Public health and molecular characterization of Campylobacteriosis

Lobna, M.A. Salem[1], Nashwa, O.Kh[1], Barakat,A.M.A[2] and Mona, S.A.[1]

1. Zoonoses Department, Faculty of veterinary Medicine, Benha University

2. Zoonoses Department, National research centre Dokki, 12622, Cairo, Egypt.

A B S T R A C T

The aim of this work is to investigate the presence of Campylobacter species. in chicken, cattle, some animal products (raw milk, kariesh cheese and yoghurt) in addition to human beings and to confirm its presence by molecular methods. 315 samples were collected from chicken, 150 samples from cattle carcasses, 122 samples from animal products and 128 human stool swabs (hospitalized patients suffering from diarrhea admitted to Toukh Central Hospital). All samples were collected from Toukh city, Kalyoubia governorate, Egypt. Campylobacter spp. were isolated from chicken samples at percentage of 27.6% from intestinal contents swabs , 27.14% from liver samples, 7.14% from breast muscles and 14.2% from thigh muscles. In cattle carcasses Campylobacter spp. were isolated with a percentage of 6% from intestinal contents swabs, 6% from liver samples and muscles (2%).Moreover the isolation rate of Campylobacter spp. from animal product samples was7.4%.11.53% from raw milk, 7.5% from kariesh cheese and 0% from yoghurt. In human Campylobacter spp. were isolated from 26 stool swabs (20.3%). Suspected strains were selected according to their biochemical testing and subjected to molecular investigations by using specific primers (mapA gene specific for C.jejuni & ceuE gene specific for C.coli) . Amplification of mapA gene of C.jejuni & ceuE gene for C.coli isolated from the above mentioned samples have shown identical fingerprints with human isolates at 589 and 462bp for C.jejuni and C.coli respectively ensuring the public health importance of the isolates. From the results of the current study, it could be concluded that Chicken, cattle and animal products are possible sources of human Campylobacter infections.

Key words: Campylobacteriosis , Public health , PCR.

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1. INTRODUCTION

Campylobacter spp. are zoonotic pathogens that are frequently isolated from a variety of animal species such as poultry, cattle, pigs, sheep, pets, wild birds and rodents (Meerburg et al., 2006).

Campylobacter spp. are important zoonotic infection of significant health hazard due to the relatively low infectious dose, the potentially serious sequelae also the association between certain Campylobacter virulence gene and the pattern of clinical infection . (Al-Mahmeed et al., 2006) .

Campylobacter spp.are common bacterial pathogens that cause gastro enteritis in humans, both in industrialized and developing countries (Coker et al., 2002). Human campylobacter infection may be due to either consumption of under cooked meat or cross-contamination of ready – to – eat food during preparation or storage (Wieczorek et al., 2012). The present study aimed to isolation and identification Campylobacter spp. in chickens, animals, animal byproducts in addition to human beings, and the use of molecular methods for confirmation of suspected Campylobacter spp.

2. MATERIALS AND METHODS

2.1- Collection of samples:

Chicken samples were taken from the intestinal contents, liver and from muscles. The samples were collected from poultry shops at Toukh city. Cattle carcasses samples were taken from 50 freshly slaughtered cattle carcasses at Toukh abattoir, .The samples were 50 swabs from intestinal content which were taken directly during evisceration, in addition to (50) liver and (50) muscles samples. Also, animal product samples (milk samples, kariesh cheese and yoghurt) were collected from different stores in the same locality. As well as, human stool swabs were collected from persons suffering from diarrhea and admitted to the governomental hospitals in the same governorate .All samples were collected in thioglycolate broth and transferred to the lab in ice box at a temperature of 4°C, for bacteriological examination.

2.2- Bacteriological examination:

Ten grams of each sample was homogenized in sterile thioglycolate broth. Broth samples were incubated at 42°C for 48 hours.Under microaerobic condition (5% O2, 10%CO2 and 85%N2). A loopful of enrichment broth were plated on semisolid thioglycolate broth (Oxoid) and incubated in microaerophilic atmosphere at 25, 37 and 42°C for 48 to 72 hours in accordance with Gebhart et al., 1985.The suspected colonies of Campylobacter spp. were identified under phase contrast microscope using (1000×) magnification power to detect their charachteristic motility and morphological charachters (Smibert, 1984). Campylobacter isolates were subcultured for purification then biochemical identification (Frost et al., 1998).

