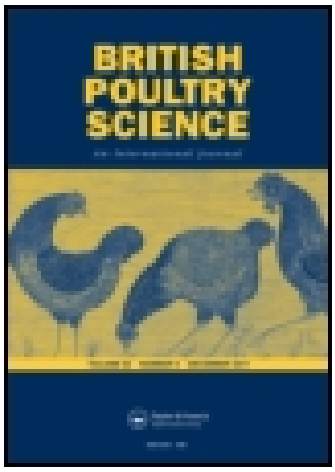

British Poultry Science
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/cbps20

Evaluation of antimicrobial resistance of Campylobacter spp. isolated from broiler carcasses

I. D. Ferro[a], T. M. Benetti[b], T. C. R. M. Oliveira[b], W. M. Abrahão[cd], S. M. S. S. Farah[e], F. B. Luciano[a] & R. E. F. Macedo[a]

[a] Graduate Program in Animal Science, School of Agricultural Sciences and Veterinary Medicine, Pontifícia Universidade Católica do Paraná, São José dos Pinhais, PR, Brazil

[b] Department of Food Science and Technology, Center for Agricultural Sciences, Universidade Estadual de Londrina, Campus Universitário, Londrina, PR, Brazil

[c] Department of Pharmacy, Division of Health Sciences, Universidade Federal do Paraná, Jardim Botânico, Curitiba, PR, Brazil

[d] Section of Food Microbiology, Central Laboratory of Paraná State, Rua Ubaldino do Amaral Curitiba, PR, Brazil

[e] Section of General Bacteriology, Central Laboratory of Paraná State, São José dos Pinhais, PR, Brazil

Accepted author version posted online: 08 Jan 2015.

To cite this article: I. D. Ferro, T. M. Benetti, T. C. R. M. Oliveira, W. M. Abrahão, S. M. S. S. Farah, F. B. Luciano & R. E. F. Macedo (2015): Evaluation of antimicrobial resistance of Campylobacter spp. isolated from broiler carcasses, British Poultry Science, DOI: 10.1080/00071668.2014.981796

To link to this article: http://dx.doi.org/10.1080/00071668.2014.981796

Publisher: Taylor & Francis & British Poultry Science Ltd

Journal: *British Poultry Science*

DOI: 10.1080/00071668.2014.981796

CBPS-2014-081

Ed. Kjaer, October 2014;

Edited Hocking 22 October 2014

Evaluation of antimicrobial resistance of C*ampylobacter* spp. isolated from broiler carcasses

I. D. FERRO[1], T. M. BENETTI [2], T. C. R. M. OLIVEIRA[2], W. M. ABRAHÃO [3,4], S. M. S. S. FARAH [5], F. B. LUCIANO[1] AND R. E. F. MACEDO[1]

[1] *Graduate Program in Animal Science, School of Agricultural Sciences and Veterinary Medicine, Pontifícia Universidade Católica do Paraná, São José dos Pinhais, PR, Brazil,* [2] *Department of Food Science and Technology, Center for Agricultural Sciences, Universidade Estadual de Londrina, Campus Universitário, Londrina, PR, Brazil;* [3] *Department of Pharmacy, Division of Health Sciences, Universidade Federal do Paraná, Jardim Botânico, Curitiba, PR, Brazil;* [4] *Section of Food Microbiology, Central Laboratory of Paraná State, Rua Ubaldino do Amaral Curitiba, PR, Brazil and* [5] *Section of General Bacteriology, Central Laboratory of Paraná State, São José dos Pinhais, PR, Brazil.*

Running title: Campylobacter antibiotic resistance in broiler

Correspondence to: Renata Macedo, Graduate Program in Animal Science, School of Agricultural Sciences and Veterinary Medicine, Pontifícia Universidade Católica do Paraná,

Accepted for publication 25thSeptember 2014

BR 376, Km 14, São José dos Pinhais, 83010-500, PR, Brazil. Email: renata.macedo@pucpr.br

Abstract. 1.

The aim of the present study was to evaluate the antimicrobial resistance of *Campylobacter* strains (*C. jejuni*, *C. coli* and *C. lari*) isolated from broiler carcasses processed in the State of Paraná, Brazil.

