

Prevalence of *Campylobacter* Species in out-Patients and Pregnant Women Attending Government Clinics in Sokoto State, Nigeria

Innocent Okwundu Nwankwo^{1,*}, Olufemi Oludayor Faleke¹, Mohammed Danlami Salihu¹, Abdullahi Alhaji Magaji¹

1. Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Usmanu Danfodiyo University Sokoto, Nigeria.

Abstract:

Campylobacter infection has been detected in poultry processors at live bird markets in Sokoto, however the prevalence of *Campylobacter* species in more diverse group of the population is uncertain. For this purpose, 292 human fecal swabs from patients in four Government Hospitals in Sokoto State were analyzed for *Campylobacter* species using culture and phenotypic typing method. The prevalence rate of 55% was revealed in the tested samples while 56% and 55% were for males and females respectively. *Campylobacter jejuni*, *C. coli* and *C. lari* had 20%, 40% and 40% in males and 38%, 35% and 27% in females respectively. The prevalence rates of 60%, 52%, 45%, 41% and 80%, were revealed in age range of (less than 1-6, 7-13, 14-20, 21-55 and 56-76) yrs respectively. Furthermore, 70% and 43% prevalence rates were recorded in pregnant and non-pregnant women of reproductive age respectively. There was no statistical significant association ($P>0.05$) between *Campylobacter* infection and age range and sex, but the statistical association between infection and pregnancy was significant ($P<0.05$). The prevalence of *Campylobacter* species in patients as found in the various hospitals has revealed the level of environmental contamination in different homes in the state and the need to include *Campylobacter* in screening routine laboratory diagnosis of gastroenteritis in humans for appropriate treatment and management especially in young children, old adult and pregnant women.

Corresponding Author: Innocent Okwundu Nwankwo, Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Usmanu Danfodiyo University Sokoto, Nigeria.

Email: kinginnoma@yahoo.com

Keywords: *Campylobacter* species, Hospitals, Nigeria, Patients, Pregnant women, Prevalence, Sokoto, Nigeria.

Running title: *Campylobacter* species in out-patients and pregnant women in Sokoto.

Received: May 20, 2016; **Accepted:** Jul 25, 2016; **Published :** Jul 31, 2016;

Introduction

Campylobacter species have been known as causative agents of veterinary disease since 1913, when two veterinary surgeons, Mcfadyean and Stockman, reported an unknown bacterium that resembled a vibrio from aborted fetuses 1. The name *Vibrio fetus* was first described as a spirillum after investigation of infectious abortion of bovines in USA 2. Winter dysentery in calves and swine dysentery have been attributed to infection caused by *Vibrio jejuni* 3.

The breakthrough in recognizing the public health and clinical importance of these '*vibrios*' as used for *Campylobacter* throughout the first half of 20th century was the isolation of *Vibrio fetus* from the blood of a pregnant woman with fever of unknown origin 4. It was later revealed that *Campylobacter enteritis* is an important human disease caused by genus *Campylobacter* which can be detected and isolated from both the blood and faeces of humans 5. They are microaerophilic, non-fermentative, non-spore forming, gram-negative and oxidase positive organism 6, 7. They are typically curved, comma or spiral shaped rods between 0.5-5µm long and 0.2-0.8 µm wide 8, 9.

Thermophilic *Campylobacter* species, mainly *C. jejuni* and *C. coli* are recognized as the most common bacteriological causes of gastroenteritis worldwide 3. Meningitis, osteomyelitis, neonatal sepsis, neuromuscular paralysis occur more in immunocompromised host such as pregnant women, AIDS patients, young and those at very old age 10,11. Young children and old adults are more prone to *Campylobacter* infection but the inability of *Campylobacter* associated diarrhoea to become pathogenic in patients greater than 6 months of age may have contributed to lesser number of cases on update of campylobacteriosis in developing countries 12, 13. In Nigeria, 16.5% prevalence rate has been reported in Lagos in children with diarrhoea 14.

In Lagos and Ogun States of South West Nigeria, 52.5% and 0.5% prevalence rates have been recorded in both children with and without enteritis and those aged between 1-36 months with diarrhoea respectively 15, 16. In Enugu State of South East Nigeria, 8.3% prevalence has been revealed in children between 1-6 months old and 93% of which were isolates of *C. jejuni* 17. In Sokoto State of North West Nigeria, 78.4% prevalence has been recorded with samples collected from exposed poultry processors 18. It has been established that *Campylobacter* can thrive well in underground water and where hygiene is compromised 19. The study therefore was conducted in four rural hospitals in the study area where environmental and personal hygiene are inadequate and source of water for consumption are questionable.

