Molecular Study of *Campylobacter jejuni* Isolated from Chicken, Dairy Cattle and Human to Determine Their Zoonotic Importance

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Abstract

Campylobacter jejuni is still the main causes of bacterial gastroenteritis worldwide. This work was done to investigate the fingerprinting of *Campylobacter jejuni* isolated from chicken, dairy cattle and human. Fecal samples were collected from 100 diarrheic chicken and dairy cattle (50 of each) as well as 50 stool samples from patients with diarrhea were subjected to standard isolation and identification of *Campylobacter jejuni.* DNA of isolates was amplified using specific primer of hippuricase gene. The prevalence of *Campylobacter jejuni* was 18(36%) chicken, 16 (32%) dairy cattle and 11(22%) patients with diarrhea. PCR analysis produced identical bands at 344 bp in all isolates, indicating the role of chicken and dairy cattle in human *Campylobacter* infection.

Introduction

Campylobacter jejuni is responsible for 99% of all cases of Campylobacteriosis (Kowalski, 2000). In developing countries, Campylobacteriosis is primarily a disease that occur among infancy, because of high levels of early exposure and acquired immunity (Oberhelman and Taylor, 2000) but in industrialized countries the epidemiology is characterized by population at all ages (Olson et al.2008) .The incubation period is about 2-4 days and clinical syndrome include diarrhea, fever, abdominal cramps and septic arthritis (Peterson, 1994). Under standing of their epidemiology is complicated by the sporadic nature of the disease, lack of representative population sampling (Uzunovic –Kamberovic, 2003) wide distribution in the environment (Hanninen et al .2000) and a high level of genetic

diversity (Dingle el al. 2002). Most outbreaks of *C.jejuni* attributed to either the consumption of raw, unpasteurized milk or contaminated water (Schidt et al. 2006). However, sporadic cases are mainly attributed to handling or consumption of under cooked poultry(Olson et al .2008) .The estimated annual campylobacter infection is 2.5 million cases in the United States and >340,000 cases in the United Kingdom (Allos , 2001 ,Kessel et al .2001) while the annual economic burden is $8 billion in the United States(Busby and Roberts , 1997) and E 500 million in the United Kingdom (Humphrey et al .2007) . The identification of *C.jejuni* based on hippurate test (Uzunovic –Kamberovic, 2001) .Hippurate hydrolysis is time consuming and sometimes difficult to interpret when the enzymatic activity is impaired under the methodological condition (Rautelin et al .1999). Therefore, different molecular strategies and genetic targets have been applied for the identification of *C.jejuni.* Examples of these include PCR by using specific primer of hippuricase (hipO) gene (Persson and Olsen ,2005) , *Campylobacter* genotyping (Sheppard et al .2009) and multilocous sequence types of *C.jejuni* isolates (de Haan et al . 2010) . The aim of this study was to determine the genetic characters specific for *C.jejuni* isolated from chicken and dairy cattle to identify the role of these animals as source of human infection.

Materials and Methods

Sampling:

One hundred animal samples were collected including fecal dropping from (50) chicken and feces (50) dairy cattle suffer from diarrhea in different farms in Toukh, Kaliobia governorate. As well as stool samples were collected from (50) patients with diarrhea inhabitant from rural area of Toukh and admitted in Toukh hospital. All samples were aseptically placed in separate sterile plastic bags and were immediately transported to the laboratory in a cooler with ice packs and processed immediately upon arrival for isolation of *Campylobacter.*

Isolation and identification of *C.jejuni:*

About 10 gm of each sample were homogenized in sterile thioglycolate broth and incubated at 42 oC For 48 hrs under microaerobic condition (5% O2, 10% CO2 and 85% N2) (Gebhart et al. 1985). A loopful of enrichment broth were plated on modified charcool cefoperazone deoxycholate agar (MCCDA) (Oxoid) and incubated in microaerophilic atmosphere at 42 oC/48 hrs (Persson and Olsen, 2005). Suspected colonies of *Campylobacter* were identified under phase contrast microscope for detection of characteristic motility and morphological character according to (Smibert, 1984). *Campylobacter* isolates were subcultured and identified by biochemical tests described by (Frost et al .1998) including growth at 25oC, at 37oC and at 43oC, growth in presence of 3.5% NaCl and 1% glycine ,motility, catalase, oxidase, H_2S production on triple sugar iron agar (TSI) agar ,sodium hippurate hydrolysis and susceptibility to nalidixic acid and cephalothin. Identified colonies were stored at -70 in nutrient broth with 15% glycerol until their use (Sheppard et al .2009).

