Journal of Food Protection, Vol. 75, No. 1, 2012, Pages 154–159
doi:10.4315/0362-028X.JFP-11-237

Research Note

Observational Study of the Prevalence and Antibiotic Resistance of *Campylobacter* spp. from Different Poultry Production Systems in KwaZulu-Natal, South Africa

LINDA A. BESTER[1]* AND SABIHA Y. ESSACK[2]

[1]*Biomedical Resource Unit and* [2]*School of Pharmacy and Pharmacology, University of KwaZulu-Natal, Private Bag x 54001, Durban 4000, South Africa*

MS 11-237: Received 12 May 2011/Accepted 21 August 2011

ABSTRACT

Campylobacter bacteria are important foodborne pathogens that cause acute diarrheal illness, and infection is often associated with contaminated poultry. In a blind observational study, the prevalence and resistance profiles of thermophilic *Campylobacter* strains collected from different poultry production systems were tested against the clinically used antibiotics ciprofloxacin, tetracycline, erythromycin, gentamicin, and streptomycin. *Campylobacter* strains were isolated from chickens in rural production systems, a free-range commercial facility, and industrially raised broiler and egg-laying chickens all situated in KwaZulu-Natal, South Africa. Isolates were collected from the chicken cecae and were identified with conventional methods and tested for antibiotic resistance with the Clinical and Laboratory Standards Institute agar dilution method. The prevalence of *Campylobacter* spp. isolates in chickens was 68% (56 samples) in rural production, 47% (140 samples) in commercial free-range broilers, 47% (133 samples) in industrial broilers, and 94% (34 samples) in industrial layer hens. Isolates from the rurally raised chickens showed significantly ($P < 0.01$) less resistance against ciprofloxacin (7.9%), erythromycin (0%), and tetracycline (21.6%) than those from commercially produced chickens. Isolates from the commercially raised chickens (free range and industrial) were highly resistant to tetracycline (98.9 to 100%). The incidence of gentamicin and streptomycin resistance was 1.6 and 11.5%, respectively, in commercial free-range broilers, 1.7 and 16.4%, respectively, in industrially raised broilers, and 12.9 and 40%, respectively, in industrially raised layers. It is possible that variations among the poultry production systems, including antimicrobial usage, result in differences in antibiotic resistance profiles in *Campylobacter*.

Bacterial stress in the gastrointestinal tract is caused by the therapeutic or prophylactic use of antibiotics during meat production, prompting the occurrence of antibiotic-resistant strains *(19)*. *Campylobacter jejuni* subsp. *jejuni* and *Campylobacter coli* have both been identified as pathogens that frequently cause acute diarrhea in humans, especially in children and the elderly *(15, 30)*. *Campylobacter* infection acquired through the oral route has been shown to have a possible connection with the development of Guillain-Barre syndrome *(18)*, and *Campylobacter* is the most frequently isolated bacterium that causes diarrhea in AIDS patients *(17, 25)*. The sources of these *Campylobacter* infections are often linked to meat products and, in particular, to poultry products *(21)*. Although the diarrhea caused by campylobacters is normally self-limiting, failure of the immune response results in the need for therapeutic intercession. The preferred first-line antibiotics are macrolides and fluoroquinolones, and thus, concern has been expressed about the health risk to humans of antibiotic-resistant *Campylobacter* strains associated with the use of antibiotics, often belonging to classes used for human therapeutic treatment, during meat and poultry production *(10, 31)*.

In South Africa, the poultry industry accounts for 43% of the national total of animal-derived products *(27)*. Commercial poultry production is undertaken by industrialized and free-range farming systems, and antibiotics are utilized both therapeutically and prophylactically. However, there is also a third farming system widespread in suburban and rural KwaZulu-Natal and, indeed, throughout sub-Saharan Africa, where antibiotic usage is either limited or absent: namely, informal small-scale family farming (henceforth referred to as rural production), in which indigenous poultry roam freely and scavenge for food themselves *(8)*.

