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Prevalence and Antibiotics Resistance of Campylobacter jejuni in Retail Chickens in Oyo State, Nigeria

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Abstract
Increasing demand and production of animal protein in Nigeria require safety assessment of associated food borne pathogens for consumer protection. Unhygienic handling of chickens during processing is a common practice at retail markets and poultry slaughter slabs in Nigeria with possibility of foodborne pathogen contamination including *Campylobacter*. Published data on *Campyloba*cter in poultry meat in Nigeria are scanty. In this study, *Campylobacterjejuni* were isolated from chicken samples obtained from retail markets in Ibadan to determine the prevalence and antibiotic susceptibility using *Campylobacter* standard culture technique. Out of 252 chicken samples collected, *Campylobacter jejuni* was isolated in 242 (96.0% prevalence) samples. Susceptibility test indicated that the isolates were 100.0% resistance to nalidixic acid, gentamicin, and erythromycin and resistance of 38.0%, 46.0%, 50.0% and 58.0% to enrofloxacin, chloramphenicol, streptomycin and tetracycline respectively. Food safety risk could result from direct contamination and cross contamination of carcasses during evisceration and unhygienic rinsing of multiple carcasses from different farms. While the high resistance to commonly used antibiotics could be due to unregulated misuse of veterinary drugs commonly practiced in Nigerian poultry production. Prudence use of antibiotics in poultry, hygienic handling and thorough cooking of poultry products will ensure food safety.
Keywords: *Campylobacter*, antibiotics resistance, chickens, food safety, Nigeria

1. Introduction

Nigerian Poultry is the fasted growing livestock sector in West Africa sub region due to high demand. Chickens are commonly consumed across every stratum of Nigeria society without religious or ethnic restrictions. Locally produced chickens are supplemented with frozen chickens illegally imported into the country. Unhygienic handling of chickens during processing is a common practice at retail markets and poultry slaughter slabs in Nigeria with possibility of foodborne pathogen contamination including *Campylobacter*.

Bacterial pathogens are the major cause of food borne diseases. Among others, common bacterial pathogens associated with poultry products include *Campylobacter species, Salmonella species* among others and according to WHO 2001 report, *Campylobacter* is the leading cause of zoonotic enteric human infections in most developed and developing countries. *Campylobacter* species are twisted Gram-negative microaerophilic bacilli (Hunt, 1992) with the thermophilic species most commonly associated with diarrhea in humans (Lastovica and Skirrow, 2000). Majority of cases of campylobacteriosis are self-limiting, however, infections may develop into severe invasive or relapsing diseases such as Guillain-Barre's syndrome,meningitis, peritonitis, pancreatitis and reactive arthritis. *Campylobacter* species colonize the gastrointestinal tract of animals such as poultry, cattle, sheep, goat, pigs and wild birds. The body temperature of birds are close to the optimum temperature for growth of thermophilic *Campylobacter,* hencehandling of raw poultry meat and eating poultry products are important risk factors for sporadic Campylobacteriosis (Vincenza *et al.,* 2007).Surveillance data from the United States and Europe indicate that 50–80% of raw retail chicken meats were contaminated with *Campylobacter* (Zhao *et al.,* 2001; Dominguez *et al.,* 2002). More so, increasing prevalence of resistant food borne bacteria is a global public health concern especially in developing countries where antibiotics are frequently and indiscriminately administered to food animals (Olatoye, 2011). Antibiotic-resistant *Campylobacter* species from animals can infect the human population via occupational exposure or through the food chain (Van den Bogaard and Stobberingh 2000).

During slaughter, the intestinal content could contaminate chicken carcasses with *Campylobacter* which are transferred along the process operations and to retail and ready to eat chicken meat (Stern and Robach, 2003). Consumers are thus exposed to *Campylobacter* infection when chicken meat is not handled hygienically, that is by cross contamination from raw chicken to ready-to-eat food including salads and/or if the meat is not properly cooked before consumption (Humphrey *et al.,* 2007; Kusumaningrum *et al.,* 2003; Rosenquist *et al.,* 2006). Despite the increasing production and consumption of chicken in Nigeria, published data on prevalence of *Campylobacter in* chicken in the country are scanty. Salihu *et al.,* 2009 reported a high prevalence of contamination with thermotolerant *Campylobacter* (81.9%) in poultry meat in Sokoto, Nigeria. Oyo State is the hub of poultry production in Nigeria, with majority of poultry breeders and broiler producers. This study was conducted to determine the incidence of contamination of chicken meats from retail poultry markets in Ibadan, Oyo State with *Campylobacter* species and antibiotic susceptibility profile of the isolates.

