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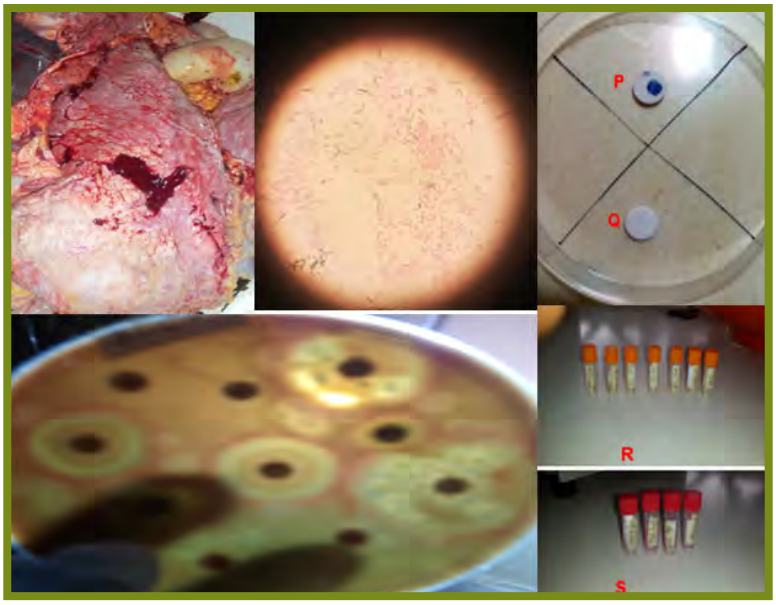

Phenotypic Characterization of *Campylobacter* Species from Ruminants Slaughtered at Major Abattoirs in Ilorin, Kwara State, Nigeria

ABSTRACT

Campylobacter is a leading cause of bacterial gastroenteritis worldwide. This study determined the phenotypic characteristics of *Campylobacter* from ruminants slaughtered in two major abattoirs in Ilorin metropolis of Kwara state, Nigeria. In total, 350 fecal samples were collected from beef cattle (n = 200) and goat (n = 150). *Campylobacter* was isolated and phenotypically characterized using standard bacteriological methods. Seventy (20%) of the samples were positive for *Campylobacter*. The isolation rate of *Campylobacter* from female animals (11.71%) was higher than that of males (8.28%), albeit there was no significant difference ($P > 0.05$). Similarly, the rate of isolation of *Campylobacter* from bovine (12.86%) was not statistically significant ($P > 0.05$) from that of caprine (7.14%) species. Only five (7.14%) of the total isolates were *Campylobacter jejuni*. All isolates were resistant to nalidixic acid and pan-susceptible to gentamicin, but there were different rates of antimicrobial resistance to other tested antibiotics. There was also high rate of resistance to cefotaxime (83%) and ampicillin (76%), and 53% of the isolates displayed multidrug resistance phenotypes. The study established 20% *Campylobacter* contamination of ruminants slaughtered in the two major abattoirs in Ilorin, and most of the isolates were multidrug resistant. Further study is recommended to molecularly characterize the species of *Campylobacter* circulating in the study area.

INTRODUCTION

Campylobacteriosis is one of the major causes of bacterial diarrheal disease worldwide (*13*). Although many species exist within the genus *Campylobacter, C. jejuni, C. coli, C. upsaliensis, C. jejuni* subspecies *doylei, C. concisus, C. lari*, and *C. mucosalis* are the species commonly cultured from fecal specimens (*27*), with almost 90% of reported cases associated with *C. jejuni* (*13*). Several other emerging species have recently been reported, including *C. curvus, C. hyointestinalis, C. insulaenigrae, C. lari, C. mucosalis, C. sputorum biovar sputorum,* and *C. ureolyticus,* all of which have been associated with gastroenteritis (*29*). *Campylobacter* species are curved, small, nonsporeforming Gram-negative rods that are microaerophiles (*23*). They belong to the *Campylobacteraceae* and are 0.2–0.9

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μm wide × 0.5–5 μm long. They are encapsulated and motile with the aid of polar flagella at one or both ends. When two or more bacterial cells are seen together under the microscope, they can form "S" or gull-winged shapes. They are very fastidious bacteria requiring various nutrients to grow *(20)*.