South Africa has no public health or food production surveillance program for *Campylobacter* spp. The objective of the present study was to evaluate the frequency of isolation and the antibiotic resistance profiles in *Campylobacter* isolates collected from each of the poultry production systems, namely, rural, commercial free-range broilers, industrialized broilers, and industrialized layers, in relation to the differing antibiotic sources and usage in each system.

MATERIALS AND METHODS

Ethical clearance was obtained from the University of KwaZulu-Natal Animal Ethics Sub-committee (015/07/Animal). Except for the rural chickens, all samples were collected randomly from abattoirs during 2008 and 2009. No data on the specific

* Author for correspondence. Tel: +27 (31) 260-7671; Fax: +27 (31) 260-7730; E-mail: besterl@ukzn.ac.za.

TABLE 1. *Prevalence of* Campylobacter *isolates collected from the different farming systems*

Farming system	No. of samples	% (no.) with isolates of:		
		C. jejuni	*C. coli*	*Campylobacter* spp.[a]
Rural chickens	56	29 (11)	71 (27)	
Commercial free-range broilers	140	48 (32)	52 (34)	
Industrial broilers	133	70 (44)	25 (16)	5 (3)
Industrial layers	34	69 (22)	9 (3)	22 (7)

[a] *Campylobacter* spp. excluding *C. jejuni* and *C. coli.*

antimicrobials used on the farms that supplied the abattoirs are available except what is known from commercial feed suppliers and legal usage. Antibiotics used in South African commercial poultry production as growth promoters and therapeutic treatment include tetracyclines (oxytetracycline and chlortetracycline) and macrolides (tylosin and kitamycin). Antibiotics indicated for therapeutic treatment only are beta-lactams (amoxycillin), quinolones (enrofloxacin and norfloxacin), and aminoglycosides (neomycin and spectinomycin) *(12, 28)*. A single large commercial free-range farm in KwaZulu-Natal province assisted with samples from an in-house abattoir for broiler chickens (5 to 8 weeks old). Samples from industrialized chickens, both broilers (5 to 8 weeks old) and layers (36 to 54 weeks old), were collected at four abattoirs situated in KwaZulu-Natal. Adult indigenous chickens were collected from rural communities in Port Shepstone, Mvoti, Maphumulo, and Shongweni, all in KwaZulu-Natal. Two adult chickens were collected from every third household in each locality. The birds were slaughtered at the Biomedical Resource Unit (a laboratory animal science unit in the University of KwaZulu-Natal), and samples collected from the cecae.

Bacterial isolation and identification. The Cape Town protocol *(14)* was used for isolating *Campylobacter* spp., but instead of isolating the organisms on antibiotic-free tryptose blood agar, a saline suspension of fecal matter collected from the ceca of each animal was passed through a 47-mm cellulose nitrate filter of 0.65-μm pore size (Sartorius Stedim Biotech, GmbH, Goettingen, Germany) onto a Butzler plate (*Campylobacter*-selective medium SR0085E, Oxoid, Ltd., Basingstoke, UK) and *Campylobacter* growth supplement SR0232E (Oxoid) containing 5% lysed horse or sheep blood. Suspected *Campylobacter* colonies were screened according to Gram staining and characteristic spiral morphology and further identified using biochemical testing based on indoxyl acetate hydrolysis, hippurate hydrolysis, growth at 42 and 24°C, and sensitivity to nalidixic acid (30 μg; Oxoid) and cephalothin (30 μg; Oxoid).

Antimicrobial susceptibility testing. *Campylobacter* strains were stored in tryptose soy broth (Oxoid) supplemented with 10% glycerol (ACE Pty., Johannesburg, South Africa) at −60°C until tested for antimicrobial susceptibility. The MIC (micrograms per milliliter) of an antibiotic required for total growth inhibition was determined by the agar dilution method of the Clinical and Laboratory Standards Institute (CLSI) *(4)*. The susceptibility breakpoints of *Enterobacteriaceae* for ciprofloxacin (Fluka AG, Buchs, Switzerland), tetracycline (Sigma-Aldrich Chemie GmbH, Steinheim, Germany), and gentamicin (Sigma) *(3)* were used, together with the proposed CLSI guideline for susceptibility of *Campylobacter* spp. to erythromycin (Sigma) *(2)*. Epidemiological cut-off values in surveillance monitoring were used for streptomycin (Sigma) *(9)*. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 served as controls as described in the CLSI standard parameters for MIC susceptibility testing. *C. jejuni* ATCC 29428 served as a growth control.