2. Rates of microbial resistance and susceptibility were assessed by both Disk Diffusion (DD) and Etest (Minimum Inhibitory Concentration) techniques. Results were expressed as percentages and the comparison of the results obtained by both methods was performed using the Chi-squared (x^2) test at a significance level of 5%. Antibiotics were tested using DD (12 antibiotics) and/or MIC (7 antibiotics) methods.

3. A total of 95.8% of the strains were resistant to at least two agents. In terms of multidrug resistance, 75% of strains were resistant to three or more groups of antibiotics. The highest rates of resistance were detected for cefalotin, ciprofloxacin, tetracycline and nalidixic acid. A high rate of susceptibility of the strains to erythromycin (95.8%) was found confirming that this is considered the agent of choice for treating campylobacteriosis. Comparison of the microbial resistance and susceptibility, as determined simultaneously by the two methods, found the techniques to be statistically equivalent for 5 out of the 6 antibiotics tested.

4. The results of this study suggest the need for adopting measures to control the use of antibiotics in broiler production to prevent multidrug resistance of *Campylobacter* strains and reduce the risk of serious human diseases caused by the consumption of contaminated chicken meat.

INTRODUCTION

In recent decades, the Brazilian production of broilers rose to be one of the most competitive activities of its national agribusiness sector. Brazil is the third largest producer and the world´s largest exporter of chicken meat (Ubabef, 2011). Among all States, Paraná is Brazil's

largest poultry meat producer. Given the growth in international trade of poultry meat, the requirements for controlling pathogens in animal breeding and meat processing have also been intensified.

Among the major causes of foodborne diseases in humans, infections with the bacterial genus *Campylobacter* spp. has gained notoriety in recent years for causing intestinal disorders worldwide, particularly for those transmitted by handling or consumption of poultry meat (Silva *et al*., 2011). Besides this fact, the *Campylobacter* genus has drawn attention of the scientific community for its resistance to antibiotics traditionally used for human therapy (Pezzotti *et al*., 2003). Antimicrobial resistance is linked to the use of antibiotics for the treatment and prevention of diseases in food animals raised under intensive farming systems and is also associated with wide use of these antibiotics for treating systemic infections and chronic diarrhoea in humans (Olah *et al*., 2006).

The indiscriminate use of these drugs, both in clinical medicine and animal production, has a major impact on public health. It reduces the number of safe antibiotics available for treating human diseases, and increases the risk of appearance of multidrug-resistant strains, which may contaminate foods, posing a serious risk to the health of those consuming these products (Olah *et al*., 2006).

The aim of the present study was to evaluate the antibiotic resistance of *Campylobacter* spp. strains isolated from refrigerated broiler carcasses to a range of drugs commonly employed for clinical treatment in humans.

MATERIALS AND METHODS

Sample collection

Sixty (60) refrigerated broiler carcasses, from 12 different commercial brands and purchased from retail stores in Curitiba, Paraná, Brazil, were assessed for the presence of *Campylobacter*

spp. Of those carcasses, 24 (40%) were positive for the presence of *Campylobater* spp. One isolate from each positive broiler carcass was submitted to antimicrobial susceptibility testing.

Strains were isolated according to the ISO 10272 protocol (ISO, 2006). Suspected colonies were assessed using the oxidase and catalase tests and identified by the API Campy system (bioMérieux, Marcy I'Etoile, France) according to the manufacturer's instructions. Results were confirmed at genus level by Real Time-PCR (polymerase chain reaction) as described by Biasi *et al.* (2011).

Antimicrobial resistance

The isolates identified as *Campylobacter* spp. were submitted to antimicrobial susceptibility testing using both the Disk Diffusion (DD) (Becton Dikson, Sparks, USA) and Gradient Diffusion (Etest) (AB Biodisk, Solna, Switzerland) methods.

Employing the MIC method (Etest), the *Campylobacter* strains were tested for their susceptibility to 7 antibiotics commonly used to treat human diseases: CI (ciprofloxacin, 0.002-32 µg/ ml), CL (chloramphenicol, 0.016-256 µg/ ml), CM (clindamycin, 0.016-256 µg/ ml), EM (erythromycin, 0.016-256 µg/ ml), GM (gentamicin, 0.016-256 µg/ ml), MP (meropenem, 0.002-32 µg/ ml) and TC (tetracycline, 0.016-256 µ/ ml).

