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Prevalence of antibiotic resistance in *Campylobacter* isolates from commercial poultry suppliers in KwaZulu-Natal, South Africa

L. A. Bester[1]* and S. Y. Essack[2]

[1]*Biomedical Resource Unit, University of KwaZulu-Natal, Private Bag X54001, Durban 4000, South Africa;*
[2]*Antibiotic Resistance Research Proto-Unit, School of Pharmacy and Pharmacology, University of KwaZulu-Natal, Private Bag X54001, Durban 4000, South Africa*

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***Objectives*: *Campylobacter jejuni* isolated from broiler and layer chickens from registered abattoirs in KwaZulu-Natal, South Africa, were tested for their susceptibility to eight antibiotics.**

***Methods*: Using agar dilution, susceptibility to eight antibiotics was determined for *C. jejuni* recovered from the caeca.**

***Results*: A total of 155 isolates were collected of which 77 were identified as *C. jejuni* (broilers $n = 56$ and layers $n = 21$). Resistance was highest to tetracycline (broilers 98.2% and layers 100%) and ceftriaxone (broilers 96.4% and layers 100%). High susceptibility was found to ciprofloxacin (broilers 91% and layers 76%) and gentamicin (broilers 98% and layers 81%). Susceptibilities to each of the antibiotics for the broilers and layers, respectively, were: 50% and 57% for erythromycin, 45% and 24% for clarithromycin, 68% and 43% for ampicillin and 64% and 48% for nalidixic acid. Statistically significant differences were detected for the MIC_{50} of gentamicin, ciprofloxacin and tetracycline between broilers and layers ($P < 0.001$) with the MIC_{90} of gentamicin also of significant difference ($P = 0.01$). Multiresistance was detected in 23% and 43% of the isolates from broiler and layer chickens, respectively.**

***Conclusions*: Mass therapy procedures used in animal husbandry have a potential impact on antibiotic resistance development in *C. jejuni*.**

Keywords: *Campylobacter jejuni*, antimicrobial resistance surveillance, food animals, growth promoters, animal reservoirs

Introduction

Although banned in the European Union, many developing countries use antibiotics sub-therapeutically as growth promoters and as prophylaxis, usually as food/water supplements. *Campylobacter* species, especially *Campylobacter jejuni* and *Campylobacter coli*, are known zoonotic bacteria causing symptomatic diarrhoea in humans.[1] Humans are normally exposed to *Campylobacter* spp. through an oral pathway by handling and consuming uncooked meat, especially poultry products, faecal contaminated water and even raw vegetables.[2] Campylobacteriosis is generally described as a self-limiting disease that rarely requires antimicrobial intervention.

The aim of this study was to determine the susceptibility profiles of *C. jejuni* collected at slaughter from commercially produced broilers and layers in KwaZulu-Natal. Antibiotic susceptibility was determined for antibiotics commonly used in human therapy for Enterobacteriaceae infections in humans, namely fluoroquinolones, quinolones, tetracyclines, macrolides, aminoglycosides and β-lactam antibiotics (cephalosporins and penicillins).

Materials and methods

Bacteria isolation

Ethical approval was granted by the University of KwaZulu-Natal (AE/Bester/06). Caecal samples were collected from commercial broiler and layer chickens soon after slaughter from four registered chicken abattoirs in KwaZulu-Natal. Samples were filtered on Butzler plates and incubated at 41.5°C in a micro-aerophilic atmosphere (CampyGen, Oxoid) for 48 h.

*Corresponding author. Tel: +27-31-2607671; Fax: +27-31-2607730; E-mail: besterl@ukzn.ac.za

Identification

Campylobacter spp. were screened using conventional methods for campylobacters, which included oxidase and catalase tests, characteristic morphology after Gram stain, hippurate hydrolysis and susceptibility to nalidixic acid and cefalotin.

Antibiotic susceptibility testing

MICs were determined by agar dilution using a modification of Columbian agar supplemented with 7% lysed horse blood and *Campylobacter* growth supplements (Oxoid). Except for erythromycin, breakpoints for ciprofloxacin, tetracycline, ceftriaxone, clarithromycin, ampicillin, nalidixic acid and gentamicin were adapted from those used for Enterobacteriaceae: ciprofloxacin, MIC $\leq$ 1 mg/L; tetracycline, MIC $\leq$ 4 mg/L; ceftriaxone, MIC $\leq$ 8 mg/L; clarithromycin, MIC $\leq$ 2 mg/L; ampicillin, MIC $\leq$ 8 mg/L; nalidixic acid, MIC $\leq$ 8 mg/L; and gentamicin, MIC $\leq$ 4 mg/L.[3] A proposed guideline was followed for erythromycin: MIC $\leq$ 8 mg/L.[4] *Escherichia coli* (ATCC 25 922) and *Pseudomonas aeruginosa* (ATCC 27 853) were used as controls. STATA was used to analyse percentile values.