Statistical analysis. Statistical validity was predetermined at 95%. Pairwise comparisons were made with Wilcoxon's rank sum test in cases where overall differences in the percentages of resistant thermophilic *Campylobacter* spp. were significant. The Stata version 10 statistical package (Stata, Inc., College Station, TX) was used for data analysis.

RESULTS

Prevalence of *Campylobacter* in the different poultry production systems. There were 38 *Campylobacter* isolates collected from 56 samples from poultry at rural production facilities, 66 isolates from 140 samples from commercial free-range chickens, 63 isolates from 133 samples from industrial broilers, and 32 isolates from 34 samples from industrial layer chickens. The frequency of thermophilic *Campylobacter* spp. isolates in KwaZulu-Natal was 68% in poultry raised in rural farming systems, 47% in commercial free-range broilers, 47% in industrial broilers, and 94% in industrial layers. The ratio of *C. jejuni* subsp. *jejuni* prevalence to *C. coli* prevalence varied between the production systems, with *C. coli* more prominent in the rural (71%) and commercial free-range broiler (52%) groups, while *C. jejuni* subsp. *jejuni* was more dominant in the industrialized broiler (70%) and layer groups (69%) (Table 1).

Antibiotic susceptibility. The results show a relationship between restricted antibiotic usage and the prevalence of *Campylobacter* antibiotic-resistant strains in poultry production. Compared to the other groups, the rural group showed the lowest occurrence of resistance (Table 2). Isolates from the commercial free-range broiler and industrial broiler and layer groups showed higher resistance to tetracycline (100, 98.9, and 100%, respectively). Except in the case of erythromycin, the industrial layer group (normally older birds, aged 36 to 52 weeks) showed more resistance than the industrial broilers (normally younger birds, aged 5 to 8 weeks), although the significance was not determined.

MIC_{50}s and MIC_{90}s. The MIC_{50}s (MICs required to inhibit growth by 50%) and MIC_{90}s for the different production systems are described in Table 3. The rural production group's MIC_{50}s were low for ciprofloxacin (0.13 μg/ml), tetracycline (0.125 μg/ml), erythromycin (0.125 μg/ml), gentamicin (0.25 μg/ml), and streptomycin (1 μg/ml). The MIC_{50}s for commercial free-range chickens for ciprofloxacin (4 μg/ml), tetracycline (64 μg/ml), and

TABLE 2. *Frequencies of antibiotic resistance of* Campylobacter *isolates collected from the different farming systems*

Antibiotic / Class / MIC breakpoint	Rural No.	Rural %	Commercial free-range broilers No.	Commercial free-range broilers %	Industrial broilers No.	Industrial broilers %	Industrial layers No.	Industrial layers %	P values
Ciprofloxacin									
Quinolones									
MIC ≤ 1	38	7.9	66	95.4	63	15.9	32	17.7	Rural vs commercial free-range broilers, $P < 0.01$; rural vs industrial broilers, $P = 0.4$; rural vs industrial layers, $P = 0.3$; commercial free-range broilers vs industrial broilers, $P < 0.01$
Tetracycline									
Tetracyclines									
MIC ≤ 4	37	21.6	66	100	63	98.9	32	100	Rural vs commercial free-range broilers, $P < 0.01$; rural vs industrial broilers, $P < 0.01$; rural vs industrial layers, $P < 0.01$; commercial free-range broilers vs industrial broilers, $P = 0.5$
Erythromycin									
Macrolides									
MIC ≤ 8	38	0	66	87.9	63	47.6	32	43.7	Rural vs commercial free-range broilers, $P < 0.01$; rural vs industrial broilers, $P < 0.01$; rural vs industrial layers, $P < 0.01$; commercial free-range broilers vs industrial broilers, $P = 0.04$
Gentamicin									
Aminoglycosides									
MIC ≤ 4	37	0	61	1.6	60	1.7	31	12.9	Rural vs commercial free-range broilers, $P = 0.9$; rural vs industrial broilers, $P = 0.9$; rural vs industrial layers, $P = 0.04$; commercial free-range broilers vs industrial broilers, $P = 0.9$
Streptomycin[a]									
Aminoglycosides									
MIC ≤ 2[b]	37	5.4	61	11.5	55	16.4	20	40	Rural vs commercial free-range broilers, $P = 0.5$; rural vs industrial broilers, $P = 0.2$; rural vs industrial layers, $P < 0.01$; commercial free-range broilers vs industrial broilers, $P = 0.6$
MIC ≤ 4[c]		0		8.2		12.7		25	Rural vs commercial free-range broilers, $P = 0.2$; rural vs industrial broilers, $P = 0.04$; rural vs industrial layers, $P < 0.01$; commercial free-range broilers vs industrial broilers, $P = 0.5$