The DD test was performed with 12 antibiotics as follows: AMC (amoxicillin + clavulanic acid, 10 µg), AMP (ampicillin, 10 µg), C (chloramphenicol, 30 µg), CIP (ciprofloxacin, 5 µg), CTX (cefotaxime, 30 µg), E (erythromycin, 5 µg), GM (gentamicin, 10 µg), KF (cefalotin, 30 µg), MEN (meropenem, 10 µg), NA (nalidixic acid, 30 µg), TE (tetracycline, 30 µg) and TOB (tobramycin, 10 µg).

Both techniques were carried out using a bacterial suspension spreaded on Müller-Hinton agar (Oxoid, Basingstoke, England) containing 5% (v/v) sheep blood (Newprov, Pinhais, Brazil) and incubated under microaerophilic conditions at 42 °C for 24 h. The suspension was adjusted to match the 0.5 McFarland turbidity using a Densimat densitometer

(bio-Mérieux, France), as recommended by the Clinical Laboratory Standards Institute (2011).

Resistance or susceptibility of isolates to the antibiotics was determined by measuring the inhibition zone diameter (mm) with the DD technique and by reading the MIC as the value where the growth inhibition ellipse intersected the strip of Etest. Interpretation of the results obtained for ciprofloxacin, erythromycin and tetracycline was performed according to the interpretive standard defined by the M45 A2 standard of the Clinical and Laboratory Standards Institute for *Campylobacter jejuni/ C. coli* (CLSI, 2011). All remaining antibiotics were interpreted using the standards defined by the M100 S21 standard for *Enterobacteriaceae* (CLSI, 2010).

A standard strain of *Campylobacter jejuni* subsp. *jejuni* ATCC 33291 (Remel, Lenexa, USA) was used as a control. The strain was cultured in Bolton broth (Oxoid, Basingstoke, England) at 37 °C for 48 h under a microaerophilic atmosphere (5% O_2, 10% CO_2 and 85% N), and tested for antimicrobial susceptibility to the same group of antibiotics in each test. Prior to its use, the strain was examined for its antimicrobial susceptibility towards all antibiotics used in this study, and to confirm the results, this test was performed 5 times.

Analysis of results

Rates of microbial resistance and susceptibility of the *Campylobacter* strains were expressed as percentages. Comparison of the results obtained for the DD versus the Minimum Inhibitory Concentration (Etest) method was performed using the Chi-squared (x^2) test at $P \leq 5\%$ level of significance.

RESULTS

From the 24 *Campylobacter* strains originally isolated, 22 (91.7%) were *Campylobacter jejuni*, 1 (4.2%) *C. coli*, and 1 (4.2%) *C. lari*.

Results using the Etest method showed resistance to 5 (71.4%) out of the 7 antibiotics tested by this method. The highest rates of resistance were found for ciprofloxacin, to which

91.7% of strains were resistant, and tetracycline with a 75.0% resistance rate. Resistance was also detected, albeit at a lower rate, to clindamycin (8.3%), erythromycin (4.2%) and gentamicin (4.2%). All *Campylobacter* isolates showed susceptibility to chloramphenicol and meropenem by the Etest method.

The DD technique was used to test 12 antibiotics and revealed bacterial resistance to 6 of them. The highest rate of resistance (95.8%) was detected towards cefalotin, followed by nalidixic acid, ciprofloxacin and tetracycline, all of which were inefficient against 75.0% of the bacteria tested. Resistance against ampicillin and chloramphenicol were 16.7% and 4.16%, respectively. Conversely, all strains showed susceptibility to amoxicillin + clavulanic acid, erythromycin, gentamicin, meropenem and tobramycin (Figure). **Figure near here**

From all *Campylobacter* isolates, 95.8% were resistant to at least 2 out of 13 antibiotics tested; where 5 isolates (21.0%) were resistant to 2 antibiotics and 18 (75.0%) showed resistance to 3 or more antibiotics. Similarly, in terms of multidrug resistance, 18 isolates (75.0%) were resistant to 3 or more groups of antibiotics.