2.3- Molecular confirmation

The identified colonies were stored at 70°c in thioglycolate broth with 15% glycerol for further molecular confirmation (Sheppard et al., 2009).

Extraction of DNA: it is applied according to QIAamp DNA mini kit instructions.

Multiplex PCR: PCR reaction contained 5 µl template DNA was performed in a total reaction volume of 25 µLcontaining PCR buffer [50 mM Tris / HCL, 10 mM KCL, 5 mM (NH4)2SO4, pH 8.3], 2.6 mM MgCL2, 260 µM dATP, dGTP and dCTP, 520 µM dUTP, 0.15 U UNG, 1.25 U Taq Polymerase, 0.2 µM mapA primers (mapA gene for C. jejuni) , mapA - F (5`- CTA TTT TAT TTT TGA GTG CTT GTG ) a n d mapA- R ( 5 ` - GCT TTA TTT GCC ATT TGT TTT ATT A) giving a 589 bp product, 0.4 uM ceuE-primers (ceuEgene for C. coli) ceuE-F (5`- AAT TGA AAA TTG CTC CAA CTA TG - 3`) and ceuE-R(5`- TGA TTT TAT TAT TTG TAG CAG CG -3`) giving a 642 bp product (Eunju and Lee ,2009).Thermocycler conditions were 94°C for 6 min, followed by 35 cycles of 94°C for 50 s, 57°C for 40 s and 72°C for 50 s and finally 72°C for 3 min. PCR product were analyzed in 1.5 % agarose gel electrophoresis under standard conditions and stained by ethidium bromide.

3. RESULTS

3.1- Occurrence of different Campylobacter spp. in the examined chicken samples:

In the current study, Campylobacter spp. were isolated from intestinal contents, liver, breast muscles and thigh muscles of the examined chicken samples with a percentage of 27.6%, 27.14%, 7.14% and 14.2% respectively (Table 1).

The isolation rate of Campylobacter spp. from the intestinal contents samples was 27.6%. Out of them 55.17% were identified as C.jejuni while 41.37% were identified as C.coli and 3.44% as C.lari. The isolation rates of C.jejuni and C.coli from liver, breast and thigh muscles were 68.4%, 100%, and 50% for C.jejuni and 31.57%, 0% and 40% for C.coli respectively. C.lari isolated from thigh muscles at percentage of 10%.

3.2- Occurrence of different Campylobacter species in the examined cattle carcasses samples:

In the current study, Campylobacter spp. isolated from cattle carcasses from intestinal contents, liver and muscle samples with rate of 6%, 6% and 2% respectively. All isolates were identified as C.jejuni except one isolate was identified as C.coli from intestinal contents sample as shown in table (2).

3.3-Occurrence of different Campylobacter species in the examined animal products samples

Also in this investigation we studied the occurrence of Campylobacter spp. in animal products such as raw milk, kariesh cheese and yoghurt. Our finding in table (3) showed that the isolation rate of Campylobacter spp. from raw milk and kariesh cheese and yoghurt was 11.53%, 7.5 and 0% respectively. C.jejuni and C.coli isolated from the above mentioned products at percentage of 44.4% and 55.6% respectively.

3.4-Risk factors of Campylobacter species in the examined human samples:-

In the current study the occurrence of Campylobacteriosis in the examined human stool swabs was 20.3% (table 4). Also the results revealed that C.jejuni and C.coli isolated at percentages of 84.6% and 15.3% respectively.

Our result revealed that Campylobacter spp. isolated from young ages at higher percentages than older ages. When age increase,the isolation rate decrease as shown in table (4). Regarding to gender of the examined humans, it was found that the isolation rates of Campylobacter spp., in males (22.2%) was higher than that of females (17.0. 2%). Also the results revealed that C.jejuni and C.coli isolated at percentages of 88.88% &11.11% in males and 75% &25% in females respectively. In respect to residence, this study showed that the prevalence of Campylobacteriosis was higher in rural areas (20.08%) than in urban areas (12. 5%). The results showed that C.jejuni and C.coli isolated at percentages of 92%& 8%in rural areas and 0% &100% in urban areas respectively. In all the examined patients there was abdomenal pain and diarrhea with history of poultry consumption.