Isolation of DNA:

DNA was prepared for PCR from 8 min. boiling colonies by using the Chelex Resin method (Bio-Rad) according to the manufacturer's instruction. The crude DNA preparation was stored at 4 oC until used.

DNA amplification reaction:

PCR reaction contained 5ul template DNA was Performed in a total reaction volume of 25 UL containing PCR buffer [50 mM Tris / HCL, 10 mM KCL , 5mM (NH4)**2** SO4, PH8.3], 2.6mM MgCL2, 260 uM dATP, dGTP and dCTP, 520 uM dUTP, 0.15 UUNG, 1.25 U Taq Polymerase, 0.2 uM hipO primers (Persson and Olsen, 2005) ,hipO – F (5` -GACT TCGT GCAG ATAT GGAT GCTT) and hipO-R(5`-GCTA TAAC TATC CGAA GAAG CCATCA) . Thermocycler conditions were 94 oC for 6 min, followed by 35 cycles of 94 oC for 50 s, 57 oC for 40 s and 72 oC for 50 s and finally 72 oC for 3 min. PCR product were analyzed in 1.5 % agarose gel electrophoresis under standard conditions and stained by ethidium bromide. The data analyzed by using Gelpro analyzer V4

Results

Table (1) Biochemical characters of suspected isolates of *C.jejuni*

Biochemical character	Results
Growth - at 25oC - at 37oC - at 43oC	 - + +
Growth in: - 3.5% NaCl - 1% Glycine	 - +
Motility	+
Catalase	+
Oxidase	+
H2S production on TSI agar	-
Sodium hippurate hydrolysis	+
Suscepibility to Nalidixic acid Cephalothin	 S R

Positive (+) Sensitive (S)
Negative (-) Resistance (R)

Table (2) the prevalence of *C.jejuni* isolated from chicken, dairy cattle and human

Samples	Number	Positive Samples	
		No	%
1- chickens	50	18	36
2-dairy cattle	50	16	32
3-Patients with diarrhea	50	11	22
Total	150	45	30

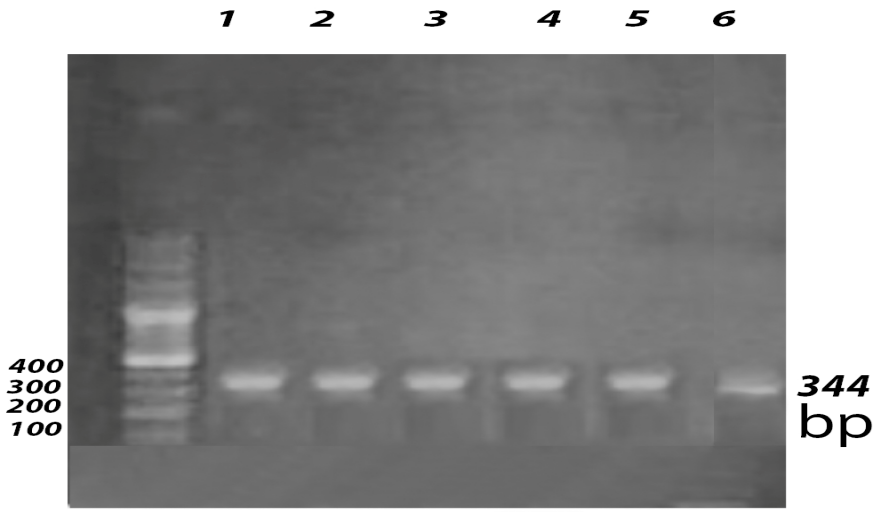

Figure (1): PCR amplification products of *C.jejuni* isolates. Lane M: a 100bp molecular size marker .Lane 1, 2, 344 bp of *C.jejuni* isolated from chicken, Lane3, 4 from dairy cattle and Lane 5, 6 from patient with diarrhea.