Among six antibiotics tested in both DD and Etest (ciprofloxacin, chloramphenicol, erythromycin, gentamicin, meropenem and tetracycline), the highest resistance rates were detected for ciprofloxacin and tetracycline. In addition, both methods were found to be statistically equivalent ($P > 0.05$) for 5 out of the 6 antibiotics examined (Table). A significant difference between the methods was only found for ciprofloxacin ($P < 0.05$), which had a higher resistance rate (91.7%) on the Etest compared with DD (75%). **Table near here**

DISCUSSION

In the international literature there are few studies comparing antimicrobial resistance of *Campylobacter* by DD and Etest. McGill *et al.* (2009) found no significant differences in antimicrobial resistance against 4 out of 6 antibiotics tested in 75 *Campylobacter* strains isolated from food and human clinical samples in Ireland. Those authors stated that either the

DD or E-test could be applied for susceptibility testing in the case of ciprofloxacin, tetracycline, nalidixic acid and chloramphenicol in *Campylobacter* spp.

Results of the present study indicated high concordance of antimicrobial resistance between the Etest and DD for chloramphenicol, erythromycin, gentamicin, meropenem and tetracycline. Thus DD could be a reliable and more cost effective technique for testing the resistance/ susceptibility of *Campylobacter* strains isolated from broilers to these antibiotics in food monitoring or surveillance programs. Luangtongkum *et al*. (2007) stated that the DD method is highly useful particularly for testing a large number of antimicrobials against a small number of isolates.

Studies found in the literature report multidrug resistance varying from 7% to 92% in *Campylobacter* isolates (Ge *et al*., 2002; Kos *et al*., 2006; Kassa *et al*., 2007; Chen *et al*., 2010; Rahimi and Ameri, 2011; Mackiw *et al*., 2012). Among the *Campylobacter* strains, 18 (75%) showed resistance to 3 or more groups of antibiotics. This finding is consistent with the fact that Gram-negative bacteria are generally more resistant to a greater number of antibiotics and chemotherapeutic agents compared with Gram-positive bacteria, because their external membrane acts as a barrier limiting access of antimicrobial agents to targets within the cell (Vaara, 1992; Nikaido, 1994).

C. lari was the only strain susceptible to all the antibiotics, a result in disagreement with Taylor and Courvalin (1988) who reported that species such as *C. lari*, *C. fetus* and *C. hyointestinalis* have a natural resistance to nalidixic acid. The same author also stated that *C. jejuni* and *C. coli* are naturally resistant to penicillins and to 1st and 2nd generation cephalosporins.

Interestingly, the isolates used in this study showed a high rate of ciprofloxacin resistance. In recent decades, numerous authors have reported an increasing prevalence of ciprofloxacin resistance in *Campylobacter* spp. strains, particularly those isolated from poultry (Sáenz *et al*., 2000; Bardon *et al*., 2009; Chen *et al*., 2010; Rahimi and Ameri, 2011;

Mackiw *et al*, 2012). The high prevalence of ciprofloxacin resistance is of great concern, since this drug is widely employed in the treatment of human gastroenteritis and recommended in cases of infection caused by macrolide-resistant *Campylobacter* (Kos *et al*., 2006). This resistance can hamper the adoption of effective therapy in cases of campylobacteriosis and may increase the prevalence of gastroenteritis caused by the consumption of poultry meat contaminated by this pathogen (Kos *et al*., 2006).

After macrolides and fluoroquinolones, tetracycline is the third agent of choice for the treatment of enteric infections caused by *C. coli* and *C. jejuni* (Gaudreau *et al*., 2008). Despite the fact that the use of tetracycline as a growth promoter for poultry in Brazil was banned in 1998 (Brazil, 2009), a high frequency of tetracycline resistance has been found for the *Campylobacter* isolates examined in this study (75%). Similarly high rates of resistance to tetracycline and nalidixic acid were previously reported by Gaudreau and Gilbert (1997), Chen *et al*. (2010) and Quin *et al*. (2011).

Although antibiotic therapy is not required in the vast majority of patients suffering with campylobacteriosis, this use is indicated for systemic or chronic infections, as well as in immunocompromised patients, where the drug of choice is erythromycin (Hariharan *et al*., 2009). In the present study, only one *Campylobacter* strain (*C. jejuni* subsp. *jejuni*) was resistant to erythromycin. This *C. jejuni subsp. jejuni* strain was also resistant to gentamicin. Andersen *et al*. (2006) reported that the occurrence of resistance to erythromycin in *C. jejuni* isolated from Danish poultry was significantly lower than the resistance rates found to tetracycline and fluoroquinolones. However, erythromycin resistance rates ranging from moderate to high in *Campylobacter* isolated from chicken were reported in studies conducted in several countries (Sáenz *et al*., 2000; Smole-Mozina *et al*., 2011; Mackiw *et al*., 2012).

