

## **Prevalence of Thermotolerant *Campylobacter* in Chicken Livers in Turkey and Antimicrobial Resistance among the *Campylobacter* Strain**

<sup>1</sup>Beytullah Kenar, <sup>2</sup>Levent Akkaya and <sup>3</sup>Yavuz Osman Birdane

<sup>1</sup>Department of Microbiology,

<sup>2</sup>Department of Food Hygen and Technology,

<sup>3</sup>Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine,  
Afyon Kocatepe University, Afyon, Turkey

**Abstract:** This study is carried out to make the comparison between the direct surface swap inoculation methods and deep tissue insertion methods after dipping the livers in alcohol. One hundred and eight campylobacter species have been isolated from 150 direct surface swabs (72%) but 30 *campylobacter species* have been isolated from 150 samples (20%) kept in 70% ethylalcohol. Eighty-six *campylobacter* out of 150 (57%) have been identified as *C. jejuni* and 22 out of 150 (14.6%) as *C. coli* from the direct swabbed samples. Similarly, 24 out of 150 (16%) *Campylobacter* have been identified as *C. jejuni* and 6 out of 150 (4%) as *C. coli* from the tissue samples kept in 70% ethylalcohol. Susceptibility percentages for gentamicin, erythromycin, amoxycillin ampicilline, tetracycline, streptomycin were 100, 95, 90, 88, 77 and 63, respectively. It has been determined that while *Campylobacter* was sensitive to most of the antibiotics, it was resistant to penicillin and trimethoprim-sulfamethoxazole. It was evaluated that the differences between direct surface swabs (108 positive) and tissue dipped in 70% ethylalcohol (30 positive) might have been resulted from cross contamination.

**Key words:** *Campylobacter* sp., incidence, isolation, identification, chicken liver, antibiotics resistance

### **INTRODUCTION**

*Campylobacters* have long been known and *Campylobacteriosis* is considered as a zoonosis today (Anonymous, 1995; Moore, 1999). *Campylobacter* infections are sometimes reported to come to an end spontaneously and sometimes reported to cause lengthy enteritis, bacteriemia, septic arthritis and other extra intestinal infections (Skirrow and Blaser, 2000).

*Campylobacters* are considered as one of the most important food borne zoonosis pathogens and they are reported to have increased their resistance recently in terms of sensitivity to antibiotics (Atabay and Corry, 1997; Ge *et al.*, 2002; Luber *et al.*, 2003). It was reported that thermophilic *Campylobacter* sp. have been isolated from chicken livers served for consumption in Turkey (Ertas *et al.*, 2004). The aim of this study is to determine the difference between direct swap inoculation method and inoculation method with alcohol treatment, to attempt to find out prevalence of *Campylobacter* in chicken livers sold by retail in the province of Afyonkarahisar and to determine susceptibility of identified *Campylobacter* sp. to some antibiotics.

It was reported that thermophilic *Campylobacter* sp. have been isolated from chicken livers served for consumption in Turkey (Ertas *et al.*, 2004).

The aim of this study is to determine the difference between direct swap inoculation method and inoculation method with alcohol treatment, to attempt to find out prevalence of *Campylobacter* in chicken livers sold by retail in the province of Afyonkarahisar and to determine susceptibility of identified *Campylobacter* sp. to some antibiotics.

### **MATERIALS AND METHODS**

Within the scope of this study, a total of 150 liver samples, 35, 35, 40 and 40 pieces in December, May, July and October of 2003, respectively, were collected from grocery stores located in the center of Afyonkarahisar. Inoculations of obtained liver samples were performed on Preston *Campylobacter* Selective Agar (CM 271 Oxoid; SR 117 Oxoid; 5% horse blood, SR 48 Oxoid) after the surfaces were swabbed. They were incubated under microaerophilic conditions (BR 39, Oxoid) for 48 h at 37 and 42°C. Then, livers were dipped in 70% alcohol for

