

ISOLATION OF CAMPYLOBACTER JEJUNI FROM POULTRY CARCASSES

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ABSTRACT

The objective of this study was to assess the incidence of Campylobacter in broiler carcasses and it was carried out on collection of 100 carcasses from a chicken abattoir. Eight bacterial agents 8% which proved morphologically and biochemically to be C. jejuni were recovered. Campylobacter jejuni isolates were biotyped as biotype 1(4 isolates) biotype 1a (3 isolates) and biotype 2 (1 isolates). The level of Campylobacter jejuni in broiler carcasses was ranging from 1.9×10 to 3.31×10 CFU per igm of carcass. This study was done to evaluate the presence of C. jejuni and identify these bacteria in the processing line of chicken abattoirs. C. jejuni resistance was increased against some antibiotics as Ampicillin, colestin, Neomycin, oxytetracycline and Novobiocine.

INTRODUCTION

Campylobacter is a common food-borne pathogen of humans that has been associated with poultry carcasses and further processed poultry products (White et al., 1997 and Saleha et al., 1998). It is generally thought that Campylobacter flows into commercial processing facilities on and within the live birds and disseminated during the various processing procedures (Saleha et al., 1998).

Campylobacter can be recovered from broiler carcasses prior to entering the scald tank or by rinsing feathered carcasses (Stern et al., 1995), or by excising or swabbing the skin (Izat et al., 1998 and Kotula and Pandya 1995).

Despite the presence of Campylobacter on the outside of broilers, emphasis is commonly on the presence and level of Campylobacter and other human pathogens in the alimentary

tract. This interest is fueled by the concern the ruptured organs, such as crop or ceca may spill contents rich in Campylobacter onto the carcass. It was reported that the crop can be broken during processing (Hargis et al., 1995).

Byrd et al., (1988) reported that Campylobacter is evident in the majority (62%) of crop samples examined on the farm just prior to catching and transport to plant. Oosterom et al., (1983) found that Campylobacter is commonly recovered in high numbers, more than $\log_{10} 6.0$ cfu/g in ceca and colon. Campylobacter had also been found on carcass skin samples, Berndtson et al., (1992) found 89% of skin samples from processed carcasses were positive for Campylobacter at about $\log_{10} 3.0$ CFU/g lower than that found in intestine samples (Oosterom et al., 1983 and Musgrove et al., 1997). However, Kotula and

Pandya (1995) recorded high levels of Campylobacter on defeathered skin prior to scalding, breast skin had higher Campylobacter populations (\log_{10} 6.9 cfu/g) than did drum or thigh skin.

MATERIAL AND METHODS

(I) Sampling of broiler carcasses:

Each one whole carcasses per slaughter batch was collected after chilling but before processing. Avoid cross-contamination during collection and transport of the carcasses. The carcasses were placed in separate sterile plastic bags to avoid cross contamination. Samples were kept at 2 to 8°C.

(II) Sample preparation:

Avoid fat and 27g tested protein were taken and placed into an empty Petri dish and further on in a stomacher bag.

About 27g tested protein were transferred into nine volumes (about 243ml) buffered peptone water (BPW) brought to room temperature before adding.

(III) Isolation and identification of Campylobacter organism:

1ml of suspension was transferred to 9ml (thioglycolate broth), each sample was incubated at 37°C for 24 hours, examined for Campylobacter growth. The suspension was investigated for detection of Campylobacter organisms as follows.

(1) Microscopical examination (Smibert, 1978):

A loopful from the suspected growth was taken and put on clean slides and covered with cover slips. These smears were examined under the phase contrast microscope using 400 magnifications for detection of the characteristic motility and morphology of Campylobacter organism.

(2) Isolation procedures (Smibert 1978):

In this method, 2 loopfuls of suspected growth were suspended in about 5ml of sterile saline solution (pH 7.4) mixed well, then aspirated by sterile syringe and filtered through a Millipore filter of pore size 0.65µm (Sartorius Co., Polycarbonat filter, Germany). The first few drops of the filtrate were discarded, then one drop of remainders were inoculated onto the surface of well-dried blood Brucella agar plates. The drop was let to be dried at 37°C for 30 minute, then streaked onto the agar surface. The plates were incubated at 37°C in microaerophilic condition (5% CO_2).