The low rate of erythromycin resistance presented in this study may be explained by the non-use of macrolides in Brazilian poultry production. Likewise, the low resistance to gentamicin may be partially attributed to its low use in poultry farming as a growth promoter

or therapeutic strategy, since this antibiotic is administered via intramuscular injection, which is impractical for large-scale use in poultry processing operations (Rodrigo *et al*., 2007).

Although gentamicin, clindamycin and ampicillin are not the primary drugs of choice for treating campylobacteriosis, they can be included as alternative drugs for the treatment of systemic infections by *Campylobacter* (Luangtongkum *et al*., 2007).

Similarly, as found in this study, low resistance rates to erythromycin, gentamicin and chloramphenicol in *Campylobacter* strains were also detected by Kos *et al*. (2006), Han *et al*. (2007) and Hariharan *et al*. (2009). Only one strain (*C. coli*) isolated in the present study showed resistance to chloramphenicol in DD test.

The disparity among the rates of antimicrobial resistance in *Campylobacter* isolates reported in different countries might be explained by the existence of specific laws restricting the use of antibiotics in animal feed as growth promoters, particularly in Europe. In developing countries, despite the existence of regulations controlling the use of antibiotics in a range of fields, including human and veterinary medicine, implementing and enforcing these regulations is still a challenge (Mackiw *et al*., 2012). Additionally, the lack of standardisation of methods for strain isolation and the lack of standardisation of cut-off values also contribute to the disparity among rates of resistance in *Campylobacter* isolates reported in different countries.

In Brazil, tetracyclines, penicillins, chloramphenicol, sulfonamides, furazolidone, nitrofurazone and avoparcin were banned as feed additives for broilers (Brazil, 2009). Nevertheless, a survey conducted in 2005 by Paraná's Ministry of Health (Secretaria de Estado da Saúde do Paraná, 2005) demonstrated that farmers still use tetracyclines (6%) as growth promoters for broilers and tetracyclines (11%), penicillin (7%) and sulfonamides (14%) as therapeutic agents, even though these are prohibited substances. The survey also showed the misuse of drugs indicated only for therapy as growth promoters, such as tiamulin (2%), ciprofloxacin (6%), olaquindox (1%) (Brazil, 2004), norfloxacin (2.7%), enrofloxacin

(33%) and carbadox (1%) (Brazil, 2005). The latter is contraindicated for broilers and is only recommended for medical or growth purposes in pigs (Secretaria de Estado da Saúde do Paraná, 2005). Currently, the growth promoters for broilers authorised for sale in Brazil are arsanilic acid, avilamycin, colistin, flavomycin, lincomycin, tylosin, virginamycin, bacitracin, spiramycin and enramycin (Brazil, 2006).

The adoption of effective measures for controlling the use of antibiotics in broiler production, along with the implementation of programmes to reduce contamination of *Campylobacter* and to detect resistant strains isolated in chicken meat, should be pursued in an effort to prevent the spread of antibiotic-resistant *Campylobacter* spp. and to reduce the risk of human diseases caused by the consumption of contaminated chicken meat.

ACKNOWLEDGEMENTS

The authors thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Ministério da Educação, Brazil, for funding Isabelle Dangui Ferro studies through a scholarship and the staff from the Central Laboratory of Paraná State (LACEN) for their collaboration with the microbiological analyses.

REFERENCES

ANDERSEN, S. R., SAADBYE, P., SHUKRI, N. M., ROSENQUIST, H., NIELSEN, N. & BOEL, J. (2006). Antimicrobial resistance among *Campylobacter jejuni* isolated from raw poultry meat at retail level in Denmark. International Journal of Food Microbiology, 107: 250-255.

BARDON, J., KOLAR, M., CEKANOVA, L., HEJNAR, P. & KOUKALOVA, D. (2009). Prevalence of *Campylobacter jejuni* and its resistance to antibiotics in poultry in the Czech Republic. Zoonoses Public Health, 56: 111-116.

BIASI, R. S., MACEDO, R. E. F., MALAQUIAS, M. A. S. & FRANCHIN, P. R. (2011). Prevalence, strain identification and antimicrobial resistance of *Campylobacter* spp. isolated from slaughtered pig carcasses in Brazil. Food Control, 22: 702-707.