A total of 100 fecal sample were obtained from diarrheic chicken and dairy cattle from different farms in Toukh , Kaliobia governorate in addition to 50 stool samples from patient with diarrhea inhabitant from rural area of Toukh and admitted to hospital .Samples were tested for *C.jejuni* using traditional bacteriological and biochemical methods , table(1) . Table (2) declared the prevalence of *C.jejuni* in the collected samples was 45 (30%) , 18 (36%) in chicken , 16(32%) dairy cattle and 11(22%) in patient with diarrhea .The result of PCR amplification of hipO gene of *C.jejuni* isolates from chicken and dairy cattle have shown identical fingerprints with human *C.jejuni* at 344bp , figure(1) .

Discussion

Campylobacter is known worldwide as a common cause of human bacterial diarrhea; however, it is commensal in the gastrointestinal tract of many domestic and wild animals, especially birds (Takamiya et. al. 2011). In this work the prevalence of *C.jejuni* depends on bacteriological and biochemical characters, was found to be 18(36%) chicken 16(32%) dairy cattle and 11(22%) human with diarrhea. Our findings were higher than *C.jejuni* isolated from 7(30%) of 23 farm chicken cloacal samples and lower than 51(68%) of 75 stool samples of patient with diarrhea in Bosnia and Herzegovina (Uzunovic – Kamberovic et al .2007) and higher than those investigated in 5(10%) fecal dropping broiler chicken and 8(16.66%) person in contact with animals.While slaughtered cattle were negative for *Campylobacter* in Giza, Egypt (Hassanain, 2011). While our results were lower than *C.jejuni* isolated from 16(44.44%) slaughtered cattle and 56(56%) patient in Egypt (Abdel-Aziz, 2006), and lower than those isolated from (49.6%) of intestinal content of broiler chicken in KawaZulu – Natal, South Africa (Bester and Essack, 2008). This may be attributed to the different in method of sampling, procedure and locality. Mishandling of raw poultry and consumption of under cooked poultry are the major risk factors for human campylobacteriosis (Altekruse et al .1999). In the present work, all isolates were biochemically identified as *Campylobacter jejuni* in chicken. This encourage (Bester and Essack, 2008) who reported that *Campylobacter jejuni* responsible for 90.8% of *Campylobacter* infection in chicken. All 16 isolates from dairy cattle were positive to biochemical characters of *Campylobacter jejuni*. This agree with (Wesley et.al.2000) who recorded that the prevalence of *Campylobacter jejuni* infection in dairy cattle is high.11 isolates from human were biochemically identified as *Campylobacter jejuni* . These enforce (HassanZadeha and Motamedifar, 2007) who found that all *Campylobacter* recovered from human stool were *Campylobacter jejuni*. Approximately 200 persons with *Campylobacter* infection may die each year in the United States

(Tauxe, 1992) .The majority of outbreaks of campylobacteriosis have been associated with consumption of raw pasteurized cow's milk (Hudson et al. 1990). While in Egypt, acute diarrhea caused by *Campylobacter* is responsible for more than 50% of deaths for children under two years of age (Ewyda, 1990). *C.jejuni* has been isolated from raw milk and milk products in Assiut, Egypt (Saad et al. 2007).

Molecular typing has enhanced many epidemiological studies including the identification of infection due to *Campylobacter* (Sails et al.2003) and determine the origin of *Campylobacter* isolates obtained from patients on the basis of their genotypes, because there is sufficient genetic variation within the bacterial population to define host or source associated genotypes (McCarthy et al . 2007) . In our study PCR amplification of *C.jejuni* isolated from chicken and dairy cattle shown identical fingerprints with human *C.jejuni* isolates, these diagnostic DNA bands of *C.jejuni* based on hippuricase gene amplified at 344bp in accordance with (Persson and Olsen, 2005). Our results agree with (de Haan et al 2010) who found *C.jejuni* in poultry and bovine in Finland and reported that poultry and bovine are equally important reservoirs for human *C.jejuni* infection. Our findings differ from those obtained in Scotland and identified poultry as the most important source of human infection (McCarthy et al. 2007). It is worth mention that previously identical *C.jejuni* isolates PFGE genotypes were found only in two cases of human and poultry meat isolates and two cases of poultry meat and farm chicken isolates in Bosnia and Herzegovina, Slovenia (Uzunovic – Kamberovic et al .2007). We can conclude that chicken and dairy cattle are possible sources of human C.jejuni infection. Efforts to prevent human illness are needed throughout each link in the food chain (Altekruse et al .1999). Prevention of infection through reduction of infection on the animal farms, changes of slaughtering procedures and increased public education and awareness could decrease the prevalence of infection.