3.5- PCR amplification of C. coli CeuE gene & C. jejuni mapA gene:-

The results showed that out of 12 biochemically suspected Campylobacter spp. isolates , by PCR , 11 isolates were confirmed as C.jejuni and 1 isolates as C.coli . Campylobacter jejuni isolates produced at 589 bps, while C.coli isolates produced at 462 bps (photograph 1).

Table (1): Occurrence of different *Campylobacter spp*. in the examined chicken samples

Type of samples	Number of examined samples	Positive *Campylobacter spp.**	Campylobacter isolates		
			*C.jejuni**	*C.coli**	*C.lari(NS)*
Intestinal contents	105	29 (27.6%)	16 (55.17%)	12 (41.37%)	1 (3.44%
Liver	70	19 (27.14%)	13 (68.42%)	6 (31.57%)	-
Breast muscles	70	5 (7.14%)	5 (100%)	-	-
Thigh muscles	70	10 (14.2%)	5 (50%)	4 (40%)	1 (10%)
Total	315	63 (20%)	39 (61.90%)	22 (34.92%)	2 (3.17%)

Table (2): Occurrence of different *Campylobacter spp*. in the examined cattle carcasses samples

Type of samples	Number of examined samples	Positive *Campylobacter spp.**	Campylobacter isolates		
			*C.jejuni**	*C.coli*	*C.lari*
Intestinal contents	50	3 (6%)	1 (33.33%)	1 (33.33%)	1 (33.33%)
Liver	50	3 (6%)	2 (66.7%)	-	1 (33.33%)
Muscles	50	1 (2%)	1 (100%)	-	-
Total	150	7 (4.67%)	4 (57.14%)	1 (14.28%)	2(28.57%)

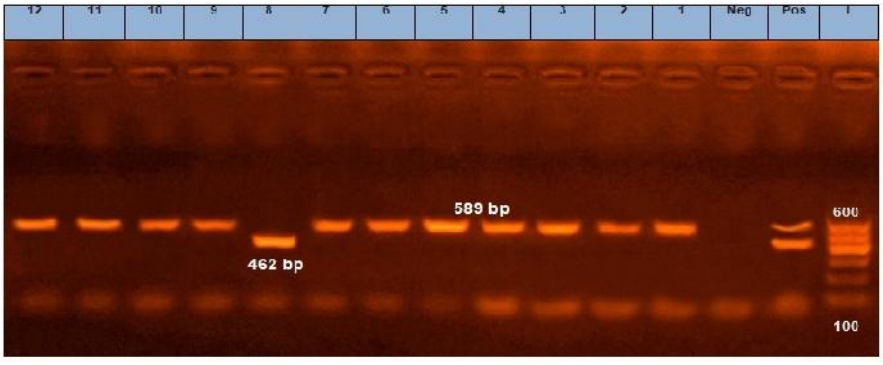
Table (3): Occurrence of different Campylobacter species in the examined animal products samples

Type of samples	Number of examined samples	Positive *Campylobacter spp.*	Campylobacter isolates	
			C.jejuni	*C.coli*
Raw milk	52	6 (11.53%)	2 (33.33%)	4 (66.67%)
Karish cheese	40	3(7.5%)	2 (66.67%)	1(33.33%)
Yoghurt	30	-	-	-
Total	122	9 (7.4%)	4 (44.4%)	5 (55.6%)

Table (4): Risk factors of *Campylobacter spp.* in the examined human samples

variable	Number of examined samples (128)	Positive Campylobacter spp. (26)		Campylobacter isolates			
				C.jejuni (22)		*C.coli (4)*	
Age		no	%	no	%	no	%
1-6	36	10	27.8	8	80	2	20
7-13	36	6	16.67	4	66.67	2	33.33
14-25	28	6	21.4	6	100	-	-
26-35	8	1	12.5	1	100	-	-
36-52	20	3	15	3	100	-	-
Gender							
Male	81	18	22.22	16	88.88	2	11.11
Female	47	8	17.02	6	75	2	25
Residence							
Rural	120	25	20.8	23	92	2	8
urban	8	1	12.5	-	-	1	100
Abdomenal pain							
Yes	128	26	20.3	22	84.6	4	15.38
No	-	-	-	-	-	-	-
Diarrhea							
Yes	128	26	20.3	22	84.6	4	15.38
No	-	-	-	-	-	-	-
Total	128	26	20.3	22	84.6	4	15.38
Bloody stool							
Yes	-	-	-	-	-	-	-
No	128						
Consumption of poultry							
Yes	128	26	20.3	22	84.6	4	15.38
No	-	-	-	-	-	-	-

