EMERGENCE OF CAMPYLOBACTERIOSIS IN ORLU, IMO STATE SOUTH EASTERN NIGERIA AND ITS ANTIBIOTIC SUSCEPTIBILITY

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Abstract:

The epidemiology of Campylobacteriosis from humans, environment and food was studied for 3 years (May 2011 to April, 2014). Stool samples were collected from patients presenting with enteric diseases (gastroenteritis and fever). Water samples were collected from River and streams within Orluand fresh meat (beef and poultry) were purchased from markets in Orlu. The samples were examined bacteriologically by culture method. In May 2011 to April, 2012, 319 stool samples, 200 beef, 180 chicken and 156 water samples were exam inedand 48 (15%) stool samples, 34 (17%) beef, 17 (9.4%) chicken and 10 (6.4%) water samples were infected with *Campylobacter jejuni*. In May 2012 to April, 2013, 338 stool samples, 215 beef, 184 chicken and 150 water samples were examined, 71 (21%) stool samples, 49 (22.8%) beef, 23 (12.5%) chicken and 13 (8.7%) water samples were infected with *Campylobacter jejuni*. In May 2015 to April, 2014, 331 stool samples, 205 beef, 189 chicken and 159 water samples were examined, 64 (19.3%) stool samples, 57 (27.8%) beef, 24 (12.7%) chicken and 18 (11.3%) water samples were infected with *Campylobacter jejuni*. Out of 988 stool samples examined in the 3 years, 183 (18.5%) were infected with *Campylobacter jejuni* and 96 (9.7%) were infected with *Salmonella* species. Out of 620 beef examined, 140 (22.6%) were infected with *Campylobacter jejuni*and 57 (9.2%) were infected with *Salmonella* species. Out of 553 chicken, 24 (12.7%) were infected with *Campylobacter jejuni*, and 20 (10.6%) were infected with *Salmonella* species. Out of 465 water samples, 41 (8.8%) were infected with *Campylobacter jejuni*, and 34 (7.3%) were infected with *Salmonella* species. Antibacterial susceptibility tests using 6 commonly used antibiotics on *Campylobacter* isolates showed that Ceftriaxone inhibited the highest number of isolates in the 3 years while Tetracycline inhibited the least number. This study has shown that Campylobacteriosis is a re – emerging infectious disease of public health importance in Orlu, Imo State, Nigeria dwellers and travellers in the area constitute the risk group.

Introduction

Campylobacter and other enteric bacteria (*Shigella*and *Salmonella*) cause acute bacillary dysentery or acute colitis often presenting with bloody diarrhea (Glickman, 1991). Schur (1991) associated Campylobacter and other enteric bacteria (*Shigella, Salmonella* and *Yersinia*) with Reactive arthritis (a Reiter's – like syndrome) which occurs 2 – 3 weeks after diarrhoea.

Materials and Methods

Test Samples

The samples used for the study are stool samples from patients presenting with enteric diseases (gastroenteritis and fever) at Imo State University Teaching Hospital, Orlu, fresh meat (beef and chicken) and water samples from Rivers and streams in Orlu.

Selection of Patients and collection of Samples

All patients presenting with enteric disease at Imo State University Teaching Hospital Orlu from May 2011 to April 2014 were approached and the objectives of the study explained to them. Their consent to participate in the study was requested for and those who indicate willingness to participate were given the research questionnaire to complete and indicate their consent in writing. On completion of the questionnaire, they were given sterile screw capped bottles and requested to produce their stool samples in it. All completed questionnaires and stool samples were labeled and transported to the Microbiology Laboratory, Imo State University Teaching Hospital Orlu for analyses.

Collection of Meat and Water Samples

Fresh meat (beef and chicken) were purchased from markets (OrieAmaEzie Market Umuna, OrieUgwu market, OrieOkporo, AforUmuaka, NkwoIsu, Nkwommiri market Amigbo, Eke Okwudor and Eke Eziachi) in Orlu. The marketers were requested to cut the meat into small pieces. They were collected in sterile screw capped bottles, labeled properly and transported to the Microbiology Laboratory IMSUTH Orlu for Analysis.