BRAZIL. (2004). Instrução Normativa n° 11 (November 24th, 2004) on the instrução para a proibição da fabricação, importação, comercialização e uso da substância química denominada Olaquindox como aditivo promotor de crescimento em animais produtores de alimentos. Diário Oficial da União**,** Brasília, Brazil. Available at http://agricultura.gov.br/animal/qualidade dos alimentos/aditivos-proibidos. Accessed 23 February 2012.

BRAZIL. (2005). Instrução Normativa n° 35 (November 24th, 2005) on the instrução para a proibição da fabricação, importação, comercialização e uso de produtos destinados a alimentação animal contendo a substância química denominada Carbadox. Diário Oficial da União, Brasília, Brazil. Available at http:// agricultura.gov.br/animal/qualidade dos alimentos/aditivos-proibidos. Accessed 23 February 2012.

BRAZIL. (2006). Instrução Normativa n° 65 (November 21th, 2006) on the Regulamento Técnico sobre os Procedimentos para a fabricação e o emprego de rações, suplementos, premixes, núcleos ou concentrados com medicamentos para animais de produção. Diário Oficial da União**,** Brasília, Brazil. Avaliable at http:// www.agricultura.gov.br/animal/qualidade dos alimentos/aditivos-proibidos. Accessed 23 February 2012.

BRAZIL. (2009). Instrução Normativa n° 26 (July 9th, 2009) on the Regulamento Técnico para a fabricação, o controle de qualidade, a comercialização e o emprego de produtos antimicrobianos de uso veterinário. Diário Oficial da União, Brasília, Brazil. Avaliable at http:// www.agricultura.gov.br/animal/qualidade dos alimentos/aditivos-proibidos. Accessed 23 February 2012.

CHEN, X., NAREN, G. W., WU, C. M., WANG, Y., DAI, L., XIA, L. N., LUO, P. G., ZHANG, Q. & SHEN, J. Z. (2010). Prevalence and antimicrobial resistance of *Campylobacter* isolates in broilers from China. Veterinary Microbiology, 144: 133-139.

CLINICAL AND LABORATORY STANDARDS INSTITUTE (CLSI). (2010) Performance Standards for Antimicrobial Testing. 21°Informational Supplement, M100 S21. 31:1.

CLINICAL AND LABORATORY STANDARDS INSTITUTE (CLSI). (2011) Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated of Fastidious Bacteria. Approved Guideline – Second Edition, M45 A2. 30:18.

GAUDREAU, C., & GILBERT, H. (1997). Comparison of Disk Diffusion and agar dilution methods for antibiotic susceptibility testing of *Campylobacter jejuni* subsp. *jejuni* and *Campylobacter coli.* Journal of Antimicrobial Chemotherapy, 39: 707-712.

GAUDREAU, C., GIROUARD, Y., GILBERT, H., GAGNON, J., & BEKAL, S. (2008). Comparison of Disk Diffusion and agar dilution methods for erythromycin, ciprofloxacin, and tetracycline susceptibility testing of *Campylobacter coli* and for tetracycline susceptibility testing of *Campylobacter jejuni* subsp. *jejuni.* Journal of Antimicrobial Chemotherapy, 52: 4475–4477.

GE, B., BODESIS, S., WALKER, R. D., WHITE, D. G., ZHAO, S., MCDERMOTT, P. F., & MENG, J. (2002). Comparison of the Etest and agar dilution for in vitro antimicrobial susceptibility testing of *Campylobacter*. Journal of Antimicrobial Chemotherapy, 50:487–494.

HAN, K., JANG, S. S., CHOO, E., HEU, S. & RYU, S. (2007). Prevalence, genetic diversity, and antibiotic resistance patterns of *Campylobacter jejuni* from retail raw chickens in Korea. Food Microbiology. 114: 50-59.

HARIHARAN, H., SHARMA, S., CHIKWETO, A., MATTHEW V. & DEALLIE, C. (2009). Antimicrobial drug resistance as determined by the E-test in *Campylobacter jejuni, C. coli,* and *C. lari* isolates from the ceca of broiler and layer chickens in Grenada. Comparative Immunology Microbiology and Infectious Diseases. 32: 21-28.

KASSA, T., GEBRE-SELASSIE, S., & ASRAT, D. (2007). Antimicrobial susceptibility patterns of thermotolerant *Campylobacter* strains isolated from food animals in Ethiopia. Veterinary Microbiology, 119: 82-87.