PCR amplification of *C. coli CeuE* gene & *C. jejuni mapA* gene



Photograph (1) PCR amplification of *C. coli CeuE* gene & *C. jejuni mapA* gene; An agarose gel electrophoresis showing. M:100 bp marker , Neg: control negative , Pos: control positive**.**

Lane 8: *C.coli* +ve sample with PCR product of 462 bps.

Lanes 1-7& 9-12 *C.jejuni* +ve samples with PCR product of 589 bp.

Lane 1.2 human samples, lane 3,4,5,6,7,8. Chicken samples lane 9.10 cattle intestine samples lane 11 raw milk samples lane 12 kariesh cheese sample.

4. DISCUSSION

Campylobacter spp. are major cause of bacterial gastroenteritis world wide (Moore et al., 2005). C.jejuni and C.coli are responsible for 90% and 10% of human enteric infection cases, respectively. (Lastovica , 2006).

In the current studyCampylobacter spp.were isolated from intestinal contents, liver, breast muscles and thigh muscles of the examined chicken samples with a percentage of 27.6%, 27.14%, 7.14% and 14.2%, respectively.

These results come in accordance with the findings of (Misawa et al., 2000) and Saad (2014). Higher isolation rate was reported by Jamshidi et al. (2008), Rahimi and Amiri(2011) and Salihu et al.(2012).While lower isolation rate was reported by Menna et al.(2005).

Poultry exposed to Campylobacter infections firstly at the farm level due to the insufficient biosecurity measures, then at markets due to contamination of carcasses during evisceration , scalding and during storage, Ellis-Iversen et al. (2009). Broiler chicken gut oftenly colonized by Campylobacters especially C.jejuni EFSA(2008).The variation in Campylobacter spp. isolation rates might be due to the difference in the study area, sanitation level during handling and processing of chicken and also due to the laboratory methodologies employed for isolation Shih (2000).The isolation rate of Campylobacter spp. from the intestinal contents samples was 27.6%. Out of them 55.17%were identified as C.jejuni while 41.37% were identified as C.coli and 3.44% as C.lari. The isolation rates of C.jejuni and C.coli from liver, breast and thigh muscles were 68.4%, 100%, and 50% for C.jejuni and 31.57%, 0% and 40% for C.coli respectively. C.lari isolated from thigh muscles at percentage of 10%. These results coincided with those reported by Stoyanchev(2004), Saad (2014) and Abdeltawab et al.(2015).

In the current study Campylobacter spp. isolated from cattle carcasses from intestinal contents, liver and muscle samples with rate of 6%, 6% and 2% respectively. All isolates were identified as C.jejuni except one isolate was identified as C.coli from intestinal contents sample table (2).

Also the occurrence of Campylobacter spp. in animal products such as raw milk , kariesh cheese and yoghurt were reported . Our finding in table (3) showed that the isolation rate of Campylobacter spp. from raw milk and kariesh cheese was 11.53% and 7.5%, respectively. These results agreed with the results obtained by Barakat et al.(2015) and Kashoma et al. (2016). But lower isolation rate was reported by Modi et al.(2015).