Water samples were collected from rivers and streams (Njaba River Okwudor, Isu and Umuaka terminals, Nwangele River, Ogidi stream Amaifeke and Ezize stream Ihioma,) in Orlu, in sterile screw capped containers. The collected water samples were transported to the Microbiology Laboratory, Imo State University Teaching Hospital, Orlu and analysed within 2 hours.

Bacteriological Examination of the Test Samples.

All the test samples (stool, water, beef and chicken) were examined bacteriologically by culture method. Each sample was cultured on MacConkey agar medium, Campylobacter selective medium, Deoxycollate citrate bile salt agar and Salmonella and Shigella agar, using streaking method as in Chesbrough (2002), Obiajuru and Ozumba (2009). All the bacterial isolates were identified using standard procedure (Chesbrough, 2002).

1ml of each water or 1g of each meat sample was processed by 2 fold serial doubling dilution using maximum recovery diluent. O.1ml of each dilution of water or meat samples was cultured on Mueller Hinton agar, Campylobavter selective agar and MacConkey agar using spread plate technique as in Obiajuru and Ozumba (2009). All the bacterial colonies that grew on the different plates were counted using bacterial colony counter, to determine the total plate count.

Antibacterial Susceptibility of *Campylobacter* isolates

All the *Campylobacter* isolated from stool, water and meat samples were examined for antibiotic susceptibility using commercially prepared antibiotic discs containing: Ceftriaxone, Ofloxacin, Levofloxacin, Ampiclox, Tetracycline and Gentamycin,

Method of data Analysis

The data obtained from this study were analysed using simple percentage and analysis of variance (AOVA) as in Philips (2002).

Result

The results of the study showed that out of 319 stool samples, 200 beef, 180 chicken and 156 water samples examined from May 2011 to April, 2012, 48 (15%) stool samples, 34 (17%) beef, 17 (9.4%) chicken and 10 (6.4%) water samples were infected with *Campylobacter jejuni*. Out of 338 stool samples, 215 beef, 184 chicken and 150 water samples examined in May 2012 to April, 2013, 71 (21%) stool samples, 49 (22.8%) beef, 23 (12.5%) chicken and 13 (8.7%) of water samples were infected with *Campylobacter jejuni*. A total of 331 stool samples, 205 beef, 189 chicken and 159 water samples were examined in May 2015 to April, 2014, and 64 (19.3%) stool samples, 57 (27.8%) beef, 24 (12.7%) chicken and 18 (11.3%) water samples were infected with *Campylobacter jejuni*. Table 1 summarizes the prevalence of campylobacteriosis and salmonellosis (May 2011 to April 2004). As shown, out of 988 stool samples examined in the 3 years, 183 (18.5%) were infected with *Campylobacter jejuni*, and 96 (9.7%) were infected with *Salmonella* species. Out of 620 beef examined, 140 (22.6%) were infected with *Campylobacter jejuni*, and 57 (9.2%) were infected with *Salmonella* species. Out of 553 chicken, 24 (12.7%) were infected with *Campylobacter jejuni*, and 20 (10.6%) were infected with *Salmonella* species. Out of 465 water samples, 41 (8.8%) were infected with *Campylobacter jejuni*, and 34 (7.3%) were infected with *Salmonella* species.

