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Multidrug resistant *Campylobacter* in faecal and carcasses of commercially produced poultry

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Poultry meat and products are major transmission routes of human campylobacteriosis. The aim of this study was to determine the numbers and antibiogram profile of *Campylobacter* isolates from slaughtered broiler and layer birds. One hundred and sixty caecal and one hundred and thirty two carcasses were randomly sampled at the Kejetia poultry slaughter, isolated on charcoal-cefoperazone-deoxycholate agar (CCDA) and confirmed by API CAMPY and their resistance profiles assessed by Kirby-Bauer disk diffusion. Prevalence was 22.5 and 21.9% in the faecal and carcasses, respectively with no significant differences. Species identified among faecal isolates were *Campylobacter jejuni* (42%), *Campylobacter coli* (28%), *Campylobacter lari* (22%) and *Campylobacter hyo-intestinalis* (8%) while 79% *C. jejuni,* 14% *C. coli*, 4% *C. jejuni sub sp. doylei*, and 3% *C. lari* were obtained from the carcasses. Resistance to the β-lactams ranged from 75 to 100%, 41 to 86% to the quinolones, 14 to 36% to the aminoglycosides, 100% to erythromycin, 97 to 100% to tetracycline, 72 to 83% to chloramphenicol and 90 to 94% to trimethoprim sulfamethoxazole. All species were sensitive to imipenem, but 100% of isolates were multidrug resistant. Contamination of carcasses with multidrug resistant strains of *Campylobacter* is a threat to handlers and consumers and of major public health issue.

Key words: Multidrug resistance, faeces, carcass, poultry, Kumasi, Ghana.

INTRODUCTION

Campylobacter, a key zoonotic pathogen is among the most commonly reported agent of enteritis in humans worldwide, with *Campylobacter jejuni* and *Campylobacter coli* accounting for almost 90% of human infections (Scallan et al., 2011). *C. jejuni* is particularly adapted to poultry, being the largest reservoir of the pathogenic species (Rizal et al., 2010). *Campylobacter* is mainly haboured in the intestinal tract of warm-blooded animals

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and birds with the caeca, colon and cloaca of birds as the main sites of colonization (Humphrey et al., 2007). Consumption and handling of raw or undercooked poultry has most often been implicated in human infections (Denis et al., 2011). *Campylobacter* enteritis is usually self-limiting warranting no antimicrobial therapy; but in severe enteritis with complications and in cases of the immune-compromised, antibiotics are necessary in which case the fluoroquinolones and erythromycin are the drugs of choice (Luangtongkum et al., 2009). Moreover increasing resistance among *Campylobacter* has been reported from different geographical regions to the drugs of choice and other relevant antibiotics used in both human and veterinary medicine (Salihu et al., 2009; Luangtongkum et al., 2009; Tambur et al., 2009). Multidrug-resistant *C. jejuni* and *C. coli* have been reported from food animals and retail meats, including poultry (Ge et al., 2003; Gebreyes et al., 2005).

The use and abuse of antimicrobial agents in veterinary medicine or as feed additives have been recognized to be a major determining factor in the growth and dissemination of resistance in most bacterial pathogens. The abuse of antibiotics in animals may cause an increase in resistance of their enteric flora. These resistant bacteria from animals can then be transferred to the human population through direct contact or as food products from animal sources (Fallon et al., 2003). Although prevalence of *Campylobacter* in poultry and poultry products is well documented globally, information on its occurrence and resistance levels in poultry is underreported in Ghana. Moreover, antibiotics are extensively used in commercial poultry production in Ghana due to myriad disease challenges faced by poultry farmers (Aning, 1995). Establishing the resultant effects of these drugs on the resistance levels in the gut flora and carcasses of these poultry birds is necessary. Therefore this study described the occurrence of *Campylobacter* in the gut content and carcasses of slaughtered birds and assessed the resistance levels of species to 13 relevant antibiotics.