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Reference

1- Abdel – Aziz, A.M. (2006) *Campylobacter jejun*i and its public health importance in Kaliobia province. Suez Canal Vet.Med.J.X (1): 275-282.

2- Allos, B.M. (2001) *Campylobacter jejun*i infection update on emerging issues and trends. Clin. Infect. Dis. 32:1201-1206.

3- Altekruse, S.; Stern, N. and Fields, I. (1999) *Campylobacter jejun*i –an emerging food borne pathogen. Emerg. Infect .Dis. 5 (1): 1-13.

4- **Bester, A.L.and Essack, Y.S (2008)** Prevalence of antibiotic resistance in *Campylobacter* isolates from commercial poultry supplies in KwaZulu –Natal. South Africa J.Antimicro. Chemother .62:1298-1300.

5- **Busby, J.C.and Roberts, T. (1997).** Economic costs and trade impacts of microbial food borne illness. World Health Stat Q 50: 57- 66.

6- de Haan, C.P.; Kivisto, R.;Hakkinen ,M.et.al.(2010) Multilocus sequence types of Finnish bovine *Campylobacter jejun*i isolates and their attribution to human infections .B.M.C. Microbiol.10:200-209 .

7- Dingle, **K.E., Colles, F.M.Ure, R. et.al. (2002)** Molecular characterization of *Campylobacter jejun*i clones: a rational basis for epidemiological investigation. Emerg. Infect.Dis .8:949-955.

8- Ewyda .E.H (1990) Prevalence of *Campylobacter* spp.in patient; with diarrhea in Assiut. M.V.Sc.Thesis. Fac. Med. Assiut Univ., Egypt.

9- Frost, J.; Oza,N. Thawaites , R.and Rowe ,B.(1998) Serotyping Scheme for *Campylobacter jejun*i and *Campylobacter coli* based on direct agglutination of heat stable antigen. J. Clin .Microbiol. 36: 336-339.

10-Gebbhart, C., Edmonds, P., word, G.; Kurtz, H, and Bernner, D. (1985) *Campylobacter* hyointestinalis spp: A new species of *Campylobacter* found in the intestine of pigs and other animals. J. Clin. Microbiol .21:715-720

11- Hanninen, M.;Perko P. and Rautelin ,H. (2000) A three – year study of *Campylobacter jejun*i genotypes in humans with domestically acquired infections and in chicken samples from the Helsinki area. J. Clin .Microbiol .38:1998 -2000.

12-Hassanain, N.A. (2011) Antimicrobial Resistant *Campylobacter jejun*i isolated from humans and animals in Egypt. Global Veterinaria 6(2):195-200.

13-**HassanZadeha, P. and Motamedifar, M. (2007)** Occurrence of Campylobacter *jejun*i in Shiraz, Southwest Iran.Med .Prin . Pract. 16:56 -62.

14- **Hudson S.J.;Sobo,A.O;Russel, K.and ligtfoot,N.F. (1990).** Jackdaws as potential source of milk – borne *Campylobacter jejun*i infection .Lancet (Brit.Ed.) 335:8698.

15-**Humphrey, T.; O,Brien,S.and Madsen,M. (2007).** *Campylobacter* as zoonotic pathogens: A food production perspective. Int. J.Food Microbiol.117:237-257.

16-**Kessel, A.S.; Gillespie, J.A.;Humphrey,T. and Ward,L. (2001)** General outbreaks of infectious intestinal disease linked with poultry, England and Wales,1992-1999. Commun. Dis. Public Health, 4:171-177

17-**Kowalski, A. (2000)** *Campylobacter jejun*i.WWW. *Campylobacter jejun*i .Com.

