The Prevalence and Antimicrobial Resistance of *Campylobacter Jejuni* and *Campylobacter coli* in Chicken Livers Used for Human Consumption in Ecuador

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Abstract: This study was carried out to determine the prevalence and antimicrobial resistance of *Campylobacter jejuni* and *C. coli* from chicken livers sold for human consumption at public markets of Loja City, Southern Ecuador. *Campylobacter* sp. was isolated from 32 (62.7%) of 51 chicken liver samples. The 22 (68.8%) yielded *C. jejuni* and 10 (31.2%) *C. coli*. Among the isolated strains, high resistant frequencies were observed with ciprofloxacin (98.3%) and tetracycline (78.1%). Resistance to ampicillin and erythromycin was 25 and 12.5%, respectively. Multi-resistance (resistance to three or more antibiotics) was found in 9 (28.1%) strains. To our knowledge, this is the first attempt to demonstrate the presence of *Campylobacter* species and their antimicrobial susceptibility patterns in foodstuff in Southern Ecuador.

Key words: Campylobacter, chicken livers, antimicrobial resistance, diarrhea, epidemiology

INTRODUCTION

The binomium *Campylobacter jejuni/C. coli* is considered worldwide as a major etiological agent in human diarrheal disease, being the leading cause of zoonotic food borne intestinal illness in industrialized countries and the second or third cause in developing countries (WHO, 2013; Fernandez, 2011).

Transmission of *C. jejuni* and *C. coli* to human beings includes consumption of contaminated water and food of animal origin, handling of raw chicken and other poultry meats or offal and direct contact with animals or their feces (WHO, 2013; Fernandez, 2011). Chicken liver could be of risk acquiring Campylobacter enteritis and several outbreaks have been reported due to the consumption of raw or under cooked liver or liver preparations such as pate or liver parfait (Whyte *et al.*, 2006; O'Leary *et al.*, 2009; Inns *et al.*, 2010; Little *et al.*, 2010; CDCP, 2013).

Besides food contamination as a risk factor for campylobacteriosis acquisition, the increasing of antimicrobial resistance rates among *Campylobacter* sp. is another issue of public health concern (Wang *et al.*, 2011; WHO, 2013; Whiley *et al.*, 2013). In South American countries, the emergence of *C. jejuni* and *C. coli* strains isolated from humans and animals, resistant to erythromycin, tetracycline, ampicillin and quinolones has been widely reported where quinolone-resistant strains reached high rates, sometimes exceeding 60% (Fernandez, 2011; Pollett *et al.*, 2012).

The aims of this study were to determine the prevalence of *C. jejuni* and *C. coli* in chicken livers for human consumption sold in public markets of Loja City, Southern Ecuador (3°59' Southlatitude S; 79°12' West longitude) and to determine the susceptibility and resistance patterns of the isolated strains to six antimicrobial drugs.

MATERIALS AND METHODS

A total of 51 chicken liver samples were obtained from the public markets of Loja City. Each sample consisting of four to five livers was ashed with 150 mL of Preston Enrichment Broth (CM 67 Oxoid enriched with 5% horse blood) and then was incubated at 42°C for 48 h in microaerophilic conditions. After that, 200 μL aliquots of the sample-enriched broth were filtrated onto Butzler agar plates using 0.45 μ filters (Fernandez *et al.*, 2007). All the plates were incubated under the same conditions as described above.

Campylobacter species were identified through their phenotypic characteristics using Gram stain, tests for catalase, oxidase, oxygen tolerance and growth at 25°C,

hippurate and indoxyl acetate hydrolysis tests and cephalothin and nalidixic acid as previously used by Fernandez and Arnas (1985) and Varela *et al.* (2007).

Susceptibility to ampicillin, amoxicillin/clavulanic acid, erythromycin, tetracycline, ciprofloxacin and gentamycin was determined by the Disk Diffusion Method following the 2014 recommendations of The European Committee on Antimicrobial Susceptibility Testing-EUCAST and the Committee for the Antibiogram of the French Society of Microbiology.

RESULTS AND DISCUSSION

Of the 51 samples, *Campylobacter* sp. was isolated from 32 (62.7%), of which 22 (68.8%) yielded *C. jejuni* and 10 (31.2%) *C. coli*.

As shown in Table 1, high resistant frequencies for *Campylobacter* sp. were observed with ciprofloxacin (98.3%) and tetracycline (78.1%) whereas 25 and 12.5% of the strains were resistant to ampicillin and erythromycin, respectively. One strain (3.1%) was resistant to gentamycin. Multi-resistance (resistance to three or more antibiotics) was found in 9 (28.1%) strains, in particular 3 strains were shown to be resistant to ampicillin, tetracycline and ciprofloxacin, 2 to erythromycin, tetracycline and ciprofloxacin, 1 to ampicillin, erythromycin, tetracycline and ciprofloxacin and 1 to ampicillin, erythromycin, tetracycline, ciprofloxacin and gentamycin.

Studies on prevalence data of Campylobacter in chicken livers have been carried out in several countries with reported isolation frequencies ranging from 15.5% in Bulgaria (Vashin *et al.*, 2009) 24% in South Africa (Bartkowiak-Higgo *et al.*, 2006), 72% in Turkey (Kenar *et al.*, 2009) to 92.9% in Chile (Fernandez and Pison, 1996) and 100% in New Zealand (Whyte *et al.*, 2006). In our study, the Campylobacter isolation rate was higher than that reported by Vashin *et al.* (2009) in Bulgaria and by Bartkowiak-Higgo *et al.* (2006) in South Africa.

