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

Innocent Okwundu Nwankwo¹, 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.
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. The name Vibrio fetus was first described as a spirillum after investigation of infectious abortion of bovines in USA. Winter dysentery in calves and swine dysentery have been attributed to infection caused by Vibrio jejuni.

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. 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. They are microaerophilic, non-fermentative, non-spore forming, gram-negative and oxidase positive organism. They are typically curved, comma or spiral shaped rods between 0.5-5µm long and 0.2-0.8 µm wide.

Thermophilic Campylobacter species, mainly C. jejuni and C. coli are recognized as the most common bacteriological causes of gastroenteritis worldwide. Meningitis, osteomyelitis, neonatal sepsis, neuromuscular paralysis occur more in immunocompromised host such as pregnant women, AIDS patients, young and those at very old age. 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. In Nigeria, 16.5% prevalence rate has been reported in Lagos in children with diarrhoea.

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 12°N to 58°N and longitudes 4.8°E to 6.54°E with annual average temperature of 28.3°C. 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 3.7 million 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=Z²p(1-p)/d² 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 x 0.20 x 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 420°C 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 Triipple 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 370°C 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 caped 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, Illela 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 respectively18, 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>
<thead>
<tr>
<th>Hospitals</th>
<th>Total sampled</th>
<th>Total+ (%)</th>
<th>C.jejuni (%)</th>
<th>C.coli (%)</th>
<th>C.lari (%)</th>
<th>X²value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amanwa Leprosium, Denge Shuni</td>
<td>158</td>
<td>79(50)</td>
<td>35(44)</td>
<td>23(29)</td>
<td>21(26)</td>
<td></td>
</tr>
<tr>
<td>Illela General Hospital Illela</td>
<td>49</td>
<td>32(65)</td>
<td>39(9)</td>
<td>13(41)</td>
<td>16(50)</td>
<td>18.321</td>
</tr>
<tr>
<td>Tambuwal General Hospital Tambuwal</td>
<td>61</td>
<td>43(70)</td>
<td>0</td>
<td>25(58)</td>
<td>18(42)</td>
<td>P=0.0004</td>
</tr>
<tr>
<td>Rabbah General Hospital Rabbah</td>
<td>24</td>
<td>6(25)</td>
<td>0</td>
<td>1(17)</td>
<td>5(83)</td>
<td>(P&lt;0.05)</td>
</tr>
<tr>
<td>Total</td>
<td>292</td>
<td>160</td>
<td>38</td>
<td>62</td>
<td>60</td>
<td></td>
</tr>
</tbody>
</table>
**Table II: Prevalence of Campylobacter species in males and females patients in Sokoto State**

<table>
<thead>
<tr>
<th>Sex</th>
<th>Total sampled</th>
<th>Total+ (%)</th>
<th>C.jejuni (%)</th>
<th>C.coli (%)</th>
<th>C. lari (%)</th>
<th>X²</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>126</td>
<td>70(56)</td>
<td>14(20)</td>
<td>28(40)</td>
<td>28(40)</td>
<td>0.00218</td>
<td>0.9628</td>
</tr>
<tr>
<td>Female</td>
<td>161</td>
<td>89(55)</td>
<td>24(27)</td>
<td>34(38)</td>
<td>31(35)</td>
<td>(P&gt;0.05)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>287</td>
<td>159(55)</td>
<td>38(25)</td>
<td>62(40)</td>
<td>59(38)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: sex was not indicated in 5 samples

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

<table>
<thead>
<tr>
<th>Age ranges (yrs)</th>
<th>Total sampled</th>
<th>Total+ (%)</th>
<th>C.jejuni (%)</th>
<th>C.coli (%)</th>
<th>C. lari (%)</th>
<th>X² Value</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early childhood</td>
<td>67</td>
<td>40(60)</td>
<td>14(35)</td>
<td>14(35)</td>
<td>12(30)</td>
<td>3.389</td>
<td>P=0.4950</td>
</tr>
<tr>
<td>Late childhood</td>
<td>48</td>
<td>25(52)</td>
<td>7(28)</td>
<td>8(32)</td>
<td>10(40)</td>
<td></td>
<td>(P&gt;0.05)</td>
</tr>
<tr>
<td>Adolescence</td>
<td>53</td>
<td>24(45)</td>
<td>0</td>
<td>12(50)</td>
<td>12(50)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young Adult</td>
<td>98</td>
<td>46(47)</td>
<td>12(10)</td>
<td>26(60)</td>
<td>18(30)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Old Adult</td>
<td>16</td>
<td>8(50)</td>
<td>0</td>
<td>3(25)</td>
<td>5(75)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>282</td>
<td>143</td>
<td>33</td>
<td>63</td>
<td>57</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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**

<table>
<thead>
<tr>
<th>Female (18-40 yrs)</th>
<th>Total sampled</th>
<th>Total+ (%)</th>
<th>C.jejuni (%)</th>
<th>C.coli (%)</th>
<th>C. lari (%)</th>
<th>X² value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnant</td>
<td>23</td>
<td>16(70)</td>
<td>2(13)</td>
<td>5(32)</td>
<td>9(56)</td>
<td>4.334</td>
<td>0.0374</td>
</tr>
<tr>
<td>Non pregnant</td>
<td>57</td>
<td>25(44)</td>
<td>6(24)</td>
<td>15(60)</td>
<td>4(16)</td>
<td></td>
<td>(P&lt;0.05)</td>
</tr>
<tr>
<td>Total</td>
<td>80</td>
<td>41</td>
<td>8</td>
<td>20</td>
<td>13</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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