Materials and Methods

Ethical approval

Ethical clearance to conduct the study was granted by the Honourable Commissioner of Health, State Ministry of Health, through the Director, Health Planning, Research and Statistics, Usman Farouk Secretariat, Sokoto, Sokoto State. Introductory letters were given to the Principal Medical Directors of Hospitals by the Hospital Service Management board. Nurses in the selected hospitals explained the purpose of the study to patients in order to mobilize volunteers. Labeled swab sticks were given to volunteers indicating their sex and ages.

The study area

The study was carried out in Sokoto State, which is geographically located in the extreme northwest of Nigeria. It lies between the latitudes 12oN to 58oN and longitudes 4.8oE to 6.54oE with annual average temperature of 28.3oC. It has four agricultural zones

and 23 Local Government Areas. It shares boundaries with Zamfara State to the East, Republic of Niger to the North and Kebbi State to the West. The State had a human population of 3.7million people with a population density of 97.7 persons per square kilometer 20.

The study design

A cross sectional study was carried out with patients found in the four hospitals selected from the 23 Local Government Areas (LGAs) of the State. Introductory letters from the hospital service management board were presented to the Principal Medical Directors of hospitals. Convenient sampling technique was used after the assigned health workers have explained the purpose of the study to the patients and such consented. Labeled swab sticks were given to volunteers and information on sex, ages and pregnancy status for the females were obtained from each individuals. The screening of faecal swabs for *Campylobacter* species was done using standard culture isolation and biochemical characterization.

Sample size determination

The minimum sample size for this study was determined by the formula $N = Z^2 p(1-p) / d^2$ 21 where N=Sample size; Z= the score for a given interval which is 1.96(S.E), at 95% confidence interval; P = known or estimated prevalence; d = (5%) level of precision. The prevalence rate of 20% was estimated and the minimum sample size (n) required was $1.962 \times 0.20 \times 0.80 / 0.052 = 245$. However, a total of 292 faecal swabs were collected and analyzed.

Transportation and processing of samples

The samples were placed in Amies transport media (CMO425, Oxoid), kept cold with the use of ice block and were transported to the Veterinary Public Health Laboratory, Faculty of Veterinary Medicine, Usmanu Danfodiyo University Sokoto for analysis. Samples were plated directly onto selective media; Modified Charcoal Cefaperazone Deoxycholate Agar

(mCCDA) and incubated at 420C for 48hrs under microaerophilic condition generated by Campygen® (Oxoid, BR0056) in the anaerobic jar 22.

Phenotypic identification of *Campylobacter* species

Identification of colonies was based on characteristic features on a mCCDA as creamy or white, moist, flat or slightly raised, extending along the streak line, or regular circular discrete colony 23. *Campylobacter* species were identified using the standard *Campylobacter* phenotypic identification test as recommended 23. Distinct colonies were gram stained and different biochemical test such as catalase, oxidase, hippurate hydrolysis and hydrogen sulphide production in triple sugar iron agar (TSI) were performed as described in the following section.

Oxidase test: Oxidase papers were used to touch the isolates. A dark purple colour along the contact portion of the paper after few seconds of contact indicates a positive reaction. *Campylobacter* species are oxidase positive.

Catalase test: A loop full of pure culture was transferred from the agar onto the surface of a clean, dry glass slide. A drop of 3% hydrogen peroxide was immediately placed onto the colony on the slide. Effervescence indicates positive reaction. *Campylobacter* species like *C. jejuni*, *C.coli*, *C. lari*, *C.hyointestinalis* are catalase positive while *C. upsailensis* is catalase negative

Hydrogen sulphide (H₂S) production test: Characteristic colony from the selective medium was touched with a straight inoculating wire. A tube of Triple Sugar Iron (TSI) agar was inoculated by stabbing the middle of the agar to within 5mm from the bottom of the tube and incubated at 370C for 48 hrs with a loose cap on the TSI agar. Blackening of the medium indicates hydrogen sulphide production.

Campylobacter jejuni and *C.lari* do not produce H₂S while *C.coli* produces H₂S.