2. Materials and Methods

2.1 Sample Collection

A total of 272 chicken meat samples were randomly and aseptically collected from major poultry retail outlets at Bodija, Mokola and Molete markets in Ibadan north local government over a period of six weeks in 2014. About 25g of each sample was placed in a sterile plastic bag containing 225ml of Amies transport medium (Oxoid CM045). The properly labeled samples were transported in a cold ice pack within 3 hrs of collection to the laboratory for analysis.

2.2 Isolation and Identification

The samples were homogenized in stomacher blender and the homogenates were incubated in anaerobic jars at 42°C for 48 hrs under microaerophilic conditions obtained using campy GEN (Oxoid). Following the incubation, a Loopful of culture was streaked onto plates of modified Charcoal Cefaperaxone Deoxycholate Agar (Oxoid, CM0739) and this was incubated at 42°C for 48 hrs. *Campylobacter* suspected colonies were sub-cultured 5% sheep blood agar, one or more times until monocultures were obtained. Curved or spiral Gram negative rods, oxidase positive were presumptively identified as *Campylobacter*. Further biochemical investigations were performed for identification (Baron *et al.,* 1994). Hippurate hydrolysis test was also performed according to the scheme described by Lior (1984). Nitrate reduction and H_2S production in triple sugar iron medium were used as additional tests for identification of the *Campylobacter species.*

2.3 Antibiotics Susceptibility Test

Susceptibility of the isolates to commonly used antibiotics was determined using Kirby-Bauer disc diffusion method on Mueller-Hinton agar (Oxoid, Basingstoke, United Kingdom) plates. Inhibition zone sizes were interpreted using standard recommendations of the Clinical Laboratory Standard Institute (CLSI, 2008). The antibiotic disks (the disk content is indicated in parentheses) contained nalidixic acid (30 µg), gentamicin (10 µg), erythromycin (15 µg), enrofloxacin (10 µg), chloramphenicol (30 µg), streptomycin and tetracycline (30 µg). *E. coli* (ATCC 25922) was used as reference strains for antibiotic susceptibility testing.

3. Results

3.1 Prevalence of Campylobacter in chickens

All the carcasses examined from the markets were contaminated with *Campylobacter*. One or more strains of *Campylobacter* species were isolated from all the 252 poultry samples collected (100.0% prevalence). While *Campylobacter jejuni*was isolated and identified from 243 poultry samples (96.4% prevalence) as shown in Table 1.

Table 1. Strains of *Campylobacterjejuni* from the positive fresh raw chicken meat samples

Campylobacter species	Bodija		Mokola		Molete		
	Number of strains	Number of positive samples	Number of strains	Number of positive samples	Number of strains	Number of positive samples	Total
Campylobacter. Jejuni	84	84	75	84	84	84	243

3.1 Antibiotics Susceptibility

The results of antibiotic susceptibility showed that the isolated C*ampylobacterjejuni* has 100.0% resistance to nalidixic acid, gentamicin, and erythromycin while it has a resistance of 38.0%, 46.0%, 50.0% and 58.0% to enrofloxacin, chloramphenicol, streptomycin and tetracycline respectively (Figure 1).