[a] Due to the lack of a CLSI breakpoint for streptomycin, the European Food Safety Authority cut-offs for *C. jejuni* subsp. *jejuni* and *C. coli* were used *(9)*.
[b] MIC for *C. jejuni* subsp. *jejuni*.
[c] MIC for *C. coli*.

erythromycin (64 μg/ml) were significantly higher ($P < 0.01$) than those for poultry from the rural system. The MIC_{50} for industrialized broilers for tetracycline (64 μg/ml) was significantly higher ($P < 0.01$) than that in the rural poultry system (0.125 μg/ml). The MIC_{50} for industrial layers for tetracycline (128 μg/ml) was significantly higher than that for poultry in the rural system ($P < 0.01$).

The MIC_{90}s for ciprofloxacin in *Campylobacter* isolates from commercial free-range broilers (8 μg/ml), industrial broilers (16 μg/ml), and industrial layers (16 μg/ml) were significantly higher ($P < 0.01$) than those in *Campylobacter* isolates from poultry in the rural system (1 μg/ml). The MIC_{90}s for tetracycline were consistently high (128 μg/ml) for all the poultry systems investigated. The MIC_{90}s for erythromycin were significantly higher ($P < 0.01$) for commercial free-range broilers (128 μg/ml), industrial broilers (128 μg/ml), and industrial layers (128 μg/ml) than for poultry in the rural system (1 μg/ml). The MIC_{90}s for gentamicin were significantly higher in *Campylobacter* isolates from industrial layers (8 μg/ml) than from poultry in the rural system (0.25 μg/ml). The MIC_{90}s for streptomycin were significantly higher in *Campylobacter* isolates from industrial layers (20 μg/ml) than from poultry in the rural system (2 μg/ml).

TABLE 3. *MICs required to inhibit 50 and 90% of the growth of the* Campylobacter *isolates collected from the different farming systems*

Antibiotic and % inhibition	MIC (μg/ml) Rural poultry	Commercial free-range broilers	Industrial broilers	Industrial layers	*P* values
Ciprofloxacin					
MIC_{50}	0.13	4	0.06	0.375	Rural vs commercial free-range broilers, $P < 0.01$; rural vs industrial broilers, $P < 0.01$; rural vs industrial layers, $P < 0.01$; commercial free-range broilers vs industrial broilers, $P < 0.01$
MIC_{90}	1	8	16	16	Rural significantly lower than free-range broilers and industrial broilers and layers, $P < 0.001$
Tetracycline					
MIC_{50}	0.125	64	64	128	Rural vs commercial free-range broilers, $P < 0.01$; rural vs industrial broilers, $P < 0.01$; rural vs industrial layers, $P < 0.01$; commercial free-range broilers vs industrial broilers, $P = 0.5$
MIC_{90}	128	128	128	128	No difference in MIC_{90}s, $P = 0.06$
Erythromycin					
MIC_{50}	0.125	64	4	4	Rural vs commercial free-range broilers, $P < 0.01$; rural vs industrial broilers, $P < 0.01$; rural vs industrial layers, $P < 0.01$; commercial free-range broilers vs industrial broilers, $P < 0.01$
MIC_{90}	1	128	128	128	Rural significantly lower than free-range broilers and industrial broilers and layers, $P < 0.001$
Gentamicin					
MIC_{50}	0.25	0.25	0.5	2	Rural vs commercial free-range broilers, $P = 0.2$; rural vs industrial broilers, $P < 0.01$; rural vs industrial layers, $P < 0.01$; commercial free-range broilers vs industrial broilers, $P < 0.01$
MIC_{90}	0.25	0.25	2	8	Rural significantly lower than industrial broilers and layers, $P < 0.001$; free-range broilers significantly lower than industrial broilers, $P < 0.001$
Streptomycin					
MIC_{50}	1	1	2	1.5	Rural vs commercial free-range broilers, $P = 0.2$; rural vs industrial broilers, $P < 0.01$; rural vs industrial layers, $P = 0.3$; commercial free-range broilers vs industrial broilers, $P < 0.01$
MIC_{90}	2	4	8	20	All MIC_{90}s significantly different, $P < 0.008$