However, it was lower than in reports coming from Turkey, Chile and New Zealand (Kenar *et al.*, 2009; Fernandez and Pison, 1996; Whyte *et al.*, 2006). In agreement with the studies of Whyte *et al.* (2006) and Kenar *et al.* (2009) *C. jejuni* was the predominant species with an isolation rate of 68.8% whereas *C. coli* was isolated in 31.2% of the studied samples. However, the opposite was found by Fernandez and Pison (1996) and Vashin *et al.* (2009) who have found that *C. coli* was most frequently isolated than *C. jejuni*.

Like chicken meat, offal could be source of Campylobacter infection (WHO, 2013). Outbreaks of Campylobacter are apparently uncommon. However, outbreaks of *Campylobacter enteritis* linked to raw or lightly cooked chicken livers (Whyte *et al.*, 2006; CDCP, 2013) or to food preparations like chicken liver pate (O'Leary *et al.*, 2009; Little *et al.*, 2010) and chicken liver parfait (Inns *et al.*, 2010) have been reported. This could be explained by the notion that chicken livers could contain a most probable number of Campylobacter >104 per liver (Whyte *et al.*, 2006) or levels ranging from 10 to >230 Campylobacter cells/100 mL of liquid exuded by livers (Fernandez and Pison, 1996), representing a potential source of human campylobacteriosis.

With regard to antimicrobial susceptibility, all the strains tested were susceptible to amoxicillin/clavulanic acid but 25% were resistant to ampicillin. Resistance to ampicillin and other β-lactam antibiotics has been reported among Campylobacter strains isolated from humans and poultry (Griggs *et al.*, 2009). Since, all the ampicillin-resistant strains found in our study were susceptible to amoxicillin/clavulanic acid, the resistance of these strains could be due to a β-lactamase production. The association amoxicillin/clavulanic acid has been proposed as a treatment choice in case of *Campylobacter enteritis* due to resistance of tested strains to the first-line antibiotics (Griggs *et al.*, 2009). Ampicillin resistance in Campylobacter strains of Southern Ecuador is likely to be an emerging problem that could require the establishment of a laboratory surveillance system to assess its real magnitude.

Table 1: Prevalence of antimicrobial resistance among *C. jejuni* and *C. coli* isolated from chicken livers used for human consumption

| | *C. jejuni* strains (n = 22) | | | | *C. coli* strains (n = 10) | | | | Global resistance* | |
| | Resistant | | Susceptible | | Resistant | | Susceptible | | | |
Antibiotics	No.	%	No.	%	No.	%	No.	%	No.	%
Ampicillin	6	27.3	16	72.7	3	30.0	7	70.0	8	25.0
Amoxicillin/Clavulanic acid	0	0.0	22	100.0	0	0.0	10	100.0	0	0.0
Tetracycline	18	81.8	4	18.2	7	70.0	3	3.0	25	78.1
Ciprofloxacin	20	90.9	2	9.1	10	100.0	0	0.0	30	93.8
Erythromycin	2	9.1	20	90.9	2	20.0	8	80.0	4	12.5
Gentamycin	0	0.0	22	100.0	1	10.0	9	90.0	1	3.1

C. jejuni + *C. coli* resistance

Only one *C. coli* strain was resistant to gentamycin. However, gentamycin-resistant strains have been isolated from chicken products (Noormohamed and Fakhr, 2012). In general terms, >99% of *C. jejuni* and 88% of *C. coli* isolates are susceptible to aminoglycosides while their resistance could be due to a modification of the antibiotic by aminoglycoside-modifying enzymes (Iovine, 2013).

Erythromycin resistance reaches 12.5% which is lower to that reported by Noormohamed and Fakhr (2012). However, since this antibiotic is the drug of choice (WHO, 2013) to treat Campylobacter enteritis, there is a necessity to create a laboratory surveillance and monitoring system for the adequate use of erythromycin in human medicine.

Tetracycline resistance was very high (78.1%) but lower to the levels obtained by Noormohamed and Fakhr (2012). The known mechanisms of tetracycline resistance in Campylobacter are alteration of tetracycline's ribosomal target and efflux and their high resistance frequency is likely due to the heavy use of this antibiotic in the past in both human and veterinary clinical practices (Iovine, 2013).

Surprisingly there was a remarkably high resistance rate to ciprofloxacin (93.8%), probably reflecting the overuse of fluoroquinolones in animals and in the poultry industry and representing an emerging worldwide epidemiological problem with implications for treatment in humans (WHO, 2013; Iovine, 2013).

All the *C. coli* strains were resistant to ciprofloxacin and always more resistant than *C. jejuni* to the other antibiotics with the exception of tetracycline. It is known that *C. coli* isolates are generally more resistant than *C. jejuni* strains (Gallay *et al.*, 2007).

Due to high Campylobacter isolation rates we found in chicken livers, a special attention must he paid to good practices in handling this foodstuff at groceries in order to minimize cross contamination with other foodstuffs, utensils and surfaces. The further studies potentially needed to assess the risk factors of Campylobacter contamination in the production chain, maintenance and sales processes of chicken livers for human consumption.

CONCLUSION

To our knowledge, this is the first attempt to demonstrate the presence of *Campylobacter* species in foodstuff in Southern Ecuador as well as their antimicrobial susceptibility patterns.

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