Fig. 1 summarizes the comparative analysis of the prevalence of campylobacteriosis in human, environment and food (meat) samples. As shown, the prevalence of campylobacteriosis in stool samples increased from 15% in 2011/2012 to 21% in 2012/2013 and decreased to 18.5% in 2013/2014. The prevalence of campylobacteriosis in water increased from 6.4% 2011/2012 to 8.7% in 2012/2012 and 11.3% in 2013/2014. The prevalence of campylobacteriosis in beef increased from 17% in 2011/2012 and 22.8% in 2012012/2013 while the prevalence of campylobacteriosis in chicken increased from 9.4% in 2011/2012 to 12.5% in 2013/2014 and 12.7% in 2013/2014. The prevalence of campylobacteriosis was highest in beef (22.6%) followed by stool (18.5%), and 11.6% in chicken.
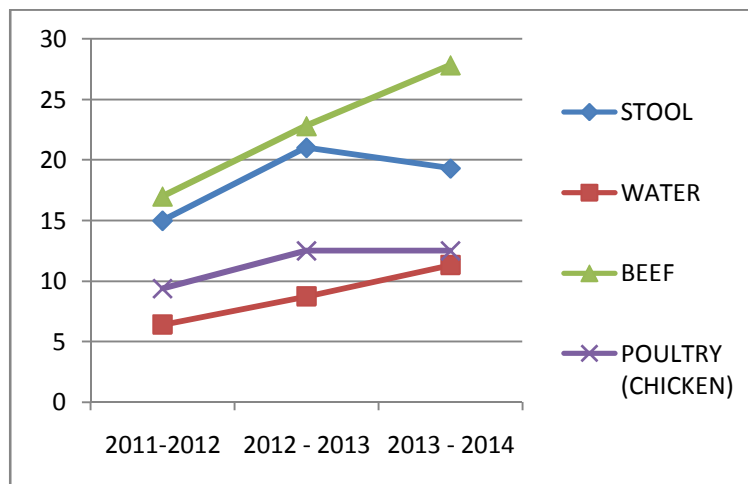
Fig. 2 shows comparative analysis of the prevalence of campylobacteriosis and salmonellosis within the 3 years of study. As shown, the prevalence of campylobacteriosis was higher than that of salmonellosis in all the samples examined..

Table 2 summarizes the total *Campylobacter* bacterial counts of water, beef and chicken samples. As shown, chicken has the highest mean *Campylobacter* count (1.21×10^5 cfu/ml) followed by beef (8.4×10^4 cfu/ml) and water had the least mean *Campylobacter* count (6.9×10^4 cfu/ml).

Table 3 summarizes the antibiotic susceptibility pattern of the *Campylobacter* isolates to selected antibiotics commonly used in Orlu and Nigeria generally. As shown, Ceftriaxone exhibited the highest susceptibility on the test isolates while tetracycline exhibited the lowest.

Table 1: PREVALENCE OF CAMPYLOBACTERIOSIS 2011 – 2014

YEAR	STOOL			WATER			BEEF			POULTRY		
	No Exam	No. Infected	(%)	No Exam	No Infected	(%)	No Exam	No Infected	(%)	No Exam	No Infected	(%)
		Campylobacter	*Salmonella*		*Campylobacter*	*Salmonella*		*Campylobacter*	*Salmonella*		*Campylobacter*	*Salmonella*
May 2011 – April 2012	319	48 (15.0)	30 (9.4)	156	10 (6.4)	13 (8.3)	200	34 (17.0)	16 (8.0)	180	17 (9.4)	14 (7.8)
May 2012 – April 2013	338	71 (21.0)	37 (10.9)	150	13 (8.7)	9 (6.0)	215	49 (22.8)	21 (9.8)	184	23 (12.5)	18 (9.8)
May 2013 – April 2014	331	64 (19.3)	29 (8.8)	159	18 (11.3)	12 (7.5)	205	57 (27.8)	20 (9.8)	189	24 (12.7)	20 (10.6)
Total	988	183 (18.5)	96 (9.7)	465	41 (8.8)	34 (7.3)	620	140 (22.6)	57 (9.2)	553	64 (11.6)	52 (9.4)