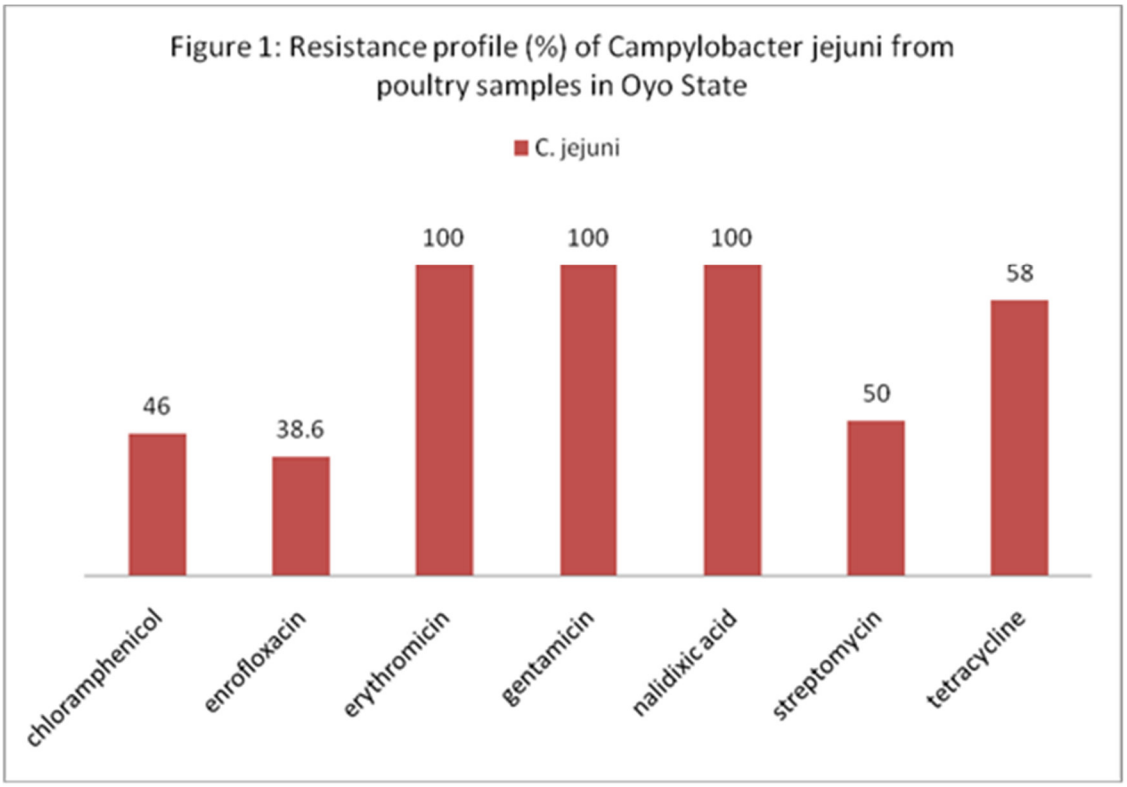

Figure 1. Antibiotic resistance profile (%) of *Campylobacter jejuni* isolated from retail chickens in Oyo State, Nigeria

4. Discussion

This study showed that all the raw chicken meats were contaminated with *Campylobacter species* which is higher than 81.9% reported in Sokoto by Salihu *et al.,* 2009 in raw poultry meat. Jozwiak *et al.,* 2006 also reported a prevalence of 100.0% in freshly slaughtered poultry carcasses in a poultry house in Hungary and in North Ireland, poultry meat at retail sale level was reported to have a prevalence of 94% (Moore *et al.,* 2002). The high contamination rate of poultry meat obtained from this study could have resulted from contamination of the carcass during evisceration procedures by ingesta from the bird's gut while the poultry meat is being processed which can lead to direct contamination of the carcasses. This could also be as a result of unhygienic practices among the meat handlers during slaughtering such as rinsing multiple carcasses in the same water and cross contamination of the meat slabs with the organism carried over from different poultry farms. More so, the chickens sold at these markets were sourced from different farms across the southwest states of Nigeria. These results also showed widespread prevalence of campylobacter in Nigerian poultry which could also be part of the normal floral of the chicken gut. Gender issues are also of concern in this result as majority of the chicken marketers were women, some of whom were breastfeeding infants during meat processing.