1 min and placed on sterile filter paper. Liver capsulas were removed by means of sterile instruments after liver surfaces were dried by sterile filter paper (Barot *et al.*, 1983). Two-three gram of liver tissue was inoculated on Preston Enrichment Broth (CM 67 Oxoid; SR 117 Oxoid; 5% horse blood, SR 48 Oxoid) and incubated under microaerophilic conditions for 24-48 h at 42°C. Following inoculation of the same on Preston *Campylobacter* Selective Agar by means of sterile swabs, they were again incubated under microaerophilic conditions for 24-48 h at 42°C. *Campylobacter* species were identified through oxidase, catalase, H<sub>2</sub>S (paper tape), sodium hippurate, nalidixic acid (30 mcg disc<sup>-1</sup>), cephalotin (30 mcg disc<sup>-1</sup>) and TTC (400 mcg mL<sup>-1</sup>) tests (Koneman *et al.*, 1992; Lior, 1984).

One out of each identified *Campylobacter* colony was taken and inoculated into Trypton Soya Broth (CM 129 Oxoid) in order to obtain pure culture. Inoculations were performed by dipping in sterile cotton swab culture of McFarland 0.5 opacity and spreading over the surface of Mueller Hinton Agar (CM 337 Oxoid). *Campylobacter* strains isolated for antibiotic sensitivity test were tested against various types of antibiotics by disc diffusion method (Bauer *et al.*, 1966). Ampicilline (AMP, 10 µg), Penicillin G (P, 10 IU), Amoxycillin-Clavulanic acid (AMC, 20:10 µg), Tetracycline (TE, 30 µg), Erythromycin (E, 15 µg), gentamicin (CN, 10 µg), Streptomycin (S, 10 µg) and trimethoprim-sulfamethoxazole (SxT, 25 µg) antibiotic discs were used for this purpose.

## RESULTS

*Campylobacter* sp. were identified in 72% (108/150) of direct surface swabs obtained from livers. About 57.34% (86/150) of identified *Campylobacter*s were *C. jejuni* and 14.66% (22/150) were *C. coli*. 15% (24/150) of *Campylobacter* sp. identified during inoculation from livers whose capsulas were extracted after being dipped in 70% alcohol were *C. jejuni* and 4% were (6/150) *C. coli*.

Identified *Campylobacter* strains were 88.0% susceptible to ampicilline, 90.0% to amoxycillin, 77.0% to tetracycline, 95.0% to erythromycin, 100% to gentamicin, 63.0% to streptomycin and 95.0% resistant to penicillin and 97.0% resistant to trimethoprim-sulfamethoxazole.

## DISCUSSION

*Campylobacter* sp. were identified in 72% of liver samples taken by direct swab method and examined in this study and it was determined that 57.34% of identified *Campylobacter* sp. were *Campylobacter jejuni* and

14.66% were *Campylobacter coli*. The rate of *Campylobacter* sp. identified in inoculations performed from livers whose capsulas were extracted after being dipped in 70% alcohol was determined as 20%. About 16.00% of identified *Campylobacter* sp. were *Campylobacter jejuni* and 4.00% were *Campylobacter coli*.