MATERIALS AND METHODS

The study was undertaken at the Kejetia central market in Kumasi, Capital of Ashanti Region of Ghana. Kejetia market is the largest open air market in Ghana, and the second largest in Africa. Ninety (90) Poultry traders, who are mostly women purchase live birds from commercial poultry farms within and outside the Metropolis, keep them in holding pens from where they are sold live or slaughtered, processed and packaged on site.

Sample collection

With permission from the traders, whole intestines were obtained intact from slaughtered broiler and layer birds into sterile ziplock bags, kept on ice packs and immediately transported to the laboratory from April, 2013 to June, 2013. In the laboratory, the caeca were cut with sterile scissors (sterilized using a burning flame) from the remaining part of the intestines. Chicken carcasses were randomly swabbed and inoculated into sterile Amies transport medium (eswab sticks, Copan, Italy) and returned on ice packs to the laboratory from May, 2013 to March, 2014. An average of 10 chicken swab and 7 faecal samples were, respectively obtained monthly and weekly for the study period.

Sample processing, isolation and identification

Caecal contents were emptied aseptically into sterile bijou bottles while swab sticks together with the transport media were aseptically transferred into bijou bottles and subsequently pre- enriched with 5 ml of blood-free Campylobacter broth (Oxoid CM0963, Denmark) each and incubated overnight at 37°C. The overnight enrichment culture was sub cultured onto mCCDA agar (Oxoid CM0689, Denmark), incubated at 42°C for 48 h using CampyGen (Oxoid CN0025A) to generate microaerophilic condition (FDA BAM, 1998). Biochemical tests including Gram stain, oxidase and catalase were performed on colonies showing typical morphology of *Campylobacter* spp. Isolates which were small, curved Gram negative bacilli, catalase and oxidase positive, were further subjected to standard phenotypic tests using API CAMPY (bioMerieux, France) to identify to species level.

Antibiotic susceptibility testing

The Kirby-Bauer disk diffusion method was carried out using Mueller-Hinton agar (Liofilchem-Italy) supplemented with 5% sheep blood. Plates were inoculated with 0.5 Mcfarland suspension and incubated microaerophilically at 48°C for 24 h (Clinical and Laboratory Standards Institute, CLSI, 2006). Drugs analysed were sourced from ROSCO (Denmark) and included: Ampicillin (10 µg/disc), chloramphenicol (30 µg/disc), ciprofloxacin (5 µg/disc), kanamycin (30 µg/disc), erythromycin (15 µg/disc), gentamicin (10 µg/disc), nalidixic acid (30 µg/disc), tetracycline (30 µg/disc), cephalexin (30 µg/disc), trimethoprim sulfamethoxazole (25 µg/disc), norfloxacin (10 µg/disc), cefotaxime (30 µg/disc) and imipenem (10 µg/disc). The recorded inhibition zones were interpreted according to EUCAST-CLSI (2013) breakpoints for *Campylobacter*. Established breakpoints by EUCAST and CLSI (2013), for enterobacteriaceae were used to interpret the results of norfloxacin, trimethoprim sulfamethoxazole, cefotaxime and kanamycin as CLSI breakpoints for these antibiotics are yet to be established for *Campylobacter*. Quality control strains of *Escherichia coli* (ATCC® 25922 ™) and *Staphylococcus aureus* (ATCC® 25923 ™) were used.

Statistical analysis

Percentages were calculated for the descriptive analysis. Associations were determined using the Chi-square test at a significance level of < 0·05. Fisher's exact test was used for expected frequencies being less than 5. All statistical tests were two-tailed. Stata 14.0 software was used for statistical analysis.

RESULTS

Prevalence of *Campylobacter* species in poultry faeces and carcasses

Of the 160 faecal content examined from Broilers (46)

Table 1. Prevalence of *Campylobacter* spp. in faecal contents and carcasses of poultry.

Poultry	No. Isolates examined		No. isolates Identified		Chi-square, df
	Faecal	Carcass	Faecal	Carcass	P-value
Layers	114	112	17 (10.6)	15 (11.3)	0.01175,1
Broilers	46	20	19 (11.8)	14 (10.6)	0.914
Total	160	132	36 (22.5)	29 (21.9)	

Values in bracket indicate percentage

Table 2. Distribution of *Campylobacter* spp. among poultry faecal isolates.