DISCUSSION

Limitations of the study. Among the limitations of our study was its blind observational nature, since only one commercial free-range farm participated in the study. In addition, the prevalence of hippurate-negative *C. jejuni* subsp. *jejuni* isolates was not confirmed with molecular identification. It is probable that a small number of such isolates would have represented organisms mistakenly identified as *C. coli* or other hippurate-negative thermophilic *Campylobacter* spp. *(24)*.

Prevalence. Woodward et al. *(32)* found that humans and a variety of animals, including wild birds, rodents, pigs, and cattle, can spread contamination of *Campylobacter* spp. In the present study, poultry in rural farming systems are often only confined to a holding area in the evenings and have interaction with other animals, for example, cattle, sheep and rodents, in the areas they scavenge freely for food sources *(8)*. In a Tanzanian study, thermophilic *Campylobacter* spp. were isolated from rural chickens significantly more often than from commercial broiler chickens (76 versus 60%, $P < 0.01$) *(20)*, in a percentage comparable to the 68% prevalence of thermophilic *Campylobacter* spp. isolated from rurally raised poultry in the present study.

The ratio of the prevalence of *C. jejuni* subsp. *jejuni* isolates to that of *C. coli* isolates in the different farming systems suggested that *C. coli* dominated in the rural and commercial free-range broilers. However, this study is only observational and, thus, further investigations will be needed to clarify this dominance. Most other studies *(11, 13)* have found *C. jejuni* subsp. *jejuni* to be typically the predominant species in poultry, as was found in the industrialized broiler and layer groups in this study. Corry and Atabay *(5)*, in a study of reports from The Netherlands and Northern Ireland, found that the prevalence of *C. coli* can often equal that of *C. jejuni* subsp. *jejuni* in live chickens and carcasses.

Resistance. In South Africa, the Department of Agriculture regulates the usage of antibiotics, prophylactically or as growth promoters, under the Stock Remedies Act No. 36 of 1947 *(7)*. Antibiotics for agricultural usage in South Africa are more freely available than in, for example, the United Kingdom, where growth-promoting antibiotics can only be obtained by prescription *(26)*. In rural settings, 87% of the family poultry systems studied in KwaZulu-Natal used traditional remedies originating mostly from plant material. In addition, the use of commercial products, and in particular, ''Terramycin'' and potassium permanganate, has also been reported *(8)*. The ciprofloxacin resistance found in the commercial production systems can be attributed to the use in these farming systems of enrofloxacin and norfloxacin. It has been reported that the use of enrofloxacin in poultry production (broilers) dramatically increased the resistance of *C. jejuni* subsp. *jejuni* isolates to ciprofloxacin *(22)*, nalidixic acid, and ofloxacin *(29)*.