18-McCarthy, N.D.; Colles, F. M.,Dingle,K.E.;et.al.(2007) Host- associated genetic import in *Campylobacter jejun*i. Emerg.Infect.Dis.13:267-272.

19-**Oberhelman, R.A. and Taylor,D.N. (2000)** *Campylobacter* infection in developing Countries .In **Nachamkin,I. Blaser ,M.J.**eds. *Campylobacter*.2nd ed.Washington D.C. A.S.M.Press:139-153.

20- Olson, C.K.; Ethelberg, S .and Tauxe, R.V. (2008) Epidemiology of *Campylobacter jejun*i infection in industrialized nations. In **Nachamkin,I. Blaser ,M.J**.eds. *Campylobacter*.2nd ed.Washington D.C. A.S.M.Press:139-153.

21- Person, S. and Olsen, K. (2005) Multiplex PCR for identification of *Campylobacter coli* and *Campylobacter jejun*i from pure cultures and directly on stool samples .J.Med .Microbiol .54:1043-1037.

22- Peterson, M.C. (1994) Clinical aspects of *Campylobacter jejun*i infections in adults. Wes. J .Med .161:148-152.

23 – Rautelin, H., Jusufovic, J. And Hanninen, M. (1999) identification of hippurate _ negative thermophilic *Campylobacter* .Diagn. Microbial. Infect.Dis. 35:9-12.
24- Saad, N.; Ahmed, A.; Abdel _ Haleem, A. and Nassife, T. (2007) Incidence of *Campylobacter* species in milk and some milk products. Assiut Vet. Med. J. 33(114): 106 – 122.
25-Sails, A.D.; Swaminathan, B. and Field,P.I.(2003) Utility of multilocus sequence typing as an epidemiological tool for investigation of outbreaks of gastroenteritis caused by *Campylobacter jejuni* .J.Clin. Microbiol.41:4733-4739.
26-Schidt, M.; Savolainen, S. and Hanninen,M.(2006) Long-lasting *Campylobacter jejuni* contamination of milk associated with gastrointestinal illness in a farming family.Epidemiol. Infect.134:401-405.
27-Sheppard, S.K.; Dallas, J.F.et.al. (2009) *Campylobacter* genotyping to determine the source of human infection.Clin.Infect.Dis.48:1072-1078.
28-Smibert, R.M. (1984) Genus *Campylobacter*.In Bergey,s Manual of Systemic Bacteriology.Ed. By **Krieg, N. and Holt**, I.9th ed.Williams and Wilkins,Baltimore pp:111-117.
29-**Takamiya,M.,Ozen,A.;Alter,T.et.al.(2011)**Genome sequence of *Campylobacter jejuni* strain 327,a strain isolated from a turkey slaughterhouse . Standards in Genomic Science, 4:113-122.
30-Tauxe, R.V. **(1992)** Epidemiology of *Campylobacter jejuni* infection in the United States and other industrialized nation. In *Campylobacter Jejuni:* Current Status and Future Trends. Am.Soc.Microbiol.Chap.2, pp: 1.
31-**Wesley, I.S.; Wells, K.; Harmon, A.: Green, L.; Schroeder-Tucker,M.and Siddique,I.(2000)**Fecal shedding of *Campylobacter* and *Arcobacter spp* in dairy cattle.Appl.Environ.Microbiol.66:19944-2000.
32-**Uzunovic-Kamberovic.S. (2001)** Changes in *Campylobacter jejuni* and *Campylobacter coli* carriage rates in the Zenica Canton of Bosnia and Herzegovina in the pre- and post war periods.J.Clin.Microbiol.39:2036-2040.
33- **Uzunovic-Kamberovic.S. (2003)** Some epidemiologic features of *Campylobacter jejuni* and *Campylobacter coli* infection in Bosnia and Herzegovina after the war .Clin .Micobiol .Infect.9:458-460.
34- **Uzunovic-Kamberovic.S.**;**Zorman,T.and Mozina,S (2007)** Role of poultry meat in sporadic *Campylobacter* infection in Bosnia

and Herzegovina :Laboratory-based study. Croat. Med.J.48 (8):842-851.