Many animals serve as reservoirs of members of the *Campylobacter* genus. The bacteria reside in the intestines of cattle, dogs, cats, pigs, sheep, goats, and even birds, causing little or no pathology. Pigs are commonly associated with *C. coli*, whereas *C. jejuni* is commonly associated with the avian and bovine species *(8, 48)*. Humans become infected either by direct contact with the feces of infected animals or through consumption of contaminated foods of animal origin *(23, 48)*. In developed countries, reported cases of *Campylobacteriosis* are estimated to be >1 million every year *(43)*. However, in less industrialized countries such as Nigeria, *Campylobacter* infections are underreported because of the absence of regular surveillance and misdiagnosis of the infection as other diarrhea-causing bacteria *(25)*. In addition to gastroenteritis, *Campylobacter* species have been incriminated in extraintestinal ailments with severe complications and sequelae such as Miller Fisher or Guillain-Barré syndromes *(47)*.

Genotypic methods used in characterization of bacteria are more precise than the traditional phenotypic methods. However, the genotypic methods are not readily available, especially in developing countries such as Nigeria. In addition, many researchers have been deterred from conducting *Campylobacter* research because of its fastidious nature, making it difficult to work with. This study aimed at determining the phenotypic characteristics of *Campylobacter* isolates from cattle slaughtered at two major abattoirs in Ilorin, Kwara state, Nigeria.

MATERIALS AND METHODS

Geographical location of study area and the study design

The study was carried out in Ilorin. Ilorin is the capital of Kwara in north central Nigeria (8°32′N, 4°35′E). It has an area of approximately 468 km^2, and it is situated in the transitional zone within the forest and the Guinean savannah regions of Nigeria *(1)*. Kwara is made up of 16 local government areas, with an estimated population of 2,588,108 *(31)*. Samples were collected from beef cattle and goats slaughtered at Abubakar Saraki Ultra-Modern abattoir, Akerebiata, and Ipata abattoir, respectively, both located at Ilorin East local government area of Kwara. These locations were purposefully selected for sampling because they are the two major abattoirs in Ilorin *(Fig. 1)*.

Sample collection

In total, 350 faecal samples were collected: beef cattle feces from Abubakar Saraki Ultra-Modern abattoir, Akerebiata (n = 200) and goats' feces from Ipata abattoir (n = 150). Ten grams of fecal sample was collected directly from the cecum of slaughtered animal into a sterile sample bag and placed into a cool box containing ice pack (4 ± 1°C). The cool box with samples was immediately transported to the Veterinary Microbiology Laboratory, University of Ilorin, for analyses. Processing of the samples was done within 24 h of collection.