Large amounts of tetracycline are used in the South African animal production system. A recent study in the Gauteng and Western Cape provinces of South Africa also reported high resistance of *Campylobacter* isolates from broiler poultry against tetracycline (95%), doxycycline (60%), and chlortetracycline (70%) *(12)*. The present study found that all the highly (>64 μg/ml) tetracycline-resistant isolates among the rural group were identified as *C. jejuni* subsp. *jejuni*. It is uncertain whether this was by coincidence and whether it has something to do with the expression of the *tet*(O) gene in *C. jejuni* subsp. *jejuni* and *C. coli*: tetracycline-resistant *C. coli* expresses the *tet*(O) gene at a chromosomal site, while the tetracycline-resistant *C. jejuni* subsp. *jejuni* expresses the gene on a conjugative plasmid *(6)*.

In a study (in this case a human study) by Putnam et al. *(23)*, *Campylobacter* isolates were obtained from stool samples collected from young children in a rural farming district of Egypt from 1995 to 2000. The study found low antibacterial resistance linked to an absence of antibiotic exposure; the isolates showed no resistance to the macrolides erythromycin and azithromycin and low resistance to ciprofloxacin, although this was observed to have increased from 17% in 1995 to 58% in 2000 *(23)*. At the time of the Putnam et al. study, fluoroquinolones were not indicated for pediatric treatment in the locality of the study but they were used in other community health contexts, and norfloxacin was used in food animal production. The investigators suggested that the increased antibiotic resistance of *Campylobacter* isolates from 1995 to 2000 may have come from exposure of the children to an antibiotic-resistant gene pool *(23)*.

The use of the macrolides tylosin and kitamycin in commercially produced free-range and industrialized poultry was reflected in the erythromycin resistance of *C. jejuni* subsp. *jejuni* and *C. coli* isolates encountered in our study. Lin et al. *(16)* noted that a single dose of tylosin given to a poultry flock had little effect on selection for erythromycin resistance in *Campylobacter* and that multiple exposures, typically the case when tylosin is used as a growth-promoting agent, were needed to establish erythromycin-resistant strains. Conversely, one would not expect to find resistance in rurally reared chickens.

This study is an expansion on the limitations of a previous study by Bester and Essack in 2008 entitled ''Prevalence of antibiotic resistance in *Campylobacter* isolates from commercial poultry suppliers in KwaZulu-Natal, South Africa'' *(1)*. The results of the present study highlight the prevalence of aminoglycoside resistance in commercially bred poultry.

In conclusion, the prevalence of *Campylobacter* isolates was higher in the rural and industrial layer chickens than in the industrial broilers and commercial free-range broilers, although study limitations do not permit extrapolation of the results as representative of the different poultry production systems. The study showed that when comparison is made between chickens from commercial production systems and rurally reared chickens, there is an association between lower antibiotic usage (in the rurally reared chickens) and reduced incidence of antibiotic-resistant *Campylobacter* isolates. The rurally reared chickens showed the lowest incidence of resistant *Campylobacter* isolates; the commercial free-range broiler and industrial broiler and layer groups showed high levels of tetracycline-resistant *Campylobacter* isolates, most likely attributable to on-going high levels of tetracycline use in commercial animal production systems, specifically, poultry production systems, in South Africa. The fact that antibiotic-resistant *Campylobacter* strains were also recovered from the rurally reared chickens is an indication that sources of antibiotic-resistant *Campylobacter* exist other than the development of resistance following therapeutic or prophylactic antibiotic treatments in animal production. A factor to consider in this regard is that the levels of hygiene are often lower in rural communities, heightening the incidence of common illnesses, and with the added prevalence of HIV/AIDS and tuberculosis, there is a likelihood of long- and short-term antibiotic use and abuse in the public health sector. The study also indicated that differing poultry production systems and antimicrobial programs can generate unique antibiotic resistance profiles.

ACKNOWLEDGMENTS

We thank the staff of the Biomedical Resource Unit and Prof. Samson Mukaratirwa and his postgraduate students for assisting with the collection of specimens from rurally produced poultry, as well as Cathy Connolly (Medical Research Council, Durban) and David Newmarch. The National Research Foundation (NRF) Thuthuka program supported the study.