Years of Study

Fig. 1:Comparative Analysis of the Prevalence of Campylobacteriosis in Human, Environment and Food samples
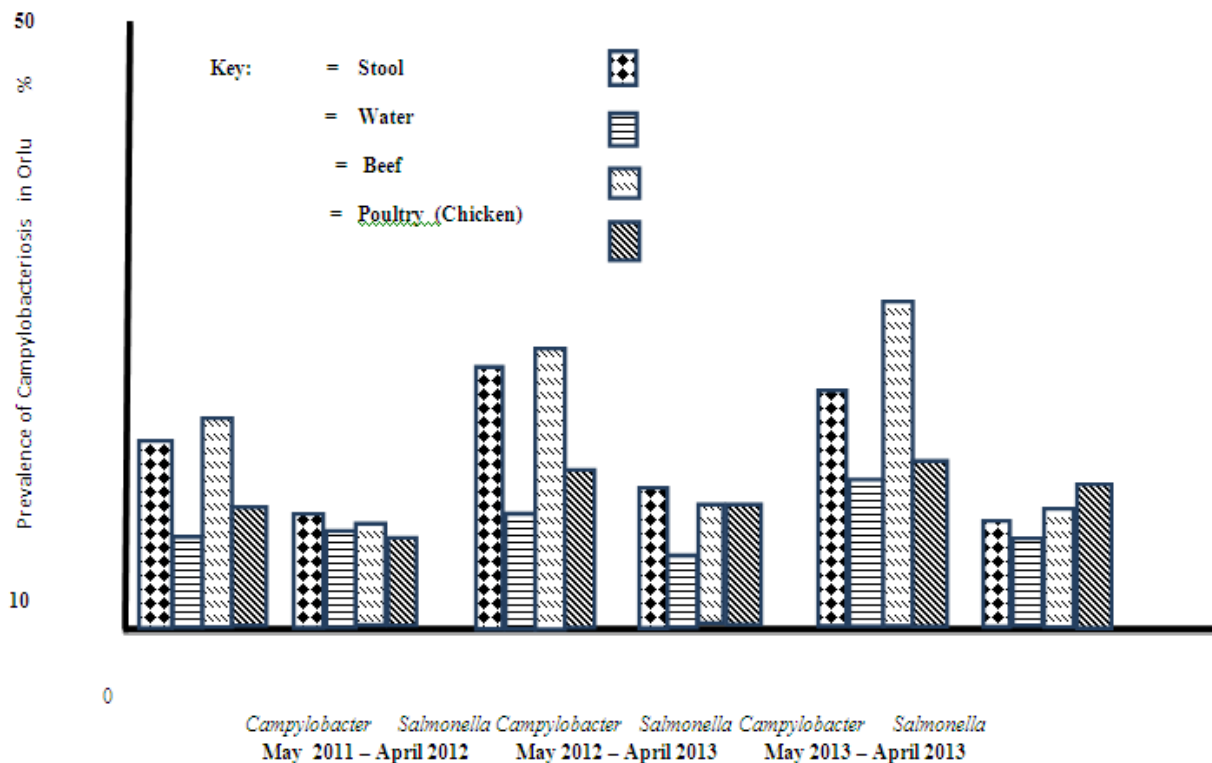
Fig. 2: Comparative Analysis of Campylobacteriosis and Salmonellosis

Table 2: Total heterotrophic *Campylobacter* count

Sample	Number Counted	Mean Colonies counted	Dilution Factor	Mean heterotrophic count (cfu/ml)
WATER	10	69	$1/10^3$	6.9×10^4
BEEF	34	84	$1/10^3$	8.4×10^4
CHICKEN	17	121	$1/10^3$	1.21×10^5