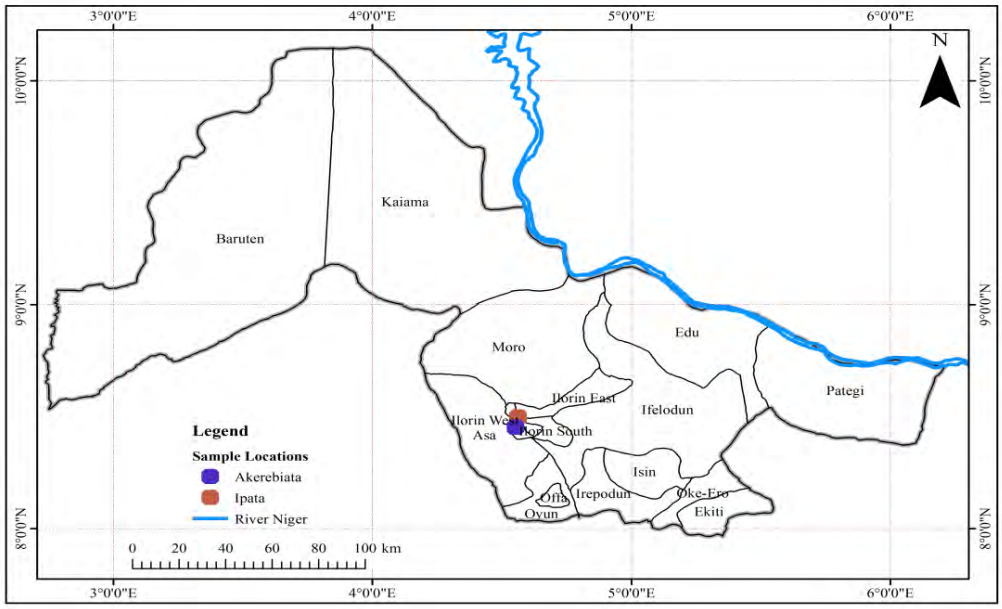

Figure 1. Map of Kwara State showing the abattoirs locaitons in Ilorin, North central Nigeria.

Isolation of *Campylobacter*

Isolation of *Campylobacter* from fecal samples was done according to NF EN ISO 10272-1: 2017 as described previously *(25, 26)*, with little modification. In brief, approximately 1 g of feces was suspended in 9 mL of thioglycolate broth supplemented with Preston *Campylobacter* selective supplement (SR0204E, Oxoid, Hampshire, UK). The sample was then incubated at 42 ± 1°C for 48 ± 2 h under microaerophilic conditions provided by CampyGen (Oxoid). After incubation, a loopful of the enriched culture was streaked onto modified charcoal cefoperazone deoxycholate agar plate (Oxoid) supplemented with Preston *Campylobacter* selective supplement (SR0204E) and incubated for 48 ± 2 h at 42 ± 1°C under microaerobic conditions (CampyGen). Distinct colonies suspected to be *Campylobacter* were selected from each modified charcoal cefoperazone deoxycholate agar plate and purified using Mueller-Hinton agar (Oxoid) supplemented with Preston *Campylobacter* selective supplement (SR0204E) that also was incubated at 42 ± 1°C for 48 ± 2 h. Pure cultures were stored at −80°C in Mueller-Hinton broth containing 30% glycerol until further analysis.

Phenotypic characterization of *Campylobacter* isolates

Biochemical tests included Gram reactions as described previously *(7)*, with minor modification: 0.1% aqueous basic fuchsin was used for counterstaining instead of safranin because it has been reported that *Campylobacter* species are not easily visualized with the safranin counterstain *(36)*. In addition, we conducted the oxidase test by using commercial oxidase disks (Hi-Media, New Delhi, India), and a color change of the disk to deep purple within 10 s was considered positive *(49)*. *C. coli* ATCC 33559 and *E. coli* ATCC 25922 were used as positive and negative controls, respectively. Catalase and urease tests also were carried out on the isolates.

A hippurate test, to differentiate *C. jejuni* from other species of *Campylobacter*, was done on the isolates as described previously *(19, 28)*. A discrete colony of presumptive *Campylobacter* isolate was inoculated into 0.2 mL of sterile distilled water in a test tube. A hippurate disc (Hi-Media) was placed in the suspension and incubated at 37°C for 2 h with gentle shaking in water bath (SHZ 82 shaker water bath). After incubation, 0.2 mL of ninhydrin reagent (Global Pharma Hamburg, Germany) was gently dropped down the side of the tube to the inoculum, and the sample was reincubated at 37°C for 10 min without shaking. *C. jejuni* is hippurate positive and turns the suspension deep purple, indicating the presence of glycine, which led to hippurate hydrolysis. *C. jejuni* ATCC 29428 and *C. coli* ATCC 33559 were used for the test and technique validations.