REFERENCES

1. Bester, L. A., and S. Y. Essack. 2008. Prevalence of antibiotic resistance in *Campylobacter* isolates from commercial poultry suppliers in KwaZulu-Natal, South Africa. *J. Antimicrob. Chemother.* 62:1298–1300.
2. Clinical and Laboratory Standards Institute. 2005. Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria. Proposed guideline M45-P. Clinical and Laboratory Standards Institute, Wayne, PA.
3. Clinical and Laboratory Standards Institute. 2008. Performance standards for antimicrobial susceptibility testing; 18th informational

supplement. CLSI document M100-S18. Clinical and Laboratory Standards Institute, Wayne, PA.

4. Clinical and Laboratory Standards Institute. 2009. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 8th ed. Approved standard M07-A8. Clinical and Laboratory Standards Institute, Wayne, PA.
5. Corry, J. E. L., and H. I. Atabay. 2001. Poultry as a source of *Campylobacter* and related organisms. *J. Appl. Microbiol.* 90:96S–114S.
6. Dasti, J. I., U. Gross, S. Pohl, R. Lugert, M. Weig, and R. Schmidt-Ott. 2007. Role of the plasmid-encoded tet(O) gene in tetracycline-resistant clinical isolates of *Campylobacter jejuni* and *Campylobacter coli*. *J. Med. Microbiol.* 56:833–837.
7. Department of Agriculture. 2008. General Notices. Notice 511 of 2008. Fertilizers, farm feeds, agricultural remedies, and stock remedies. Act, 1947 (Act no. 36 of 1947). *Government Gazette,* 30 April 2008. Available at: http://www.info.gov.za/view/DownloadFileAction?id=80982. Accessed 21 September 2011.
8. Dhlamini, S. O. 2002. Family poultry studies in KwaZulu-Natal. Part 1. On-farm survey of family poultry in Makhuzeni sub-ward. Part 2. Dried bread waste as a replacement for maize in the diet of caged laying hens. M.Sc. thesis. University of Natal, Pietermaritzburg, South Africa.
9. European Food Safety Authority. 2008. Harmonised monitoring of antimicrobial resistance in *Salmonella* and *Campylobacter* isolates from food animals in the European Union. *Clin. Microbiol. Infect.* 14:522–533.
10. Grugel, C., and J. Wallmann. 2004. Antimicrobial resistance in bacteria from food-producing animals. Risk management tools and strategies. *J. Vet. Med. B* 51:419–421.
11. Gruntar, I., M. Ocepek, J. Avbersek, J. Mićunović, and M. Pate. 2010. A pulse-field gel electrophoresis study of the genetic diversity of *Campylobacter jejuni* and *Campylobacter coli* in poultry flocks in Slovenia. *Acta Vet. Hung.* 58:19–28.
12. Jonker, A. 2009. Antimicrobial susceptibility in thermophilic *Campylobacter* species isolated from pigs and chickens in South Africa. M.Sc. dissertation. University of Pretoria, Pretoria, South Africa.
13. Kramer, J., J. Frost, F. Bolton, and D. Wareing. 2000. *Campylobacter* contamination of raw meat and poultry at retail sale: identification of multiple types and comparison with isolates from human infection *J. Food Prot.* 63:1654–1659.
14. Lastovica, A. 2006. Emerging *Campylobacter* spp.: the tip of the iceberg. *Clin. Microbiol. News* 28:49–55.
15. Lastovica, A. J. 2006. Antibiotic resistance patterns of *Campylobacter jejuni, C. concisus* and *C. upsaliensis* isolates from paediatric patients in Cape Town, South Africa, 1998–2005, poster C-038. 106th Gen. Meet. Am. Soc. Microbiol. American Society for Microbiology, Washington, DC.
16. Lin, J., M. Yan, O. Sahin, S. Pereira, Y.-J. Chang, and Q. Zhang. 2007. Effect of macrolide usage on emergence of erythromycin-resistant *Campylobacter* isolates in chickens. *Antimicrob. Agents Chemother.* 51:1678–1686.