(3) Bacteriological identification (Kwialck et al., 1990):

3.1. Motility test:

For motility detection, a drop from the incubated enrichment thioglycollate broth was examined under phase-contrast microscope for motility detection and S shape character of campylobacter organisms.

3.2. Colony characters and morphology:

Sheep blood Brucella agar was used and suspected colonies of Campylobacter organisms were stained by Gram's stain for staining affinity and organism morphology.

3.3. Oxygen requirement:

Each isolate was subcultured on two blood agar plates. One plate was incubated aerobically and the other micro-aerophilic by using gas pack jar at 37°C and 42°C for 72h., then examined for growth.

3.4. Biochemical identification:

Isolates of Campylobacter were identified biochemically according to Carter, (1984).

(4) Sensitivity of Campylobacter isolates to antibiotics was studied according to **Peckham, (1984)**.

RESULTS AND DISCUSSION

The incidence of Campylobacter infection in broiler carcasses was carried out by collection samples from 100 carcasses among chicken abattoirs where its incidence was 8%, while other incidence percentage recorded were 12% by **Bryan and Doyle (1995) and Berrang et al., (2001)**. The variation in percentages especially in high value due to the high contamination by Campylobacter in the processing plant where the final results in contamination of the end product was about 49% and 80% respectively (**Oosterom et al., 1983 and Roesenquist et al., 2006**).

Campylobacter identification:

Eight isolates were identified morphologically on culture basis as Campylobacter colonies were small, moist and transparent. Cover slide hanging drop method showed darting movement, Gram's stained preparations showed negative curved rods and or spirals. There were similar result described by **Levina, (1964) and Pckham, (1984)**.

The biochemical identification (Table 2) of 8 isolates showed no variation in biochemical activities of *C. jejuni*. Similar procedure was carried out by **Fletcher and Platridge, (1964); Neill et al., (1984) and Ezzat et al., (1991)**. The obtained results showed that only 2 isolates were H₂S negative using lead acetate strips. Similar observations were reported by **Fletcher and Plastidge (1984)**.

Biotyping of the identified *C. jejuni* (Table 3) isolates revealed 4 strains of biotype 1, 3 strains biotype 1a and 1 strain belonged to biotype 2. This was based on hippurate hydroly-

sis, DNA hydrolysis and H₂S production. Similar procedures were carried out by **Loir, (1984); Prescott and Bruin, (1981); Smibert (1978) and Adayel, (1993)**.

In Table (4), the presence and the level (from carcasses) of Campylobacter were 8 broiler carcasses from 100 broiler carcasses being Campylobacter positive with number ranging from 1.9 x 10 to 3.31 x 10 CFU per carcass. Similar results of **Johannessen et al., (2007)** which recorded that Campylobacter number were 2.6 x 10 CFU per carcass.

The antibiogram to *C. jejuni* isolates showed high sensitivity to Gentamycin, Trimethoprim and Flumequine. The high sensitivity of the isolated *C. jejuni* to Gentamycin was similar to findings of **Bradbury and Munroe (1985)**. Intermediate sensitivity to Kanamycin and Carbenicillin were noticed to the isolated *C. jejuni* strains where they were sensitive to Kanamycin (**Diker and Yardimci 1989**). All the isolated strains were resistance to Ampicillin and Colxacillin. Similar results were obtained by **Zien (1989) and Ezzat et al., (1991)**.

CONCLUSION

It can conclude that the carcasses from Campylobacter positive broiler ones were heavily contaminated with Campylobacter from cecal content. Carcasses might play an important role in the transmission of Campylobacter jejuni to human being. These results emphasize the importance to improving control measures and both hygiene and sanitary condition in chicken abattoirs.

