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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 one of 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 chickens 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 primers of hippuricase gene. The prevalence of *Campylobacter jejuni* was 18(36%) in chicken, 16 (32%) in dairy cattle and 11(22%) in 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.

Key words: Campylobacter *Jejune* · Chicken · Dairy Cattle · Human And PCR

INTRODUCTION

Campylobacter jejuni is responsible for 99% of all cases of Campylobacteriosis [1]. In developing countries, Campylobacteriosis is primarily a disease that occurs among infancy, because of high levels of early exposure and acquired immunity [2] but in industrialized countries the epidemiology is characterized by population at all ages [3].The incubation period is about 2-4 days and clinical syndrome include diarrhea, fever, abdominal cramps and septic arthritis [4]. Under standing of their epidemiology is complicated by the sporadic nature of the disease, lack of population sampling [5], wide distribution in the environment [6] and a high level of genetic diversity [7]. Most outbreaks of *C. jejuni* are attributed to either the consumption of raw, unpasteurized milk or contaminated water [8]. However, sporadic cases are mainly attributed to handling or consumption of under cooked poultry [3]. The estimated annual campylobacter infection is 2.5 million cases in the United States and >340,000 cases in the United Kingdom [9, 10] while the annual economic burden is $8 billion in the United States [11] and E 500 million in the United Kingdom [12].

The identification of *C. jejuni* is based on hippurate test [13]. Hippurate hydrolysis is time consuming and sometimes difficult to interpret when the enzymatic activity is impaired under the methodological condition [14]. 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 [15], *Campylobacter* genotyping [16] and multi locous sequence typinges of *C. jejuni* isolates [17]. 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 droppings from (50) chicken and feces (50) from dairy cattle suffering from diarrhea in different farms in Toukh, Kaliobia governorate. As well as stool samples collected from (50) patients with diarrhea inhabiting from rural area of Toukh and were admitted in Toukh hospital.

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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 °C For 48 hrs under micro aerobic condition (5% O2, 10% CO2 and 85% N2) [18]. A loopful of enrichment broth were plated on modified charcool cefoperazone deosoxycholate agar (MCCDA) (Oxoid) and incubated in microaerophilic atmosphere at 42°C /48 hrs [15]. Suspected colonies of *Campylobacter* were identified under phase contrast microscope for detection of characteristic motility and morphological character according to Smibert [19]. *Campylobacter* isolates were sub cultured and identified by biochemical tests described by Frost *et al.* [20] including growth at 25°C, at 37°C and at 43°C, growth in presence of 3.5% NaCl and 1% glycine, motility, catalase, oxidase, H2S 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 and Dallas [16].

Isolation of DNA: DNA was prepared for PCR from 8 min. boiling of identified colonies by using the Chelex Resin method (Bio-Rad) according to the manufacturer's instruction. The crude DNA preparation was stored at 4°C 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, pPH 8.3], 2.6mM MgCL2, 260 uM dATP, dGTP and dCTP, 520 uM dUTP, 0.15 UUNG, 1.25 U Taq Polymerase and 0.2 uM hipO primers [15]-F (5`-GACT TCGT GCAG ATAT GGAT GCTT) and hipO-R(5`-GCTA TAAC TATC CGAA GAAG CCATCA)]. Thermocycler conditions were 94 - for 6 min, followed by 35 cycles of 94 - for 50 s, 57 - for 40 s and 72 - for 50 s and finally 72 - for 3 min. PCR product were analyzed in 1.5 % agarose gel electrophoresis under standard conditions and stained by ethidium bromide. The data were analyzed by using Gelpro analyzer V4.

RESULTS

Table (1) shows the prevalence of *C. jejuni* in the collected samples was 45 (30%), 18 (36%) in chicken, 16(32%) dairy cattle and 11(22%) in patients 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).

Table 1: Biochemical characters of suspected isolates of *C.jejuni*

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

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

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

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

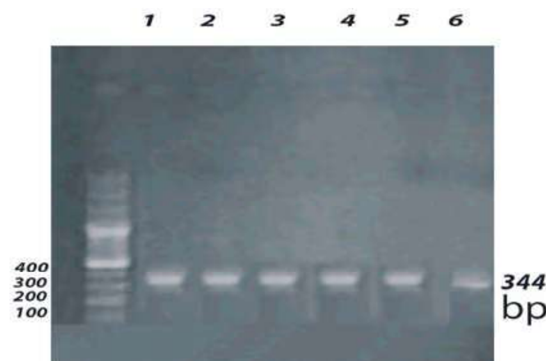

Fig. 1: PCR amplification products of *C. jejuni* isolates. Lane M: a100bp molecular size marker. Lanes 1 and, 2,344 bp of positive samples for *C. jejuni* isolated from chicken, lanes 3 and 4, positive samples from dairy cattle and lane 5 and 6 positive samples from patient with diarrhea.

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 [21].

In this work the prevalence of *C. jejuni* depend on bacteriological and biochemical characters, was found to be 18(36%) in chicken, 16(32%) in dairy cattle and 11(22%) in human with diarrhea. Our findings were higher than *C. jejuni* isolated from farm chicken cloacal samples 7 (30%)of 23 and lower than stool samples of patients with diarrhea 51 (68%)of 75, in Bosnia and Herzegovina [22] and higher than those investigated in fecal dropping broiler chicken 5(10%)of 50, and persons in contact with animals 8(16.66%)of 50,While slaughtered cattle were negative for *Campylobacter* in Giza, Egypt [23]. While results are lower than *C. jejuni* isolated from 16(44.44%) slaughtered cattle and 56(56%) patients in Egypt [24] and lower than those isolated from (49.6%) of intestinal contents of broiler chicken in KawaZulu-Natal, South Africa [25]. This may be attributed to the different in methods of sampling, procedures and locality. Mishandling of raw poultry and consumption of under cooked poultry are the major risk factors for human campylobacteriosis [26].

In the present work, all isolates were biochemically identified as *Campylobacter jejuni* in chicken. This encourage [25] who reported that *Campylobacter jejuni* is responsible for 90.8% of *Campylobacter* infection in chicken. All 16 isolates from dairy cattle were positive to biochemical characters of *Campylobacter jejuni*. This agrees with Wesley *et al.* [27] who recorded that the prevalence of *Campylobacter jejuni* infection in dairy cattle is high. 11 Eleven isolates from human were biochemically identified as *Campylobacter jejuni*. These enforce Mention authors' names??[28] 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 [29]. The majority of outbreaks of campylobacteriosis have been associated with consumption of raw pasteurized cow's milk [30]. While in Egypt, acute diarrhea caused by *Campylobacter* is responsible for more than 50% of deaths for children under two years of age [31]. *C. jejuni* has been isolated from raw milk and milk products in Assiut, Egypt [32]

Molecular typing has enhanced many epidemiological studies including identification of infection due to *Campylobacter* [33] and determination of 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 [34].

In our study PCR amplification of *C. jejuni* isolated from chicken and dairy cattle shown identical fingerprints with human *C. jejuni* isolates, thisese diagnostic DNA bands of *C. jejuni* based on hippuricase gene amplified at 344bp is in accordance with Person and Olsen [15]. Our results agree with Person and Olsen [15] 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 [34]. 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 [22]. 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 [26]. 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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