Studies in relation to thermophilic *Campylobacter*s and chicken livers have been conducted in many countries (Atabay and Corry, 1997; Boukara *et al.*, 1991; Dominguez *et al.*, 2002; Fernandez and Pison, 1996; Yildiz and Diker, 1992). Diker (1987) reported that 56% of *Campylobacter* sp. was isolated from chicken livers. Boukara *et al.* (1991) reported that they performed 21.07% (9.9/47) isolation from lesional necrotic livers and 12% (6/50) isolation from non-lesional livers. Dominguez *et al.* (2002) reported they isolated 49.50% of thermophilic *Campylobacter* sp. in their study performed on chicken samples. Barot *et al.* (1983) identified *Campylobacter jejuni* in 48% of (56/117) 117 chicken livers collected from 15 different grocery stores in their study. They reported that 30.7% (36/117) of *Campylobacter jejuni* positive livers were identified by the help of surface inoculations, 15.3% (18/117) *Campylobacter jejuni* were identified both from surface and tissue of samples which were first kept in 70% alcohol and that 1.7% (2/117) were identified by tissue. They reported that contamination of these livers was due to unhygienic conditions and bare hand contact and that rate of contamination varied from one grocery store to another. Stoyanchev (2004) reported in a study conducted in slaughterhouses that *Campylobacter* sp. contaminated 53.3% of meat and giblets. Yildiz and Diker (1992) reported they identified 90% *Campylobacter* sp. from livers and giblets. In a study performed with broilers in Malaysia, Saleha (2002) reported 72.6% of identified *Campylobacter* sp. and also reported that dominant strain was *C. jejuni*. In a study on chicken organs (gizzard and livers) (Shih, 2000) identified 100% *Campylobacter* sp. in grocery stores and 60% in supermarkets and reported that less *Campylobacter* sp. were generated in supermarkets compared to retail stores. Fernandez and Pison (1996) reported they identified 92.9% (126/117) of *Campylobacter* sp. in chicken samples and that 78.6% (92/117) of identified strains were *Campylobacter coli* and 21.4% (25/117) were *C. jejuni*. Kramer *et al.* (2000) reported they identified 83.3% of *Campylobacter* sp. contamination in chicken meat and *C. jejuni* was the dominant strain with a rate of 77.3%. Properties of culture media used, microorganism load, ecological structure, antibiotic use are among the reasons for different findings of the above-mentioned researchers which may influence isolation rates.

When antibiotic susceptibility of identified strains in this study were examined, it was determined that strains were 100% susceptible to gentamicin, 95.0% to erythromycin, 90.0% to amoxycillin, 88.05 to ampicilline, 77.0% to tetracycline and 63.0% to streptomycin. On the other hand, they were 95.0% resistant to penicillin and 97.0% to trimethoprim-sulfamethoxazole.

In a research carried out in terms of antibiotic sensitivity, Sackey *et al.* (2001) reported that isolated *Campylobacter* sp. were resistant to ampicilline and tetracycline and sensitive to erythromycin, penicillin, amoxycillin and nalidixic acid. Hinrichsen and Gottschalk (2004) reported that *Campylobacter* strains isolated from liver samples were strongly resistant to penicillin, tetracycline, erythromycin and streptomycin and 10 *Campylobacter* strains were resistant, 13 were resistant at an intermediate level and approximately 16 were sensitive to trimethoprim. Lubet *et al.* (2003) tested 6 antibiotics on 430 *C. jejuni* and 79 *C. coli* strains in their studies. They reported that all isolates were sensitive to gentamicin and 6.1% resistant to erythromycin and they reported no resistance to ampicilline in any *C. coli* strain according to results of their study in 1991 and various rates of resistance as per species according to results of their study in 2001. Stoyanchev (2004) reported in a study performed in Bulgaria that it was 53.3% contaminated with *campylobacter* sp. Saleha (2002) reported that 76 *Campylobacter* sp. strains which were isolated were all resistant to tetracycline and 82.9, 33.2, 26.3 and 22.4% resistant to streptomycin, ampicilline, gentamicin and erythromycin, respectively. Ge *et al.* (2002) reported in their study which was performed by the Agar Dilution Method that the highest resistance was to tetracycline by 82.2%, then to nalidixic acid by 21.5% and to erythromycin by 17.0% and also reported that no isolated strain was resistant to erythromycin.