KOS, V., KEELAN, M., & TAYLOR, D. (2006). Antimicrobial susceptibilities of *Campylobacter jejuni* isolates from poultry from Alberta, Canada. Antimicrobial Agents and Chemotherapy, 50: 778-780.

LUANGTONGKUM, T., MORISHITA, T. Y., EL-TAYEB, A. B., ISON, A. J. & ZHANG, Q. (2007). Comparison of antimicrobial susceptibility testing of *Campylobacter spp.* by the agar dilution and the agar Disk Diffusion methods. Journal of Clinical Microbiology, 45: 590-594.

MACKIW, E., KORSAK, D., RZEWUSKA, K., TOMCZUK, K. & ROZYNEC, E. (2012). Antibiotic resistance in *Campylobacter jejuni* and *Campylobacter coli* isolated from food in Poland. Food Control, 23: 297-301.

MCGILL, K., KELLY. L., MADDEN, R. H., MORAN. L. & CARRO, C. (2009). Comparison of Disk Diffusion and epsilometer (E-test) testing techniques to determine antimicrobial susceptibility of *Campylobacter* isolates of food and human clinical origin. Journal of Microbiological Methods, 79: 238-241.

NIKAIDO, H. (1994). Prevention of drug access to bacterial targets: permeability barriers and active efflux. Science, 264: 382-388.

OLAH, P. A., DOETKOTTB, C., FAKHRA, M. K. & LOGUEA, C. M. (2006). Prevalence of the *Campylobacter* multi-drug efflux pump (CmeABC) in *Campylobacter* spp. isolated from freshly processed Turkeys. Food Microbiology, 23: 453-460.

PEZZOTTI, G., SERAFIN, A., LUZZI, I., MIONI, R., MILAN, M. & PERIN, R. (2003). Occurrence and resistance to antibiotics of *Campylobacter jejuni* and *Campylobacter coli* in animals ad meat in northeastern Italy. International Journal of Food Microbiology, 82: 281-287.

QUIN, S. S., WU. C. M., WANG. Y., JEON, B., SHEN, Z. Q., WAN, Y., ZHANG, Q. & SHEN, J. Z. (2011). Antimicrobial resistance in *Campylobacter coli* isolated from pigs in two provinces of China. International Journal of Food Microbiology, 146: 94-98.

RAHIMI, E.,& AMERI, M. (2011). Antimicrobial resistance patterns of *Campylobacter* spp. isolated from a raw chicken, turkey, quail, partridge and ostrich meat in Iran. Food Control, 22: 1165-1170.

RODRIGO, S., ADESIYUN, A., ASGARALI, Z. & SWANSTON, W. (2007). Antimicrobial resistance of *Campylobacter* spp. isolated from broilers in small poultry processing operations in Trinidad**.** Food Control, 18: 321-325.

SÁENZ, Y., ZARAZAGA, M., LANTERO, M., GASTANARES, M. J., BAQUERO, J. & TORRES, C. (2000). Antibiotic resistance in *Campylobacter* strains isolated from animals, foods, and humans in Spain in 1997–1998. Antimicrobial Agents and Chemotherapy, 44: 267-271.

SECRETARIA DE ESTADO DA SAÚDE DO PARANÁ. (2005). Levantamento do uso e comercialização de medicamentos veterinários em frango de corte. Programa Estadual de Controle de Resíduos de Medicamentos Veterinários em Alimentos de Origem Animal. Paraná, Brazil. Available at http://www.saude.pr.gov.br/arquivos/File/vigilancia%20sanitaria/Relatorio%20levantamento%20frango.pdf. Accessed 18 February 2012.

SILVA, J., LEITE, D., FERNANDES, M., MENA, C., GIBBS, P. A. & TEIXEIRA, P. (2011). *Campylobacter* spp. as a food borne pathogen: a review. Frontiers in Microbiology, 2: 200.

SMOLE-MOZINA, S., KURINCIC, M., KLANCNIK, A. & MAVRI, A. (2011). *Campylobacter* and its multi-resistance in the food chain. Trends in Food Science & Technology. 22: 91-98.

TAYLOR, D. & COURVALIN, P. (1988). Mechanisms of antibiotic resistance in *Campylobacter* species. Antimicrobial Agents and Chemotherapy, 32: 1107-1112.