An antimicrobial susceptibility test was done according to Kirby Bauer disk diffusion assay *(4)* by using Mueller-Hinton agar (Oxoid) supplemented with 10% sheep red blood cells. Nine different antimicrobials (Hi-Media) were used for the test: ampicillin 10 µg (AMP 10), ceftazidime 30 µg (CAZ 30), ciprofloxacin 5 µg (CIP 5), ceftriaxone 30 µg (CTR 30), cefotaxime 30 µg (CTX 30), cefoxitin 30 µg (CX 30), gentamicin 10 µg (GEN 30), nalidixic acid 10 µg (NAL 10), and tetracycline 10 µg (TET 10). Inoculum of fresh isolate was prepared in 5 mL of sterile distilled water. The turbidity of the inoculum was adjusted to 0.5 McFarland standard corresponding to 1.8×10^5 CFU/mL. The inoculum was flooded on 10% blood Mueller-Hinton agar and allowed to stand for 3 min. The excess inoculum was then discarded and the plate was drained for approximately 3–5 min. Antimicrobials were then dispensed on the isolate-seeded blood Mueller-Hinton agar by using a disc dispenser (Oxoid). The plates were incubated at 42°C for 24 h under microaerophilic conditions. The diameters of the zones of inhibition were measured and interpreted as resistance, intermediate, or sensitive according to Clinical and Laboratory Standards Institute *(6)*.

RESULTS

Of the 350 samples collected from the two abattoirs sampled in Ilorin, 70 (20%) were biochemically positive for *Campylobacter*. The isolation rate of *Campylobacter* from female animals (11.71%; n = 41) was higher than that from males (8.28 %; n = 29); however, there was no statistically significant difference ($P > 0.05$) between the rates. Similarly, the rate of isolation of *Campylobacter* from bovine species (12.86%; n = 45) was not significantly different from that of ovine species (7.14%; n = 25; $P > 0.05$) *(Table 1)*. The biochemical characterization revealed that all of the 70 (20%) isolates were Gram-negative curved rods, glucose and lactose non fermenters, and urease negative and oxidase positive, whereas 8 (4%) produced H_2S in the lead acetate paper. The hippurate hydrolysis test revealed that 7.14% (n = 5) of the total isolates are presumptively identified as *C. jejuni*, whereas 92.86% (n = 65) are other species of *Campylobacter* *(Table 2)*. Different rates of antimicrobial resistance were observed among the isolates, with all the isolates displaying resistance to nalidixic acid, whereas they were pan-susceptible to gentamicin *(Table 3)*. The isolates similarly displayed a higher rate of resistance to the β-lactam antibiotics: cefotaxime (83%) and ampicillin (76%). Thirteen percent of isolates (9; 3 *C. jejuni* and 6 *Campylobacter* species) displayed resistance to two antibiotics, whereas 26% (26; 4 *C. jejuni* and 22 *Campylobacter* species) showed resistance to three antimicrobials. Fifty-three percent (37; 9 *C. jejuni* and 28 *Campylobacter* species) of all the isolates displayed multidrug resistance phenotypes *(Table 4)*.

DISCUSSION

Campylobacter infection is one of the major causes of human gastroenteritis, and foods of animal origin have been incriminated as the major source of human infection *(9, 25, 41)*. *Campylobacter* species also are associated with abortion and reproductive failure in different species of animals, including cattle and sheep *(38, 39)*. Campylobacteriosis

TABLE 1. Isolation rate of *Campylobacter* from ruminants slaughtered at major abattoirs in Ilorin, Kwara state, Nigeria

Animal	Sex	No. of samples	No. positive (%)	*P*-value
Bovine	Male	12	4 (1.14)	0.35
	Female	188	41 (11.71)	
	Subtotal	200	45 (12.86)	
Caprine	Male	145	25 (7.14)	0.31
	Female	5	0 (0.00)	
	Subtotal	150	25 (7.14)	
	Total	350	70 (20.00)	0.18

[a]$P > 0.05$.