17. Manfredi, R., A. Nanetti, M. Ferri, and F. Chiodo. 1999. Fatal *Campylobacter jejuni* bacteraemia in patients with AIDS. *J. Med. Microbiol.* 48:601–603.
18. McCarthy, N., and J. Giesecke. 2001. Incidence of Guillain-Barré syndrome following infection with *Campylobacter jejuni*. *Am. J. Epidemiol.* 153:610–614.
19. McEwen, S. A., and P. J. Fedorka-Cray. 2002. Antimicrobial use and resistance in animals. *Clin. Infect. Dis.* 34(Suppl. 3):S93–S106.
20. Mdegela, R. H., H. E. Nonga, H. A. Ngowi, and R. R. Kazwala. 2006. Prevalence of thermophilic *Campylobacter* infections in humans, chickens and crows in Morogoro, Tanzania. *J. Vet. Med. B* 53:116–121.
21. Nachamkin, I. 2003. *Campylobacter* and *Arcobacter,* p. 902–914. *In* P. R. Murray, E. J. Baron, J. H. Jorgensen, M. A. Pfaller, and R. H. Yolken (ed.), Manual of clinical microbiology. American Society for Microbiology, Washington, DC.
22. Payot, S., A. Cloeckaert, and E. Chaslus-Dancla. 2002. Selection and characterization of fluoroquinolone-resistant mutants of *Campylobacter jejuni* using enrofloxacin. *Microb. Drug Resist.* 8:335–343.
23. Putnam, S. D., R. W. Frenck, M. S. Riddle, A. El-Gendy, N. N. Taha, B. T. Pittner, R. Abu-Elyazeed, T. F. Wierzba, M. R. Rao, S. J. Savarino, and J. D. Clemens. 2003. Antimicrobial susceptibility trends in *Campylobacter jejuni* and *Campylobacter coli* isolated from a rural Egyptian pediatric population with diarrhea. *Diagn. Microbiol. Infect. Dis.* 47:601–608.
24. Rautelin, H., J. Jusufovic, and M. L. Hänninen. 1999. Identification of hippurate-negative thermophilic campylobacters. *Diagn. Microbiol. Infect. Dis.* 35:9–12.
25. Samie, A., J. Ramalivhana, E. O. Igumbor, and C. L. Obi. 2007. Prevalence, haemolytic and haemagglutination activities and antibiotic susceptibility profiles of *Campylobacter* spp. isolated from human diarrhoeal stools in Vhembe district, South Africa. *J. Health Popul. Nutr.* 25:406–413.
26. Sarmah, A. K., M. T. Meyer, and A. B. Boxall. 2006. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VA's) in the environment. *Chemosphere* 65:725–759.
27. South African Poultry Association. 2010. Report of the management: chairmans' report 2010. Available at: http://www.sapoultry.co.za/pdf/2011%20MANAGEMENT%20REPORT%20-avi.pdf. Accessed 27 June 2011.
28. Swan, G., C. Carrington, A. Du Plessis, and A. Wellington (ed.). 2007. MIMS: index of veterinary specialities, vol. 45. Johncom Media Investments Ltd., Johannesburg, South Africa.
29. Takahashi, T., K. Ishihara, A. Kojima, T. Asai, K. Harada, and Y. Tamura. 2005. Emergence of fluoroquinolone resistance in *Campylobacter jejuni* in chickens exposed to enrofloxacin treatment at the inherent dosage licensed in Japan. *J. Vet. Med. B* 52:460–464.
30. Takkinen, J., A. Ammon, O. Robstad, T. Breuer, and the *Campylobacter* Working Group. 2001. European survey on *Campylobacter* surveillance and diagnosis. *Euro Surveill.* 8:207–213.
31. Van den Bogaard, A. E., and E. E. Stobberingh. 1999. Antibiotic usage in animals: impact on bacterial resistance and public health. *Drugs* 58:589–607.
32. Woodward, L., J. O'Brien, and B. Pearce. 2005. *Campylobacter* and poultry. Enhanced biodiversity: a risk to food safety?, p. 45. Proceedings of the 1st Scientific FQH conference, Frick, Switzerland, 28 and 29 November 2005.