UBABEF. (2011). Associação Brasileira de Produtores e Exportadores de Frangos. Available at: http://www.abef.com.br . Accessed: 09 November 2011.

VAARA, M. (1992). Agents that increase the permeability of the outer membrane. Microbiological Reviews, 56: 395-411.

FIGURE LEGEND

Figure. *Rates of microbial resistance, susceptibility and intermediate reaction of* Campylobacter *spp. strains (n = 24) by the Disk Diffusion method. AMC (amoxicillin + clavulanic acid, 10 µg), AMP (ampicillin, 10 µg), KF (cefalotin, 30 µg), CTX (cefotaxime, 30 µg), CIP (ciprofloxacin, 5 µg), C (chloramphenicol, 30 µg), E (erythromycin, 5 µg), GM (gentamicin, 10 µg), MEN (meropenem, 10 µg), NA (nalidixic acid, 30 µg), TE (tetracycline, 30 µg) and TOB (tobramycin, 10 µg).*

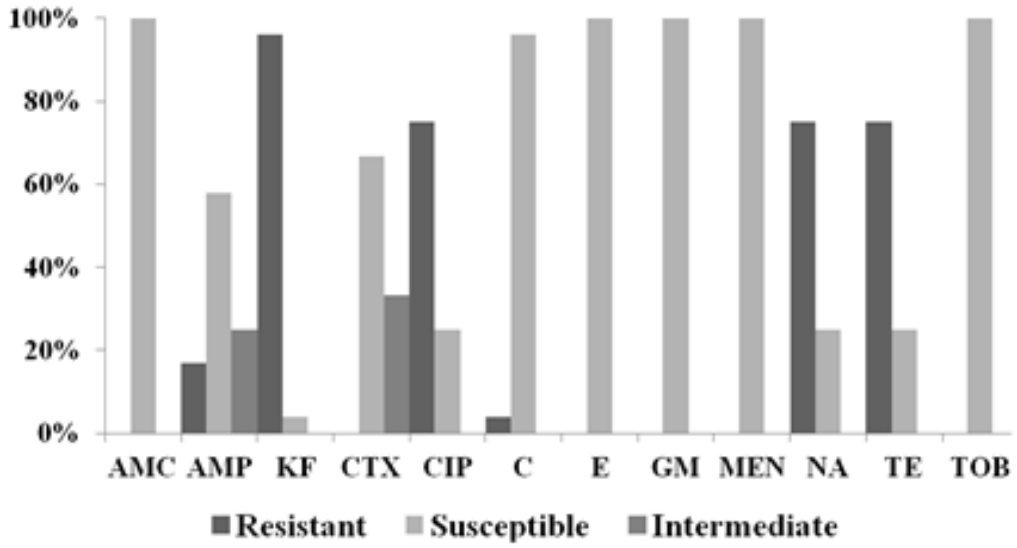

Table. *Comparison of frequency (%), number of Campylobacter strains, and concordance rate (%) of antimicrobial resistance and susceptibility by Disk Diffusion (DD) and Etest methods*

Antibiotic[1]	Resistant			Susceptible		
	DD, % (n)	Etest, % (n)	Concordance, %	DD, % (n)	Etest, % (n)	Concordance, %
CI *	75.0% (18)	91.7% (22)	81.8	25% (6)	4.2% (1)	16.7
CL **	4.1% (1)	0.0% (0)	- [2]	95.8% (23)	100% (24)	95.8
EM **	0.0% (0)	4.0% (1)	-	100% (24)	95.8% (23)	95.8
GM **	0.0% (0)	4.0% (1)	-	100% (24)	95.8% (23)	95.8
MP	0.0% (0)	0.0% (0)	100	100% (24)	100% (24)	100
TC	75.0% (18)	75.0% (18)	100	25.0% (6)	25.0% (6)	100

* $P = 0.04$; ** $P = 0.31$ for overall x^2 test of DD vs. Etest methods.

[1]CI (ciprofloxacin, 0.002-32 µg/ ml), CL (chloramphenicol, 0.016-256 µg/ ml), EM (erythromycin, 0.016-256 µg/ ml), GM (gentamicin, 0.016-256 µg/ ml), MP (meropenem, 0.002-32 µg/ ml) and TC (tetracycline, 0.016-256 µ/ ml).

[2] Insufficient number of results for analysis.