TABLE 2. Biochemical characteristics of *Campylobacter* isolates from ruminants slaughtered at major abattoirs in Ilorin, Kwara state, Nigeria

Animal	No. positive	Biochemical tests carried out on isolates[a]									Detection (%)
		GRA	URE	OXI	GLU	LAC	CAT	H_2S	HIP[b] (%)		
									+c	–	
Bovine	45	G-ve¯	–	+	–	–	+	+	3 (4.28)	42 (61.43)	0.45
Caprine	25	G-ve¯	–	+	–	–	+	+	2 (2.86)	23 (31.43)	0.25
Total	70								5 (7.14)	65 (92.86)	0.70

[a]GRA, Gram reactions; G-ve¯, Gram-negative curved rods; URE, urease; OXI, oxidase; GLU, glucose; LAC, lactose; CAT, catalase; H_2S, hydrogen sulfide.

[b]HIP, hippurate: –, negative; +, positive.

[c]*C. jejuni.*

TABLE 3. Frequency of resistance of *Campylobacter* species isolated from ruminants slaughtered at abattoirs in Ilorin, Kwara state, Nigeria

Antimicrobial agent	No. of resistant isolates (%) (n = 70)	*C. jejuni* (n = 5) (%)		Other *Campylobacter* spp. (n = 65) (%)	
		Caprine (n = 2)	Bovine (n = 3)	Caprine (n = 23)	Bovine (n = 42)
Ampicillin	53 (76)	1 (50)	2 (67)	12 (52)	38 (90)
Ciprofloxacin	18 (26)	0 (0)	3 (100)	6 (26)	9 (21)
Cefoxitin	12 (17)	1 (50)	1 (33)	6 (26)	4 (9.5)
Cefotaxime	58 (83)	2 (100)	3 (100)	17 (74)	36 (86)
Nalidixic acid	70 (100)	2 (100)	3 (100)	23 (100)	42 (100)
Ceftriaxone	20 (29)	0 (0)	2 (67)	6 (26)	12 (29)
Ceftaxidime	25 (36)	1 (50)	1 (33)	11 (48)	12 (29)
Tetracycline	14 (20)	2 (100)	1 (33)	6 (26)	5 (12)

TABLE 4. Resistance profiles of *Campylobacter* species isolated from ruminants slaughtered for at major abattoirs in Ilorin, Kwara state, Nigeria

S/No.	Resistance profile[a]	Species of *Campylobacter* (n)	No. of classes of antimicrobials resisted	Multidrug resistance status
1	AMP-NAL	*Campylobacter* spp. (5)	2	Absent
2	AMP-CX	*C. jejuni* (2) *Campylobacter* sp. (1)	2	Absent
3	CIP-NAL	*C. jejuni* (1)	1	Absent
4	AMP-CIP-CX	*Campylobacter* spp. (12)	3	Present
5	AMP-CAZ-NAL	*C. jejuni* (4) *Campylobacter* spp. (2)	3	Present
6	CAZ-CIP-CX-NAL	*Campylobacter* spp. (8)	2	Absent
7	AMP-CIP-CTR-CAZ-NAL-TET	*C. jejuni* (1) *Campylobacter* spp. (5)	4	Present
8	AMP-CAZ-CTX-CTR-NAL-TET	*Campylobacter* sp. (1)	4	Present
9	AMP-CIP-NAL-TET	*C. jejuni* (3)	3	Present
10	AMP-CIP-CX-CTX-CAZ-CTR-NAL-TET	*C. jejuni* (1) *Campylobacter* spp. (4)	5	Present
11	AMP-CIP-CTX-CAZ-CTR-NAL	*Campylobacter* spp. (4)	4	Present