Kramer *et al.,* 2000 reported that more than one *Campylobacter* species may be found in the same sample, this is in line with the isolation of more than one strain of *Campylobacter* from most of the raw poultry meat samples in this study. The isolation of *Campylobacter* species in this study is of serious public health concern, because *Campylobacter jejuni* and other thermophilic *Campylobacter* species such as *Campylobacter coli* and *Campylobacter lari* have been implicated in causing human disease (Hanninen, 2000).The spectrum of Campylobacteriosis can range from mild to severe, particularly in the immuno-suppressed, and may exhibit neurological and rheumatologic sequelae (Allos *et al.,* 2009).

Also, antibiotic resistance profile of the isolated *Campylobacter* revealed a high percentage of the organisms showing multiple drug resistance to commonly used antibiotics. In this study, the isolates exhibited 100.0% resistance to nalidixic acid, gentamicin and erythromycin while resistance to enrofloxacin was 39.0%. *Campylobacter* with resistance to ciprofloxacin or other fluoroquinolones, Macrolides and Chloramphenicol, amino glycosides, tetracycline and quinolones, have been reported (Moore *et al.,* 2006). Of utmost importance is the resistance to drug like erythromycin (a drug of choice for treat of *Campylobacteriosis* in humans) which could be due to the abuse or over use of such drugs in livestock production. Antimicrobial resistance has become a global public health concern in recent years (Isenbarger *et al.,* 2002; Nachamkin *et al.,* 2002). Antibiotic-resistant *Campylobacter* species from animals can infect the human population via occupational exposure or through the

food chain (Van den Bogaard and Stobberingh 2000). This, increasing prevalence of resistance among clinically important bacterial pathogens like *Campylobacter* is of public health importance because people's consumption of raw or unpasteurized bovine milk, undercooking of poultry meat and cuts on meat processor exposes such people to this pathogenic organisms. The occurrence of human *Campylobacter* gastroenteritis has been largely attributed to the consumption of contaminated food of animal products (Salihu *et al.,* 2010).

5. Conclusions

This study indicated that most of the chickens produced for human consumption in Oyo State were contaminated with food borne pathogen such as *Campylobacter.* The organisms could have carry over from different poultry farms transported to retail markets. Also the unhygienic handling and processing of such animal products as well as consumption of undercooked poultry meat could be the cause of human campylobacteriosis. Farm to fork food safety mechanisms including prudence use of antibiotics and operation of Hazard Analysis Critical Control Points (HACCP) are advocated. Poultry farmers and middlemen such as those selling chicckens in the market should be enlightened on hygienic poultry meat processing and handling methods as well as the public health importance of campylobacteriosis thereby ensuring food safety. Regulatory control of antibiotic use in animals will enable effective management of the progressive increase in antimicrobial resistance among *Campylobacter* species in human and animal health.

Refrences

Allos, B.M., Blaser, M.J.: In Mandell, Douglas & Bennett's (2009). "Principles and Practice of Infectious Diseases" 7th edition. Edited by Mandel GL. Philidelphia, PA: Elsevier Publishing Co. Chapter 216.

Allen, K. J., and Griffiths, M.W. (2001). "Use of luminescent *Campylobacter jejuni* ATCC 33291 to assess eggshell colonization and penetration in fresh and retail eggs", *Journal of Food Protection* 64, 2058-2062.

Hänninen, M.L., Perko-Mäkelä, P., Pitkälä, A. and Rautelin, H., (2000). "A three-year study of *Campylobacter jejuni* genotypes in humans with domestically acquired infections and in chicken samples from the Helsinki area", *Journal of Clinical Microbiology* 38, 1998-2000.

Humphrey, T., O'Brien, S. and Madsen, M. (2007). "*Campylobacter*s as zoonotic pathogens: a food production perspective", *International Journal of Food Microbiology* 117, 237-257.

Hunt, M.J. (1992). "Isolation of thermophilic *Campylobacter* species from foods and water", FDA Bacteriological Analytical Manual. 7th ed. AOAC International, Arlington VA USA.

Isenbarger, D.W., Hoge, C.W., A., Pitarangsi, C., Vithayasai, N., Bodhidatta, L., Hickey, Srijan, K.W. and Phung, D.C. (2002). "Comparative antibiotic resistance of diarrhoeal pathogens from Vietnam and Thailand", 1996–1999. *Emerging Infectious Diseases* 8, 175-180.