It was determined that *Campylobacter* sp. identified in this study were only resistant to penicillin and trimethoprim-sulfamethoxazole among antibiotics and sensitive to others at various rates. The results of study are compatible with findings of Hinrichsen and Gottschalk (2004) in terms of resistance to penicillin and trimethoprim-sulfamethoxazole; findings of Lubet *et al.* (2003) in terms of susceptibility to gentamicin, ampicilline and erythromycin; findings of Ge *et al.* (2002) in terms of susceptibility to gentamicin and 83.0% sensitivity to erythromycin; findings of Sackey *et al.* (2001) in terms of sensitivity to erythromycin, amoxycillin and nalidixic acid and findings of 10 in terms of resistance to tetracycline and erythromycin. However, extended use of the same antibiotic may be a reason for differential resistance in terms of common antibiotics used. The difference among antibiotic sensitivities of *Campylobacters* identified in

this study and antibiotic sensitivities determined in these studies (Ge *et al.*, 2002; Hinrichsen and Gottschalk, 2004; Lubet *et al.*, 2003; Sackey *et al.*, 2001; Saleha, 2002; Stoyanchev, 2004) may be due such reasons as extended use of the same antibiotic, infections related to different regional stereotypes and beta lactamase enzymes existing in some microorganisms.

## CONCLUSION

The studies carried out indicate that chicken livers available in the market have been contaminated by *Campylobacter* species at various rates. The difference between direct surface swab inoculations in chicken livers and tissue inoculations after dipping in 70% alcohol may be due to cross contamination. One should pay careful attention to hygienic conditions in order to minimize cross contamination and Hazard Analysis and Critical Control Points (HACCP) in production, processing and distribution processes should be improved in an aim to reduce the rate of cross contamination. Consumers should be warned not to consume undercooked or uncooked foods of animal origin, to use quality water, to ensure that instruments used in the kitchen and other places are not contaminated by microorganisms and to keep pets away from kitchens.

## REFERENCES

- Atabay, H.I. and J.E.L. Corry, 1997. The prevalence of *campylobacters* and *arcobacters* in broiler chickens. J. Applied Microbiol., 83: 619-626. DOI: 10.1046/j.1365-2672.1997.00277.x. PMID: 9418023.
- Barot, M.S., A.C. Mosenthal and V.D. Bokkenheuser, 1983. Location of *Campylobacter jejuni* in Infected Chickens Livers. J. Clin. Microbiol., 17 (5): 921-922. DOI: 0095-1137/83/050921-02\$02.00/0.
- Bauer, A.W., W.M.M. Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., 45 (4): 493-496. ISBN: 1-55581-169-8. PMID: 5325707.
- Boukara, L., S. Messier and Y. Robinson, 1991. Isolation of *Campylobacter* from livers of broiler chickens with and without necrotic hepatitis lesions. Avian Dis., 35 (4): 714-717. PMID: 1786003.
- Diker, S.K., 1987. Isolation of *Campylobacter* sp. from various animals and evaluation of zoonotic aspects. Microbiol. Bul., 21 (4): 268-273. PMID: 3503968.
- Dominguez, C., I. Gomez and J. Zumalacarregui, 2002. Prevalance of *Salmonella* and *Campylobacter* in retail chicken meat in Spain. Int. J. Food Microbiol., 72: 165-168. DOI: 10.1016/S0168-1605(01)00638-9. PMID: 11843408.