[a]AMP, ampicillin; NAL, nalidixic acid; CX, cefoxitin; CIP, ciprofloxacin; CAZ, ceftazidime; CTX, cefotaxime; CTR, ceftriaxone; TET, tetracycline.

may therefore hamper animal production in addition to its deleterious effects on public health. The result of this study showed that the isolation rate of *Campylobacter* from ruminants slaughtered in Ilorin metropolis corroborate results of previous studies *(32, 33, 40)*, but are lower than that of Olabode et al. *(34)*, whose rate of isolation was 68% from cattle slaughtered at Gwagwalada abattoir, Abuja. A lower rate (3.54%) was reported in sheep in Kaduna state, Nigeria *(37)*. Similarly, lower rates (between 1.2 and 14.9%) have been recorded in other countries, including Tanzania, Belgium, Finland, and Canada *(5, 12, 15, 25)*. Long ago, higher rates were recorded in industrialized nations such as Denmark, Norway, Italy, the United States, Finland, and the United Kingdom, where rates of 23, 30.5, 53.9, 34.1, 31.1, and 62%, respectively, were recorded *(2, 3, 17, 21, 30, 35)*, albeit the rates of isolation have dropped, probably as a result of improved hygiene and biosecurity in farms and the environment. Several variables may be responsible for the differences in isolation rates in different regions, and these include, but are not limited to, geographical zone, feed type, system of rearing, and method of slaughtering *(25)*. The detection of *Campylobacter* in ruminants slaughtered for human consumption is of public health significance because it may serve as source of infections to humans directly during processing of the carcass or indirectly through the consumption of improperly processed fecally contaminated meat or meat products *(13, 23, 25, 42)*. Future studies could examine the presence of the pathogen on carcass or finished products. The study also revealed lower isolation rates in male animals (8.28%) than in female animals (11.41%), although the difference was not statistically significant and corroborates a previous report by Ngulukun et al. *(32)*, but does not agree with Kashoma et al. *(25)* and Salihu et al. *(40)*, both of which recorded higher isolation rates in male than in female animals. This difference might be because of a few male animals being sampled in the current study, as it has been reported that larger sample size can lead to the probabilities of obtaining more isolates on culture *(1)*. A low rate of *Campylobacter* isolation in male compared with female animals in this study also might be due to the heat used in processing male animals (male animals are processed by burning) in the abattoirs because *Campylobacter* species are known to be sensitive to temperatures higher than 70°C *(13, 23, 25, 41)*. It also is reported that air drying of carcasses reduces the prevalence of *Campylobacter (16)*. Campylobacteriosis has been reported to have sexual dimorphism (a bias toward infection of young males rather than young females), which is more pronounced at early stages of life and male animals are more prone to the disease than female animals. The reason for this is unclear, but has been attributed to physiological differences between male and female animals *(45)*.

In this study, it was presumptively observed that *Campylobacter* species other than *C. jejuni* were predominant in this study and that only 7.14% of the total isolates were biochemically identified as C. jejuni. This does not agree with previous studies that reported a high rate of isolation of *C. jejuni* in food

animals such as cattle and poultry, whereas *C. coli* is more common in pigs *(15, 17, 18)*, although the need to identify the isolates molecularly is essential in further studies. This variation may be because of various factors such as period of sampling, sampling location, age of the subject sampled, and geographical locations of the study *(22, 24)*. For example, a higher rate of *C. coli* was documented in samples of feces from cattle in the southern United States than samples from cattle from northern, western, and eastern areas *(41)*.