Poultry	No. isolates Identified	*Campylobacter spp.* identified			
		C. jejuni	*C. coli*	*C. lari*	*C. hyointestinalis*
Layers	17	8 (47)	4 (23)	3 (18)	2 (12)
Broilers	19	7 (37)	6 (32)	5 (26)	1 (5)
Total	36	15 (41.6)	10 (27.7)	8 (22.2)	3 (8.3)

Values in bracket indicate percentage.

Table 3. Distribution of *Campylobacter* spp. among poultry carcass isolates.

Poultry	No. isolates identified	*Campylobacter* spp. identified			
		C. jejuni	*C. doylei*	*C. coli*	*C. lari*
Layers	15	13 (87)	0 (0)	1 (7)	1 (6)
Broilers	14	10 (72)	1 (7)	3 (21)	0 (0)
Total	29	23 (79.3)	1 (3.4)	4 (13.7)	1 (3.4)

Values in bracket indicate percentage.

Of the 160 faecal content examined from Broilers (46) and Layers (114); 36 (22.5%) were confirmed as *Campylobacter* spp. Of the 132 poultry carcasses processed, 29 (21.9%) were positive for *Campylobacter spp.* (Table 1). No significant difference were observed in the isolation rates from faecal and carcass samples (p= 0.914). Four main species and one subspecies of *Campylobacter* were isolated from the samples. *Campylobacter jejuni* and *C. coli* were the dominant species followed by *C. lari* with C. *jejuni* subsp. *doylei* being the least. Faecal samples accounted for 41.6% for *C. jejuni*, 27.7% for *C. coli*, 22.2% for *C. lari* and 8.3% for *C. hyointestinalis* (Table 2) and in their carcasses, 79.3% for *C. jejuni,* 13.7 % for *C. coli*, 3.4% for *C. lari* and 3.4% for *C. jejuni* subsp*. doylei* (Table 3).

Antibiotic resistance patterns of poultry isolates

Resistance among faecal isolates to erythromycin and Ampicillin was 100% each and to tetracycline, trimethoprim sulfamethoxazole (SXT) and chloramphenicol were 97, 94 and 72%, respectively. Against the cephalosporins resistance was 86% to cephalexin and 75% to cefotaxime. Resistance to the quinolones was 86% to ciprofloxacin, 78% to nalidixic acid and 44% to norfloxacin. Against the aminoglycosides resistance was 36% to kanamycin and 14% to gentamicin. No resistance (0%) was observed against imipenem (Table 4). Carcass isolates showed 100% resistance each to tetracycline, erythromycin and Ampicillin, 90 and 83%, respectively to trimethoprim sulfamethoxazole (SXT) and chloramphenicol. Against the cephalosporins resistance was 100% to cephalexin and 97% to cefotaxime. Resistance to the quinolones was 59% to ciprofloxacin, 55% to nalidixic acid and 41% to norfloxacin. Resistance to aminoglycosides was 24% to gentamicin and 21% to kanamycin. No (0%) resistance was observed against imipenem (Table 4).

Species specific resistance profile of poultry isolates

Campylobacter jejuni resistance ranged from 87.2 to100% to the β-lactams, 23.1 to 28.2% to the

Table 4. Antibiotic resistance and susceptibility patterns of *Campylobacter* species from poultry.

Antibiotic	Faecal n=36			Carcass n=29		
	% S	% I	% R	% S	% I	% R
Nalidixic acid	22	NA	78	45	NA	55
Norfloxacin	31	25	44	45	14	41
Ciprofloxacin	6	8	86	14	27	59
Ampicillin	0	0	100	0	0	100
Cefotaxime	17	8	75	0	3	97
Cephalexin	14	NA	86	0	NA	100
Kanamycin	39	25	36	65	14	21
Gentamicin	78	8	14	62	14	24
Erythromycin	0	NA	100	0	NA	100
Tetracycline	3	0	97	0	0	100
Chloramphenicol	8	20	72	3	14	83
SXT	6	0	94	7	3	90
Imipenem	75	25	0	83	17	0