No Campylobacter spp. was isolated from yoghurt samples. This may be due to the milk used in preparation of yoghurt was pasteurized milk or due to the acidity of yoghurt is sufficient to inhibit the growth of the microorganism. Actually, C.jejuni and C.coli isolated from the above mentioned products at percentage of 44.4% and 55.6%, respectively. This observation in agreement with Barakat et al.(2015) and Saad et al. (2007) .Whyte et al.(2004) reported that 1 of 62 raw milk samples were positive for C.coli. Raw milk is persumed to be contaminated by bovine feces, however, direct contamination of milk due to bovine mastitis has been recorded. About 12% of raw milk samples from dairy farms were contaminated with C.jejuni. USDA (2008).The high occurrence of Campylobacter spp. in traditional dairy products could be due to environmental contamination which occur from infected animal wastes or due to unsanitary food production and storage practices or may be due to the use of un pasteurized milk .Campylobacter infection in human are usually charachterized by self limiting watery / bloody diarrhea , abdominal pain , nausea and fever ; however , sever neurological sequelae, bacteremia and other extra intestinal complications may develop infrequently (Blaser andEngberg,2008) .

In the current study the occurrence of Campylobacteriosis in the examined human stool swabs was 20.3% (table 4). This result is nearly similar to the results reported by Hussain , 2011, while lower results reported by Awadallah et al.(2014)and Kang et al. (2006) and higher percentages reported by Abushahba et al.(2018).Also the results revealed that C.jejuni and C.coli isolated at percentages of 84.6% and 15.3% respectively at all examined ages .These results was nearly similar to the results obtained by Abd el tawab et al.(2015). Lower results recorded by Saad(2014) .The variation in prevalence of Campylobacter spp. in humans within different countries might be due to different reasons including health status (diarrheic or apparently healthy) , age , nutritional state , level of hygiene and sanitation , study season , contact with animals , geographical factors and different habits of meal (AbdEL-Baky et al.,2014) .

Our result revealed that Campylobacter spp. isolated from young ages at higher percentages than older ages.When age increase, the isolation rate decrease as shown in table (4). Our finding is statistically insignificant. This result is similar to the result obtained by Coker et al., 2002. Infants were at high risk of contracting Campylobacteriosis than adult due to their impaired immunity, especially in developing countries (Coker et al., 2002). Regarding to gender of the examined humans, it was found that the isolation rates of Campylobacter spp., in males (22.2%) was higher than that of females (17.0. 2%) . Also the results revealed that C.jejuni and C. coli isolated at percentages of 88.88% &11.11% in males and 75% & 25% in females respectively The result is statistically insignificant but it indicate that males are more subjected to infection than females. Nearly similar results are recorded by Abushahba etal., 2018. In respect to residence, this study showed that the prevalence of Campylobacteriosis was higher in rural areas (20.08%) than in urban areas (12. 5%). The results showed that C.jejuni and C.coli isolated at percentages of 92%& 8%in rural areas and 0% &100% in urban areas respectively. This result agreed with Abushahba et al. (2018). The higher rate in rural areas may be due to lack of hygienic measures and precautions during handling of live poultry or due to close proximity to birds. In all the examined patients there was abdomenal pain and diarrhea with history of poultry consumption.This results are in agreement with Blaser et al. (1983). However, no bloody stool noticed in any of the examined samples. These results disagree with Abushahba etal. (2018). The absence of blood in the examined stools may be due to the early life production of IgG antibodies against Campylobacter in developing countries (Blaser et al., 1986)

In this study, according to the multiplex PCR methods and the amplification parameters as specified by Wang et al. (2013) a series of optimization reactions were carried out according to QIAamp DNA mini kit instruction.

Probe based PCR reactions targeting mapA and ceuE genes specific for C.jejuni and C.coli were used during the current study. The results showed that out of 12 biochemically suspected Campylobacter spp. isolates , by PCR , 11 isolates were confirmed as C.jejuni and 1 isolates as C.coli . Campylobacter jejuni isolates produced at 589 bps, while C.coli isolates produced at 462 bps.

5. CONCLUSION

Chicken, cattle and animal products are possible sources of human Campylobacter infections. The highest isolation rate of Campylobacter spp. was from intestinal content swabs of chickens. The presence of Campylobacter in raw milk and milk products indicates that raw milk consumption is hazardous and proper pasteurization of milk

and adaptation of hygienic condition is necessary to protect human beings from this zoonotic pathogen. Reduction of infection on the animal farms and control of Campylobacter infection in poultry would reduce the risk of human exposure to Campylobacter and decrease the prevalence of infection. Increasing the public education and awareness could decrease the prevalence of infection. PCR is a useful molecular tool for identification of Campylobacter spp.

6. REFERENCES

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