C. jejuni among the confirmed isolates was identified using the hippurate hydrolysis test 24. A small amount of pure culture was inoculated in 0.4 ml of 1% sodium hippurate (1 g of sodium hippurate and 99 ml of distilled water) in a tube. The tube was capped and incubated for 2 h at 37°C. Then, 0.2 ml of 2% ninhydrin solution was added and re-incubated for further 15 min at 37°C. The development of a purple-violet colour identified the isolate as *C. jejuni*. Antibiotic sensitivity test was determined using the disc diffusion method to further differentiate the species 25. The antibiotic discs used were; nalixidic acid (30 mg), cephalothin (30 mg) and metronidazole (30 mg).

Statistical analysis

The results obtained were presented in tables, and percentages. Chi-square (X²-test) was used to determine any significant statistical association between *Campylobacter* infection and hospitals, sex, age ranges and pregnancy.

Results

Out of the total 292 patients sampled, 160 (55%) were positive for *Campylobacter* infection. The prevalence rates in the hospitals revealed 43 (70%), 6 (25%), 32 (65%) and 79 (50%) in Tambuwal, Rabbah, Illella and Amanawa Leprosium/General Hospitals respectively (Table I). Out of the positive samples, 70 (56%) were males while 89 (55%) were females. In the males, 28 (40%), 28 (40%) and 14 (20%) were *C. coli*, *C. lari* and *C. jejuni* respectively while *C .jejuni*, *C. coli*

and *C. lari* had 24 (27%), 34 (38%) and 31 (35%) in the females respectively (Table II). The prevalence rate of 60% was recorded in the early childhood age range less than 1-6 yrs, while 80% was recorded in old adult age range of 56-76 yrs. The prevalence rates of 52%, 45% and 41% were recorded in late childhood age range (7-13) yrs, adolescence age range (14-20) yrs and young adult age range (21-55) yrs respectively (Table III). Using the reproductive age range (18-40) yrs in females, the prevalence rates of 16 (70%) and 25 (43%) were obtained in pregnant and non-pregnant women respectively. *C. lari* was 9 (56%) followed by *C .coli* and *C. jejuni* which had 5 (32%) and 2 (13%) in the pregnant women respectively while *C. coli* recorded higher prevalence rate of 15 (60%) than 6 (24%) and 4 (16%) by *C. jejuni* and *C. lari* in non-pregnant women respectively (Table IV).

Discussion

The presence of *Campylobacter species* has been established among patients that presented themselves for different medical attention in the General Hospitals in Sokoto State with an overall prevalence rate of 55%. The prevalence rate in this study was in disagreement with 78.4%, and 87% recorded by Salihu in (2009) and Saenz et al., (2000) in Nigeria and Spain respectively 18, 26. This disparity was expected since the samples used in those studies were from risk group while the samples used in this study were obtained from diverse groups in the general population. The different prevalence rates as recorded in the Government Hospitals were reflection of general environmental, food and water contamination in the communities since the

Table I: Prevalence of *Campylobacter* species in the selected Government hospitals in Sokoto state

Hospitals	Total sampled	Total+ (%)	C.jejuni (%)	C.coli (%)	C.lari (%)	X ² value
AmanwaLeprosium, DengeShuni	158	79(50)	35(44)	23(29)	21(26)	
Illella General Hospital Illella	49	32(65)	3(9)	13(41)	16(50)	18.321
Tambuwal General Hospital Tambuwal	61	43(70)	0	25(58)	18(42)	P=0.0004
Rabbah General Hospital Rabba	24	6(25)	0	1(17)	5(83)	(P<0.05)
Total	292	160	38	62	60	

Table II: Prevalence of *Campylobacter* species in males and females patients in Sokoto State

Sex	Total sampled	Total+ (%)	C.jejuni (%)	C.coli (%)	C. lari (%)	X ²	P value
Male	126	70(56)	14(20)	28(40)	28(40)	0.00218	0.9628
Female	161	89(55)	24(27)	34(38)	31(35)		(P>0.05)
Total	287	159(55)	38(25)	62(40)	59(38)		

Note: sex was not indicated in 5 samples

Table III: Prevalence of *Campylobacter* species in patients according to different age ranges

Age ranges (yrs)	Total sampled	Total + (%)	C.jejuni (%)	C.coli (%)	C.lari (%)	X ² Value	p Value
Early childhood (1-6)	67	40(60)	14(35)	14(35)	12(30)		
Late child hood (7-13)	48	25(52)	7(28)	8(32)	10(40)	3.389	P=0.4950
Adolescence (14-20)	53	24(45)	0	12(50)	12(50)		(P>0.05)
Young Adult (21-55)	98	46(47)	12(10)	26(60)	18(30)		
Old Adult (56-76)	16	8(50)	0	3(25)	5(75)		
Total	282	143	33	63	57		

Note: Age was not indicated in 10 samples.