Table 3: Antibiotic Susceptibility of *Campylobacter* Isolate

Year of Isolation	Source of Isolate	No Exam	Number of Isolates Susceptible (%)					
			Ceftriax	Ofloxaci	Levoflox	Ampiclox	Tetracycl	Gentam
2011 - 2012	Stool	48	41 (85.4)	36 (75)	39 (81.3)	14 (29.2)	11 (22.9)	25 (52.1)
	Water	10	10 (100)	8 (80)	6 (60)	6 (60)	5 (50)	7 (70)
	Beef	34	30 (88.2)	31 (91.2)	30 (88.2)	19 (55.9)	20 (58.8)	26 (76.5)
	Poultry	17	16 (94.1)	15 (88.2)	13 (76.5)	10 (58.8)	12 (70.6)	`5 (29.4)

2012 - **2013**	**Stool**	**71**	68 (95.8)	55 (77.5)	59 ()83.1	43 (60.6)	25 (35.2)	34 ()47.9
	Water	**13**	13 (100)	10 (76.9)	9 (69.2)	9 (69.2)	6 (46.2)	8 (61.5)
	Beef	**49**	40 (81.6)	45 (91.8)	42 (85.7)	23 (46.9)	15 (30.6)	29 (59.2)
	Poultry	**23**	20 (87)	17 (73,9)	21 (91.3)	14 (60.9)	9 (39.1)	11 (47.8)
2013 - **2014**	**Stool**	**64**	59 (92.2)	56 (67.5)	59 (92.2)	34 (53.1)	21 (32.8)	45 (70.3)
	Water	**18**	13 (72.2)	15 (83.3)	16 (88.9)	11 (61.1)	8 (44.4)	13 (72.2)
	Beef	**57**	51 (89.5)	46 (80.7)	49 (86)	32 (56.1)	17 (29.8)	44 (77.2)
	Poultry	**24**	20 (83.3)	19 (79.2)	20 (83.3)	12 (50)	9 (37.5)	13 ()54.2

Discussion

Campylobacteriosis is an enteric bacterial infection which causes diarrhea, dysentery, Reiter's – like syndrome, Psoriatic arthritis amongst others. In Nigeria, campylobacteriosis is not common and rarely included in routine diagnoses of patients. Major attention is given to typhoid fever caused by *Salmonella* species. In May – June, 2010, 7 patients presenting with gastroenteritis in Community Medicine and General out - patient departments of Imo State University Teaching Hospital Orlu were diagnosed of campylobacteriosis. In February, 2011, 3 HIV – positive patients with severe gastroenteritis in Community Medicine Department and 2 pregnant women in ante natal clinic were diagnosed of campylobacteriopsis. Following these observations, we mounted a 3 – years clinical surveillance for campylobacteriosis in the hospital.

The study has established the existence of campylobacteriosis which had not been in existence in the area. The emergence of campylobacteriosis in Orlu is of public health importance. People at rick, who are likely to acquire the infection include dweller and travellers to the area. Clinicians in the area should take the advantage of this study and include campylobacteriosis check routine checks for patients with gastroenterititis and fever. Forbes and Jackson (1994) associated *Campylobacter,* with secretory diarrhoea characterized by copious fluid stools and dehydration. Patients with these and related symptoms should be examined for campylobacteriosis.

The present study recorded high prevalence of campylobacteriosis in meat (beef and chicken) sold in various markets in Orlu as well as in water samples from Rivers and streams in the area. The disease is a food borne infection acquired by ingesting contaminated food or water. Thus the contaminated Rivers and streams as well as contaminated meat sold in the various markets are the likely sources of the emergence of campylobacteriosis in Orlu. Public health authorities in the area should take advantage of this study to inspect and monitor meat sold to the public in various markets and abattoirs. Also, poultry farmers in the area should be examined routinely and their poultry birds inspected regularly for infection. These amongst other measures will help to control the infection.

The antibiotic susceptibility test of the isolates showed that the commonly used antibiotics in the area are effective against the bacteria. Thus in case of infected people, these antibiotics stand a greater chance of proffering treatment and/or cure. In the rural areas wher there are limited diagnostic facilities and lack of trained personnel primary health care providers may include these antibiotics in their routine drugs for patients with symptoms of the infection pending the possibility of definitive diagnosis and confirmation.

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