identify the specific *Campylobacter* species involved and educate the public especially the abattoir workers and women on the need to conduct proper hygienic practices during meat and meat products handling is thus suggested.

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How to cite this article:

Olabode, H.O.K., S. Mailafia, M.E. Ogbole, G.R. Okoh, C.I.C. Ifeanyi, H.O. Onigbanjo and Ugbaja, I.B. 2017. Isolation and Antibiotic Susceptibility of *Campylobacter* species from cattle offals inGwagwalada abattoir, Abuja-FCT Nigeria. *Int.J.Curr.Microbiol.App.Sci*. 6(4): 324-333. doi: https://doi.org/10.20546/ijcmas.2017.604.035