- Ertas, H.B., B. Çetinkaya, A. Muz and H. Öngör, 2004. Genotyping of broiler-originated *Campylobacter jejuni* and *Campylobacter coli* isolates using fla typing and random amplified polymorphic DNA methods. Int. J. Food Microbiol., 94 (2): 203-209. DOI: 10.1016/80168-1605(03)00312-X.
- Fernandez, H. and V. Pison, 1996. Isolation of thermotolerant species of *campylobacter* from commercial chicken livers. Int. J. Food Microbiol., 29 (1): 75-80. DOI: 10.1016/0168-1605(95)00023-2. PMID: 8722188.
- Ge, B., S. Bodies, R.D. Walker, D.G. White, S. Zaho, P.F. McDermott and J. Meng, 2002. Comparison of the Etest and agar dilution for *in vitro* Antimicrobial susceptibility testing of *Campylobacter*. J. Antimicrob. Chemother., 50: 487-94. DOI: 10.1093/jac/dkf162. PMID: 12356792.
- Hinrichsen, N. and J. Gottschalk, 2004. Microbiology Research of *Campylobacter jejuni* on poultry. <http://student.uaa.alaska.edu/ashnh1/body.html>.
- Anonymous, 1995. Institute of Food Science and Technology (IFST) current hot topics. Foodborne *Campylobacteriosis* and How to safeguard Against it, pp: 1-5. <http://www.ifst.org/hottop3.htm>.
- Koneman, E.W., S.D. Allen, W.R. Dowell and H.M. Sommers, 1992. Color Atlas and Text Book of Diagnostic. 4th Edn. In: Lippincott, J.D. (Ed.). Philadelphia. ISBN: 0397512015.
- Kramer, J.M., J.A. Frost and D.R. 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 (12): 1654-1659. PMID: 11131886.
- Lior, H., 1984. New extended biotyping scheme for *Campylobacter jejuni*, *Campylobacter coli* and *Campylobacter laridis*. J. Clin. Microbiol., 20 (4): 636-640. PMID: 6490850. PMCID: PMC271400.
- Luber, P., J. Wagner, H. Hahn and E. Bartelt, 2003. Antimicrobial resistance in *Campylobacter jejuni* and *Coli* strain isolated. In 1991 and 2001-2002 from poultry and human. Antimicrob. Agent Chemother. (AAC), 47 (12): 3825-3830. DOI: 10.1128/AAC.47.12.3825-3830.2003. PMID: 14638490. PMCID: PMC29-6193.
- Moore, J.E., 1999. *Campylobacter* enteritis in human: Sources of infection and modes transmission. The Soc. Food Hyg. Technol. Hygien Rev., pp: 1-5. [http://www.sofht.co.uk/isfht/irish\\_99\\_enteritis.htm](http://www.sofht.co.uk/isfht/irish_99_enteritis.htm).
- Sackey, B.A., P. Mensah, E. Collison and E.S. Dawson, 2001. *Campylobacter*, *Salmonella*, *Shigella* and *E. coli* in live and dressed poultry from metropolitan Accra. Int. J. Food. Microbiol., 71: 21-28. <http://cat.inist.fr/?aModele=afficheN&cpsidt=14110446>.
- Saleha, A.A., 2002. Isolation and characterization of *Campylobacter jejuni* from broiler chicken in Malaysia. Int. J. Poult. Sci., 1 (4): 94-97. <http://www.pjbs.org/ijps/fin12.pdf>.
- Shih, D.Y.C., 2000. Isolation and Identification of Enteropathogenic *Campylobacter* sp. from Chicken Samples in Taipei. J. Food Prot., 63 (3): 304-308. <http://www.ncbi.nlm.nih.gov/pubmed/10716556>. PMID: 10716556.
- Skirrow, M.B. and M.J. Blaser, 2000. Clinical aspects of *Campylobacter* infection. 2nd Edn. *Campylobacter*. In: Nachamkin, I. and M.J. Blaser (Eds.). ASM Pres, Washington DC, USA., pp: 69-88. ISBN: 1-65581-165-5.
- Stoyanchev, T.T., 2004. Detection Of *Campylobacter* Using Standard Culture and Pcr of 16s Rrna Gene in Freshly Chilled Poultry and Poultry Products in a Slaughterhouse. Turk. J. Sci., 2 (3): 59-64. [http://www.uni-sz.bg/tsj/Volume2\\_3/Stoyanchev,%20T..pdf](http://www.uni-sz.bg/tsj/Volume2_3/Stoyanchev,%20T..pdf).
- Yildiz, A. and S.K. Diker, 1992. *Campylobacter* contamination in chicken carcasses. Turk. J. Vet. Anim. Sci., 16: 433-439.