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 x 10 to 3.31 x 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 Amplcillin, 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 proeessing 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 Campylobaeter on the outside of broilers, emphasis is commonly on the presenee 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 eeca may spill eontents rich in Campylobaeter onto the carcass. It was reported that the erop can be broken during proeessing **(Hargis et al., 1995)**.

Byrd et al., (1988) reported that Campylobacter is evident in the majority (62%) of erop 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 log10 6.0 cfu/g in eeca and colon. Campylobaeter had also been found on carcass skin samples, **Berndtson et al., (1992)** found 89% of skin samples form proeessed careasses were positive for Campylobacter at about log10 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 (log10 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 plaeed in separate sterile plastic bags to avoid cross eontamination. 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 suspention was investigated for detection of Campylobacter organisms as follows.

(1) Microscopical examination (Smibert, 1978):

A loopful form the suspected growth was taken and put on clean slides and covered with cover slips. These smears were examined under the phase contrast mieroscope 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 0.65um (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% Co2).

(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 Campylobaeter organisms were stained by Gram's stain for staining affinity and organism morphology.

3.3. Oxygen requirement:

Eaeh 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 bioehemieally aceording to Carter, (1984).

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

RESULTS AND DISCUSSION

The incidence of Campylobacter infection in broiler carcasses was carried out by collection samples from 100 careasses 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. Grain'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 Plastridge, (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 hydrolysis, DNA hydrolysis and H2S production. Similar proeedures were carried out by **Loir, (1984); Prescott and Bruin, (1981); Smibert (1978) and Adayel, (1993)**.

In Table (4), the presence and the level (from careasses) 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, Trimethobrim 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 notieed 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_2S 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_2S 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

عـــزل الكامبيلوبكـتر جـوجـونـاى من لحـــوم الدواجـــن

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معهـد بحـوث صحـة الحيـوان - الدقى

فى هذه الدراسـة تـم تجـميع ١٠٠ عـينة من لحوم دواجـن التـــين من المجـازر الخـاصـة بالدواجـن، وجمد نسـبـة العـزل من الكامـبـيلوبكتر من هـذه العـينات ٨٪، وكـانت ٨ معـزولات من الكامـبـيلوبكتر تم التعـرف عليـهـا من المورفـولوچى والاختـبارات البـيوكـيميـائية على أنهـا كـمبـيلوبكتر جوجـونـاى، وتم تقسـيم هـــذه المعـزولات بالطـرق الكيـمـيـائيـة إلى ٤ بـيوتايب، ٣ بـيوتايب ومعـزولـة واحـدة بيوتـايب ٢، وكـان عـدد الكامبيلوبكتر فى اللحـوم المصابـة تتراوح بـين ٩ر١ × ١٠ إلى ٣ر٣١ × ١٠ لكـل جـرام من الذبيحـة.

ومـن هـذه الدراسـة يتضح أن وجود ميكـروب الكامبيلوبكـتر وتصنيفها إلى كامبيلوبكـتر جـوجـونـــاى، ولذلك يجـــب المحافظة على عـــدم تلـوث لحـــوم الدواجـــن فى المجـازر من التلـوث بالميكـروب من الأمـعـــاء والحـويصلـة وخـصـوصـــاً أن ميكروب الكامـبـيلوبكتر مقـــاوم لكثـيـر من المضـــادات الحيـويـة مـثل الأمبـــلين والكولـــتين والنيـوميـــين والأوكســى تتراسـيكلين والنوفوبـايـــوجـــين.