A varying degree of resistance was displayed to the different antimicrobials tested, and this may be because of the use and abuse of different antimicrobials without restrictions either for prophylaxis, treatment, or as growth promoters in livestock production in most developing countries of Africa and Asia *(23–25, 42)*. The rate of resistance to ciprofloxacin (26%) in this study is relatively lower than those of previous studies *(46, 50)*, but higher than that documented by Kashoma et al. *(24)*. The high rate of resistance to the fluoroquinolone ciprofloxacin is worrisome because this drug is the drug of choice recommended for treatment of Campylobacteriosis and salmonellosis in Nigeria, and it is not commonly in use in livestock production. The high resistance to this drug in this study may suggest that the isolates originate from humans during the processing of carcases, although further analyses are necessary to validate this hypothesis. Furthermore, because enrofloxacin is commonly used in poultry and fish production, selective pressure builds up as a result of resistance by *Campylobacter* isolates from poultry and fish that may be transmitted to cattle farms via fomites, insects, or even farm workers *(25, 44)*. Similarly, all the isolates were resistant to nalidixic acid, and similarly high resistance to nalidixic acid has been documented previously *(24, 46, 50)*. Although sensitivity to antibiotics is no longer of much value in identification of bacteria, resistance to nalidixic acid has been one of the major characters that was hitherto used in the identification of *Campylobacter* species *(7)*. In addition, a higher number of the isolates in the current study showed resistance to ampicillin (76%) and cefotaxime (83%), and this finding corroborates previous reports *(24, 25, 46, 51)*. The high rate of resistance to β-lactam antibiotics is not surprising because *Campylobacter* species have been reported to produce β-lactamase, which inhibits the actions of β-lactams including penicillin and cephalosporin via reduced affinity of the antibiotics to bind to penicillin binding proteins or the inability of the drugs to penetrate the outer membrane pores *(24)*. The isolates were pan-susceptible to gentamicin, and this finding agrees with the previous study by Kashoma et al. *(25)*. The resistance to tetracycline in this study is in agreement with Kashoma et al. *(25)*, although higher rates of tetracycline-resistant *Campylobacter* isolates were reported previously *(46, 50)*.

The isolates displayed 11 different resistant phenotypes with ampicillin-ciprofloxacin-cefoxitin being the most common, and 37 (53%) of the isolates exhibited multidrug resistance phenotypes. This is worrisome because most human infections originate from ingestion of contaminated foods of animal origin *(1, 10, 11, 14)*. This showed that the need for promulgation of policies that control use of antimicrobials in animal production in Nigeria, like other developing countries, is inevitable.

CONCLUSIONS

This study highlighted high rate of *Campylobacter* isolation (20%) from ruminants slaughtered at the two major abattoirs in Ilorin metropolis, and most of the isolates displayed multiresistance to commonly used antimicrobials. Further study is recommended to characterize the different species of *Campylobacter* circulating in Kwara. There is need by the health authorities in Nigeria to promulgate policy to control use of antimicrobials in animal production to curtail the spread of resistance *Campylobacter* among animals and humans.

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In Memory

William S. LaGrange

IAFP expresses our deepest sympathy to the family of Dr. William S. "Bill" LaGrange, who passed away in January 2022 in Ames, Iowa. Dr. LaGrange joined IAFP in 1957 and retired in 2000 as Extension Specialist in Food Science from the Department of Food Science and Human Nutrition at Iowa State University in Ames after a 40-year career on its faculty (following in the footsteps of his father, who was a professor of animal husbandry at Iowa State College).

Throughout his long tenure at ISU, Dr. LaGrange offered extension programs to help with food safety, regulatory requirements, quality testing methods, product packaging, and facility management, working "with everyone from the food plant executives to the folks cleaning the floors."

Dr. LaGrange received the IAFP Honorary Life Membership Award in 2006 and the Educator Award in 1992. He served as Scientific Editor of Food Protection Trends *from 1996–2003.*

Dr. LaGrange received his Ph.D. from ISU in 1959. He is survived by his wife, three daughters, a step-daughter, and four grandchildren. His interests included nature, running, tennis, officiating at ISU track meets, traveling, and creating works of stained glass.

IAFP will always have sincere gratitude for Dr. LaGrange's long-time contributions to the Association and the profession.