Results

Of the 155 samples, 77 were identified as *C. jejuni*, of which 56 were collected from broilers and 21 were collected from layers. Table 1 shows the percentage resistance and MIC_{50} and MIC_{90} results with significant differences between broilers and layers evident for ciprofloxacin, tetracycline and gentamicin. Multiresistance, described as resistance to four or more antibiotics, was detected in 23% and 43% of the isolates from broiler and layer chickens, respectively.

Discussion

Fluoroquinolones and macrolides are the drugs of choice for early treatment of campylobacteriosis.[5] The prevalence of ciprofloxacin resistance is a cause for concern, especially for the layer chickens (24%) that do not have as intensive exposure to antibiotics as do the broilers. Approximately half the isolates from the broilers (50%) and layers (47%) were resistant to erythromycin.

Even though layer chickens are not continuously exposed to growth promoter antibiotics in their feed, in the long run the therapeutic use of some drugs does have the potential to affect the susceptibility of *C. jejuni* when one considers the nature of poultry production. Normal commercial production methods make it difficult to treat individual animals mainly because of the large numbers of animals grouped together; thus, mass medication is the only option. However, addition of antimicrobial agents to water or feed may result in individual animals receiving inadequate curative doses or excessive prophylactic doses.[6] Such production practices may result in reservoirs of resistant *C. jejuni* and possibly other zoonotic pathogenic bacteria.

Cui *et al.*[7] examined *Campylobacter* originating from conventionally and organically reared chickens in the USA and showed similar levels of resistance to tetracycline (78%), erythromycin (46%) and ciprofloxacin (8%). In contrast, a Belgian study showed lower levels of resistance in broiler and layer chickens, respectively, for: tetracycline, 34.4% and 20%; and erythromycin, 6.3% and 8.6%. The study also showed a higher level of ciprofloxacin resistance in their broiler and layer chickens: 44.2% and 27.6%, respectively.[8] These fluctuations in resistance to common antibiotics differ from country to country and are reflective of local legislations that regulate the use of antimicrobials for animal production. The termination or more efficient regulation of veterinary antibiotics resulting in decreased resistance in zoonotic bacteria has been reported by several studies globally.[9] Unfortunately, South Africa has not taken any stance regarding the use of antibiotics in animal feed. The use of antibiotics, either sub-therapeutically or as growth promoters, in animal feeds is legislated under Act 36 of 1947 as *Stock Remedies* and is regulated by the Department of Agriculture.[10]

Children are particularly vulnerable to campylobacterioses through zoonotic acquisition. Clinical *Campylobacter* isolates collected at the Red Cross Children's Hospital in Cape Town, South Africa, from the period 1998 to 2005 have shown a steady increase in resistance to antibiotics: resistance to ciprofloxacin increased from 1.4% to 29%; to erythromycin from 3.4% to 7.2%; to nalidixic acid from 5.7% to 41%; and to ceftriaxone from 3.6% to 24.6%. For the first time, multiresistant

Table 1. Percentage resistance and MIC_{50} and MIC_{90} results for *C. jejuni* isolates collected from broiler and layer chickens in KwaZulu-Natal, South Africa

	% resistance		MIC_{50} (mg/L)			MIC_{90} (mg/L)		
Antibiotic	broilers	layers	broilers	layers	*P*	broilers	layers	*P*
Ciprofloxacin	8.9	23.8	0.031	0.25	<0.001	0.5	64	0.06
Tetracycline	98.2	100	64	128	<0.001	128	128	NA
Erythromycin	50	42.9	12	4	0.6	128	128	NA
Ceftriaxone	96.4	100	32	32	0.9	128	64	NA
Clarithromycin	55.4	76.2	16	64	0.3	128	128	NA
Ampicillin	32.1	57.1	8	16	0.07	16	32	0.9
Nalidixic acid	35.7	52.4	4	16	0.8	128	128	NA
Gentamicin	1.8	19	1	2	<0.001	2	8	0.01

NA, not applicable.

Campylobacter isolates were also observed in South Africa.[11] Steady increases in fluoroquinolone and macrolide resistance and increased penicillin resistance were also observed in the Venda district, South Africa, from 2002 to 2007; resistance to ciprofloxacin has increased from 8% to 13%, to erythromycin from 25% to 53%, and to gentamicin from 8% to 17.3%. However, fewer clinical isolates were resistant to tetracycline (27%) and ceftriaxone (7%), which showed very high resistance among both broiler and layer chickens in this study.[12] The Venda district study did not specify the *Campylobacter* species, but it is accepted that the *Campylobacter* species mostly responsible for acute diarrhoea in humans is *C. jejuni*.

Unlike European surveillance programmes, such as DANMAP, the prevalence and antibiotic resistance of *Campylobacter* infections in South Africa are not monitored and no surveillance programme exists.[1] The importance of such bacteria is overshadowed by the existence of more severe diseases, for example, HIV infections, malaria, tuberculosis and salmonellosis.

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Transparency declarations

None to declare.

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