KEY: S=sensitive; I=intermediate; R=resistant; NA= intermediate not available.

aminoglycosides, 38.5 to 69.5% to the quinolones, and 100% each to erythromycin and tetracycline, 84.6% to chloramphenicol and 92.3% to trimethoprim sulfamethoxazole. *C. coli* strains showed resistance of 85.7 to 100% to the β-lactams, 0 to 28.6% to the aminoglycosides, 0 to 64.3% to the quinolones, 100% to erythromycin, 92.9% to tetracycline, 64.3% to chloramphenicol and 92.9% to trimethoprim sulfamethoxazole. Resistance among *C. lari* strains was 100% to all antibiotics with the exception of norfloxacin, chloramphenicol, trimethoprim sulfamethoxazole and aminoglycosides where resistance of 77.8, 55.6, 88.9 and 33.3% each was, respectively observed (Table 5). Differences in resistance rates among the different species to the various antibiotics was not statistically significant ($p > 0.05$), with the exception of norfloxacin and nalidixic acid ($p < 0.001$). Resistance to 3 or more antibiotics was defined as multidrug resistance (MDR) in this study and 100% was observed among the faecal and carcass isolates (Table 6).

DISCUSSION

Campylobacter contamination of commercially produced poultry birds slaughtered in Kejetia, a suburb of Kumasi, Ghana, was 22.5%, which was expected because *Campylobacter* are frequent colonizers of the intestinal tracts of birds especially poultry and falls within the reported global range of 10 to 90% (Jacob-Reitsma et al., 1994; Newell and Fearnley, 2003). This study recorded rate is however higher than the 14.1% earlier reported by Sackey et al. (2001) but lower than the 43.6% by Abraham et al. (1990) in Ghana. In South Africa, 47% has been reported in commercial and industrial broilers and 94% in industrial layers, 51.5% has been described in Nigeria, 63.8% in Cote d'Ivoire, 83.1% in Ireland and 87.2% in Poland (European Food Safety Authority, EFSA, 2010; Bester and Essack, 2012; Salihu et al., 2012; Bernadette et al., 2012; Wieczorek et al., 2012). Countries with low *Campylobacter* colonization rates in poultry has been attributed to limited small-scale poultry farms with high biosecurity levels which are measures lacking in our subregion (Johnsen et al., 2006).

Campylobacter spp. are common contaminants of poultry carcasses with prevalence of 20 to 100% established in fresh chicken from several countries (Dominguez et al., 2002; Jorgensen et al., 2002; Son et al., 2007). This study recorded contamination levels of 21.9% which is much lower than the 100% reported by Jozwiak et al. (2006) in Broiler chicken in Hungary. Similarly, 58.9, 69 and between 70.7 to 91.4% have been reported in Poland, Iran and Malaysia, respectively (Tang et al., 2009; Wieczorek et al., 2012; Bagherpour et al., 2014). The contamination of the poultry carcasses in this study could be as a result of the lack of a well-structured processing plant (facility), slaughtering in open air with inadequate rinsing and washing facilities and poor environmental hygiene.

Campylobacter jejuni was the dominant species; (42, 79%), followed by *C. coli* (28, 14%), from the faeces and carcasses, respectively. This finding affirms the dominance of *C. jejuni* in poultry and poultry products (Jorgensen et al., 2002; Son et al., 2007; Salihu et al., 2012). Nevertheless, the high recovery of the thermophilic *Campylobacters* in comparison with the non-thermophiles could be imputed to the selective nature of the CampyGen gas generating system which optimizes the growth of

Table 5. Species specific resistance profile of poultry isolates.