Table IV: Prevalence of *Campylobacter* species in pregnant and non-pregnant women of reproductive age range in Sokoto State

Female (18-40 yrs)	Total sampled	Total+ (%)	C.jejuni (%)	C.coli (%)	C.lari (%)	X ² value	p value
Pregnant	23	16(70)	2(13)	5(32)	9(56)	4.334	0.0374
Non pregnant	57	25(44)	6(24)	15(60)	4(16)		(P<0.05)
Total	80	41	8	20	13		

infection is usually transmitted by contact with faeces of animal and human and ingestion of contaminated food and water 19. In these areas, the absence of pit latrine and good source of drinking water may have contributed to the spread of infection 19. The prevalence rates for both male and female patients indicated no sex preference in *Campylobacter* infection in agreement with the findings of Samuel et al., (2004) which recorded similar rates among males and females 27.

Young children and old adults are said to be more prone to *Campylobacter* infection and the high prevalence rates recorded in age range (56-76 and less than 1-6) yrs which represent the old adult and early childhood age groups respectively have revealed that low level of immunity may play role in infection 28. Furthermore, the prevalence rate in pregnant women of reproductive age (18-40) yrs compared with the rate in non-pregnant ones emphasized the factor played by immunity as low immunity is usually observed during pregnancy. Most *Campylobacter* infections in pregnant women are mild and self-limited with no severe adverse consequences for the mother or baby 29. However, neonatal sepsis and death can occur if a woman is infected during the third trimester of pregnancy as babies can be infected during birth if the mother is excreting *Campylobacter* at the time of delivery 30. Neonates experience only benign infection but may develop severe enteritis or meningitis 31. In study area, some of the women attending ante-natal clinics admitted to have once experienced abortion and infection with *C. jejuni* is said to be associated with spontaneous abortion or premature labour in early pregnancy 30. There is need for improved public health awareness campaign, food hygiene and environmental sanitation. *Campylobacter* species should be included in the screening routine laboratory diagnosis of gastroenteritis in humans for appropriate treatment and management especially in young children, old adults and pregnant women.

Conclusion

The presence of *Campylobacter species* in patients presented for treatment in the various hospitals have been established in the study area. The established prevalence rates have further revealed the level of environmental contamination in different homes in the state and the need for improved public health awareness campaign and environmental sanitation. Collaborative approach between medical and veterinary professions should be encouraged toward diagnosis, treatment, prevention and control of *Campylobacter* infection.

Acknowledgement

The research was funded by ION being part of his PhD thesis. The corporation and assistance of Chief Medical Officers and some nurses of the hospital used for sample collection were highly appreciated by the authors. We also thank the Laboratory staff of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Usmanu Danfodiyo University Sokoto, Nigeria, for their assistance throughout the course of the study.

Competing Interests

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

Authors' contributions

ION developed the research proposal, collected data, performed the laboratory analyses and drafted the manuscript. MDS contributed substantially to the conception and design of the study. OOF and AAM supervised the study and approved the experimental protocol.