Table (1): Culture characteristics of suspected *Campylobacter* isolates from broiler carcasses.

Isolate No.	Growth temperature			Anaerobic growth	Growth in 5% oxygen	Motility
	25°C	37°C	42°C			
12	-	+	+	-	+	+
15	-	+	+	-	+	+
20	-	+	+	-	+	+
24	-	+	+	-	+	+
43	-	+	+	-	+	+
55	-	+	+	-	+	+
73	-	+	+	-	+	+
82	-	+	+	-	+	+

Table (2): Biochemical identification of suspected *Campylobacter* isolates from broiler carcasses.

Isolate No.	Catalase test	Oxidase test	Glycine tolerance	NaCl tolerance 3-5%	H ₂ S production on lead acetate	Hippurate hydrolysis
12	+	+	+	-	+	+
15	+	+	+	-	+	+
21	+	+	+	-	-	+
24	+	+	+	-	+	+
43	+	+	+	-	+	+
55	+	+	+	+	-	+
73	+	+	+	-	+	+
82	+	+	+	-	+	+

Table (3): Biotyping of *C. jejuni* isolates from broiler carcasses.

Case No.	Hippurate hydrolysis	Rapid H ₂ S test	DNA hydrolysis	Biotypte 1	Biotype 1a	Biotype 2
12	+	-	+		1a	
15	+	+	-			2
21	+	-	-	1		
24	+	-	-	1		
43	+	-	-	1		
55	+	-	+	0	1a	
73	+	-	-	1		
82	+	-	+		1a	

Table (4): *Campylobacter* counts, recovered from broiler carcasses from different Apa Hoird.

Replication	12	15	21	24	43	55	37	82
Mean log ₁₀ cfu/g of sample	2.93	3.31	2.8	3.1	2.7	2.75	2.1	1.9

Table (5): Results of *in vitro* sensitivity testing of isolates of *C. jejuni*.

Antimicrobial agent	Disc potency	Standard sensitivity zone	Susceptibility
Gentamycin	10 ug	>15 <19	+++
Trimethobrim	1.25 +	> 11 < 15	++
Flumequine	23.5ug	> 13 < 18	++
Kanamycin	30mg	> 11 < 15	+
Canbenicillin	30mg	> 11 < 13	+
Nobiocin	100mg	> 15 < 18	-
Ampicillin	30mg	> 15 < 18	-
Colstine	10mg	> 11 < 13	-
Neomycin	30mg		-
Oxytetracycline	30mg	> 13 < 16	-
	30mg	> 15 < 18	

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الملخص العربى

عزل الكامبيلوبكتري جوجوناي من لحوم الدواجن

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معهد بحوث صحة الحيوان - الدقى

فى هذه الدراسة تم تجميع ١٠٠ عينة من لحوم دواجن التسمين من المجازر الخاصة بالدواجن، وجد نسبة العزل من الكامبيلوبكتري من هذه العينات ٨٪، وكانت ٨ معزولات من الكامبيلوبكتري تم التعرف عليها من المورفولوجى والاختبارات البيوكيميائية على أنها كامبيلوبكتري جوجوناي، وتم تقسيم هذه المعزولات بالطرق الكيميائية إلى ٤ بيوتايب، ٣ بيوتايب ومعزولة واحدة بيوتايب ٢، وكان عدد الكامبيلوبكتري فى اللحوم المصابة تتراوح بين ١٩ × ١٠ إلى ٣١ × ١٠ لكل جرام من الذبيحة.

ومن هذه الدراسة يتضح أن وجود ميكروب الكامبيلوبكتري وتصنيفها إلى كامبيلوبكتري جوجوناي، ولذلك يجب المحافظة على عدم تلوث لحوم الدواجن فى المجازر من التلوث بالميكروب من الأمعاء والحويصلة وخصوصاً أن ميكروب الكامبيلوبكتري مقاوم لكثير من المضادات الحيوية مثل الأمبسلين والكولستين والنيوميسين والأوكسى تتراسيكلين والنوفويمايوجين.