Antibiotic	*C. jejuni* n=39	*C. coli* n=14	*C. lari* n=9	P-value
	(%) resistance	(%) resistance	(%) resistance	
Nalidixic acid	15 (38.5)	1 (7.4)	9 (100)	<0.001
Norfloxacin	19 (48.7)	0 (0)	7 (77.8)	<0.001
Ciprofloxacin	27 (69.2)	9 (64.3)	9 (100)	0.128
Ampicillin	39 (100)	14 (100)	9 (100)	-
Cefotaxime	34 (87.2)	12 (85.7)	9 (100)	0.506
Cephalexin	39 (100)	12 (85.7)	9 (100)	0.029
Kanamycin	11 (28.2)	4 (28.6)	3 (33.3)	0.954
Gentamicin	9 (23.1)	0 (0)	3 (33.3)	0.089
Erythromycin	39 (100)	14 (100)	9 (100)	-
Tetracycline	39 (100)	13 (92.9)	9 (100)	0.175
Chloramphenicol	33 (84.6)	9 (64.3)	5 (55.6)	0.097
SXT	36 (92.3)	13 (92.9)	8 (88.9)	0.934

SXT=Trimethoprim sulfamethoxazole.

Table 6. Multidrug resistance among *Campylobacter* spp. from poultry.

Poultry			p-value	chi-square
Faecal	36	36(100.0)	1.0000	-
Carcass	29	29(100.0)		
Total	65	65(100.0)		

Multidrug resistance defined as resistance to 3 or more antibiotics

thermophilic *Campylobacters* but inhibits the growth of non-thermophiles by not producing hydrogen enriched atmosphere, which is required by the non-thermophilic *Campylobacters* (Workman et al., 2005).

Resistance was commonly observed against Ampicillin, tetracycline, chloramphenicol, erythromycin, trimethoprim sulfamethoxazole, cefotaxime, ciprofloxacin and nalidixic acid with moderate and no resistance against gentamicin and imipenem, respectively. The resistance levels in our study were comparable to work from different countries (Sukhapesna et al., 2005; Tang et al., 2009; Usha et al., 2010; Mansouri-najand et al., 2012; Kovalenko et al., 2014) although lower resistance have also been reported by Fallon et al. (2003). High resistance was generally observed among *C. jejuni* and *C. coli* isolates to the various antibiotics with no significant differences in the resistance levels with the exception of nalidixic acid and norfloxacin. However, *C. coli* isolates were highly susceptible (0% of resistance) to norfloxacin and gentamicin. There are varied literature reports of resistance patterns of *C. jejuni* and *C. coli* strains; while some authors established higher resistance among *C. jejuni* (Tambur et al., 2009), others found higher resistance among *C. coli* (Jonker and Picard, 2010) and in some studies no difference in resistance were observed among the two species (Uzunovic et al., 2009; Ewnetu and Mihret, 2010). Moreover, no specific reasons have been cited for the differences in resistance among the two species (Luangtongkum et al., 2009).

Multidrug resistance of 100% was established in both faecal and carcass isolates which agrees with work in Malaysia by Tang et al. (2009) who recorded 100% MDR in poultry isolates. MDR rates of 35, 75 and 97% in poultry have, respectively been reported in Malaysia, Nigeria and Thailand (Sukhapesna et al., 2005; Akwuobu et al., 2010; Mansouri-najand et al., 2012). The observed high resistance rates against most of the assayed drugs may be explained by the reality of numerous disease outbreaks which frequently threaten the Ghanaian poultry industry (Aning, 1995) resulting in widespread use and abuse of antibiotics for prophylaxis and treatment of diseases and as growth promoters. The non-observance of withdrawal periods of antibiotics by Ghanaian poultry farmers which leaves residues in the poultry products with public health implications has also been reported by Turkson (2002). Chicken meat is a primary source of human campylobacteriosis, therefore the presence of antimicrobial-resistant *Campylobacter* in raw chicken meat constitutes a risk for consumers considering the high drug resistance found among isolates in this study.

Conclusion

Campylobacter species were present in the faecal and carcass samples of poultry birds (broilers and layers) at the Kejetia poultry slaughter in Kumasi. Contamination of carcasses by multidrug resistant *Campylobacter* strains poses risk to handlers and consumers. The abuse of antibiotics in poultry cannot be ignored deliberating the

high resistance levels documented against most of the commonly used antibiotics. Therefore constant education, surveillance and monitoring of antibiotic usage in poultry have become necessary.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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