Jozwiak, A., Reichart, O. Laczay, P. (2006). "The occurrence of *Campylobacter* species in Hungarian broiler chickens from farm to slaughter", *Zoonoses and Public Health* 53, 291-294.

Kramer, J.M, Frost, J.A, Bolton, F.J, Wareing, D.R (2000). "*Campylobacter* contamination of raw meat and poultry at retail sale: identification of multiple types and comparison with isolates from human infection", *Journal of Food Protection* 63:1654-1659.

Kusumaningrum, H.D, Riboldi, G, Hazeleger, W.C and Beumer, R.R (2003). "Survival of food borne pathogens on stainless steel surface and cross-contamination to foods", *International Journal of Food Microbiology* 85, 227-36.

Lastovica, A.J. and Skirrow, M.B. (2000). "Clinical significance of *Campylobacter* and related species other than *Campylobacter jejuni* and C. *Coli"* In Nachamkin I. and BlaserM.J. (eds.), *Campylobacter*, 2nd ed. P. 89-120, American Society for Microbiology, Washington, D.C.

Moore, J. E, Barton, M.D, Blair, I.S, (2006). "The epidemiology of antibiotic resistance in *Campylobacter*". *Microbes and Infections* 8, 1955–1966.

Moore, J.E., Wilson, T.S., Wareing, D.R., Humphrey, T.J., Murphy, P.G. (2002). "Prevalence of thermophilic *Campylobacter* spp. in ready-to-eat foods and raw poultry in Northern Ireland", Journal of Food Protection 65, 1326-1328.

Nachamkin, I., Szymanski, C., Blaser, M. (2008). "Campylobacter", 3rd ed. ASM Press, American Society for Microbiology. Washington, DC.

Olatoye, I.O. (2011) "Antibiotics use and resistance patterns of *Salmonella* spp in poultry in Ibadan Nigeria", Tropical Veterinarian 29, 28-35.

Rosenquist, H., Sommer, H., Nielsen, N., Christensen, B., (2006). "The effect of slaughter operations on the contamination of chicken carcasses with thermotolerant *Campylobacter"*. International Journal of Food Microbiology*,* 108, 226-232.

Salihu, M.D., Junaidu, A.U., Magaji, A.A. and Rabiu, Z.M., (2010). Study of *Campylobacter* in Raw Cow Milk in Sokoto State, Nigeria. British Journal of Dairy Science 1, 1-5.

Salihu, M.D., Junaidu, A.U., Magaji, A.A., Abubakar, M.B., Adamu, A.Y. and Yakubu, A.S. (2009). Prevalence of *Campylobacter* in poultry meat in Sokoto, Northwestern Nigeria. Journal of Public Health and Epidemiology 1, 41-45.

Stern, N.J., Robach, M.C. (2003). "Enumeration of *Campylobacter* spp. in broiler feces and in corresponding processed carcasses", Journal of Food Protection, 66, 1557-1563.

Van den Bogaard, A.E. and Stobberingh, E.E. (2000). Epidemiology of resistance to antibiotics links between animals and humans. International Journal of Antimicrobial Agents 14, 327–335.

Vincenza, P., Gabrielia, P., Paolo, C., Christina, M.C., Iorgio, P., Daniela, M., Francesco, P., Daniel, P.M. (2007). "Thermotolerant *Campylobacter* in poultry meat marketed in the Abruzzo and Molise regions of Italy: prevalence and contamination levels", Veterinaria Italiana, 43, 167-174.

WHO (2001). "The increasing incidence of human Campylobacteriosis", Report and proceedings of a WHO Consultation of Experts, Copenhagen, Demark (21-25 November 2000).

Zhao, C., Ge, B., De Villena, J., Sudler, R., Yeh, E., Zhao, S., White, D.G., Wagner, D. and Meng, J. (2001). "Prevalence of *Campylobacter* spp., *E. Coli* and *Salmonella* serovars in retail chicken, turkey, pork and beef from the Greater Washington, D. C., area. Journal of Applied Environmental Microbiology, 67, 5431-6.