References

1. McFadyean J, Stockman S. (1913) cited by Butzler, JP. 2004. *Campylobacter*, from obscurity to celebrity. *Clin Microbiol Infect.* 10: 868-876.
2. Smith T. (1919) cited by Butzler, JP. (2004) *Campylobacter*, from obscurity to celebrity. *Clin Microbiol Infect.* 10: 868-876.
3. Jones FS, Orcutt M, Little RB. (1931) *Vibrios* (*Vibrio jejuni*, n.sp) associated with intestinal disorder of cows and calves. *J Exp Med.* 53:853-863.
4. Vincent R, Dumas J, Picard A. (1947) In Butzler, JP. (2004) *Campylobacter*, from obscurity to celebrity. *Clin Microbiol Infect.* 10:868-876.
5. Butzler JP, Skirrow MB. (1979) *Campylobacter enteritis*. *Clinical Gastroenterology.* 8: 737-765.
6. Corry JL, Atabay HT, Forsythe SJ, Mansfield LP. (2002) Culture media for the isolation of *Campylobacters*, *Helicobacters* and *Arcobacters*. In: culture media for food Microbiology. Elsevier Science, 271-315.
7. Snelling WJ, Matsuda M, Moore JE, Dooley JS. (2005) *Campylobacter jejuni*, *Lett Appl Microbiol.* 41:297-302.
8. Abdul R, Brain M, John GA. (1998) Effect of low-osmolality Nutrient media on Growth and Culturability of *Campylobacter* spp. *Environ Microbiol.* 64 (12): 4643-4649.
9. Park SF. (2002) The physiology of *Campylobacter* species and its relevant to their role as food borne pathogens. *Int J Food Microbiol.* 17: 177-188.
10. Chui DW, Owen RL. (1994) AIDS and the gut. *J Gastroenterol Hepatol.* 9(3): 291-303.
11. Vandenberg O, Klein A, Souayah H. et al. (2003) Possible *Campylobacter jejuni* Osteomyelitis in a 14month old child. *Int J Infect Dis.* 7:164-165.
12. Rao MR, Naficy AB, Savarino SJ. et al. (2001) Pathogenicity and convalescent excretion of *Campylobacter* in rural Egyptian children. *Am J Epidemiol.* 154: 166-173.
13. Coker AO, Isokpehi RD, Thomas BN. et al., (2002) Human campylobacteriosis in developing countries. *Emerg Infect Dis.* 8:237-244.
14. Coker AO, Adefeso AO. (1994) The changing patterns of *Campylobacter jejuni/coli* in Lagos, Nigeria after ten years. *East Afri Med J.* 74: 437-440.
15. Alabi SA, Coker AO, Dosumlt-Ogunbi O, Odugbemi T. (1986) Biotype and serogroup distribution of *Campylobacter* isolates from children in Nigeria. *J Clin Microbiol.* 9: 856-859
16. Adekunle O.C., Coker A.O. & Kolawole D.O. 2009. Incidence, isolation and characterization of *Campylobacter* species in Osogbo. *J Bio Med.* 1: 24-27.
17. Ohanu ME, Offune J. (2009) The prevalence of *Campylobacter* in childhood diarrhea in Enugu State of Nigeria. *J Commun Dis,* 41(2): 117-120.
18. Salihu MD. (2009) Epidemiological studies of *Campylobacter* in food animals in Sokoto State', Doctoral thesis. Usmanu Danfodiyo University Sokoto. 1-132.
19. Ugboma AM, Salihu MD, Magaji AA, Abubakar M.B. (2013) Prevalence of *Campylobacter* species in ground water in Sokoto, Sokoto State, Nigeria. *Vet World* (6): 285-287.
20. NPC. (2006) Sokoto State census statistical tables, Abuja, NPC, 1-10
21. Thrusfield M. (1999) Estimation of disease prevalence. In: *Veterinary Epidemiology.* 2nd ed. Oxford: Blackwell Science, 232-237.
22. Butzler J.P. (2004) *Campylobacter*, from obscurity to celebrity. *Clin Microbiol Infect.* 868-876.\

23. Atabay HL, Corry JEL. (1998) The isolation and prevalence of Campylobacters from the dairy using a variety of methods. *J Appl Microbiol.* 84: 733-740.
24. On SL, Holmes B. (1992) Assessment of enzyme detection tests useful in identification of campylobacteria. *J Clin Microbiol.* 30(3), 746-755.
25. Bauer AW, Kirby WM, Sherris JC, Turck M. (1966) Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol.* 45: 493-499.
26. Saenz Y, Zarazaga M, Lantero M et al., (2000) Antibiotic resistance in Campylobacter strains isolated from Animal, Foods and Humans in Spain, in 1997-1998. *Antimicrob Agents Chemother.* 44: 267-271.
27. Samuel MC, Vugia DJ, Shallow S. et al., (2004) Epidemiology of sporadic Campylobacter infection in United States and declining trend in incidence food net 1996-1999. *Clin Infect Dis,* 38(3): 165-169.
28. Friedman C.R., Hoekstra R.M., Samuel M. et al. (2004) Risk factors for sporadic Campylobacter infection in the United State: A case-control study in FoodNet sites. *Clin Infect Dis.* 38(3), 5285-5296.
29. Wong SN, Tam AY, Yuen KY. (1990) Campylobacter infection in the neonate: case report and review of the literature. *Pediatr Infect Dis.* 9: 665-669.
30. Simor AE, Ferro S. (1990) Campylobacter jejuni infection occurring during pregnancy. *Euro Clin Microbiol Infect Dis.* 9:142-144.
31. Goossens H, Henocque G, Kremp L. Et al., (1986). Noscomial outbreak of Campylobacter jejuni meningitis in newborn infants. *Lancet,* 2:146-149.