Phenotypic Characterization of *Clostridium Species* in Sheep Meat

Mahmoud E. El-Sayed[1], Islam Z. Mohamed[2], and Islam M. Khalifa[3]

[1] *Department of Bacteriology, Immunology and Mycology, Faculty of Veterinary Medicine, Suez Canal University.* mezzat05@yahoo.com

[2,] *Animal Health Research Institute, Dokki, Giza*

islamshereef@yahoo.com

3 Salhia, Sharkia. ekhalifa84@gmail.com *(Corresponding author)*

Abstract

The existence of *Clostridium species* in sheep meat has a public health importance. The current preliminary study was aimed to investigate the prevalence of various *Clostridium species* in sheep meat. Thirty meat samples were randomly collected from butcher's markets at Ismailia province. The collected samples were subjected to the bacteriological examination; moreover, the enumeration of anaerobic count was performed. The total count of anaerobes was $6x10^3$ C.F.U./ g; in addition, the prevalence of *C. bifermentans, C. sporogens, C. subterminal,* and *C. perfringens* was10%, 43.3%, 30%, 30% sheep meat samples, respectively. *C. perfringens* is considered one of the main spoilage organisms of meat leading to contaminations of great amount of decomposed meat, which ending with economic losses; bad hygienic conditions in slaughterhouses are the main cause for meat contamination with *Clostridium species*, since the gastrointestinal tract of slaughtered animals is their natural habitat, the abattoir processing represent a great source for meat surface contamination by these pathogens.

Keywords: *C. bifermentans, C. sporogens, C. subterminal, C. perfringens*, Sheep meat

Introduction

Meat is an excellent source of high-quality protein and rich in minerals and vitamins which are essential for human health, power and vitality. Meat may affect the public health due to their contamination with spoilage microorganisms which are responsible for objectionable changes as well as the presence of pathogenic organisms which may lead to either food infection or food intoxication ***(Libby,***

1975; Hanninen, 1980 and FAO/WHO, 1983). Microbiological quality of fresh meat has been receiving attention all over the world due to the occurrence of significant outbreaks of foodborne illness affecting consumers. Quality maintenance is important not only for consumer health protection but also to assure uniformity in fresh meat shelf-life, ***(Widders, 1995).*** Meat is considered an important source of proteins and essential amino acids. Due to this rich composition, it offers a highly favorable environment for the growth of pathogenic bacteria. The microbiological contamination of carcasses occurs mainly during processing and handling, such as skinning, evisceration, storage and distribution at slaughter houses and retail establishments ***(Gill, 1998 and Abdalla et al., 2009).***

Anaerobic bacteria such as *Clostridia* constitute an important group of the microorganisms which are responsible for many public health hazards. They are able to survive the relatively high temperature by production of their resistant spores ***(Barnes, 1985).*** *Clostridium perfringens* is one of such group which has great effect on human health as food poisoning organism. The majority of foodborne diseases caused by *C. perfringens* result from the consumption of meat and meat products ***(Lynch et al. 2006; Wahl et al. 2013).***

The abattoir process is considered the great source of meat surface contamination by *C. perfringens* organisms either internally from animal after slaughtering as postmortem invasion from gastrointestinal tract or externally from contaminated hands, skin of animals, water, soil and processing equipment. Foodborne pathogens are the leading causes of illness and death in developing countries costing billions of dollars in medical care, medical and social costs ***(Fratmico et al., 2005).***

Due to the rising incidence of food borne infections, there is an urgent need for control and/or prophylaxis for food poisoning outbreaks associated with meat. It depends greatly on investigating the causative agents in carcasses, eliminating them to ensure food safety and to protect public health from microbial contamination of food ***(Al Khaldi et al. 2004 and Albini et al. 2008).***

The current preliminary study was aimed to investigate the prevalence of various *Clostridium species* as well as monitoring the total anaerobic count in sheep meat.

Material and Methods

1- Samples collection:

A total of 30 Meat samples were randomly collected from butcher's markets at Ismailia province. Samples were collected separately in sterile plastic bags, and then were labeled and given serial numbers. They were kept in an Ice box and transferred quickly to the laboratory with minimum of delay for anaerobic bacteriological examination according to ***Cruickshank et al., (1975).***

2-Processing of collected samples:

a) Preparation of homogenate *(ICMSF. 1978).*

Twenty five grams of each sample were removed aseptically using a sterile forceps and scissors from different points of the sample then placed in sterile polyethylene bag to which 225 ml of sterile peptone water 0.1% were added aseptically; the content was homogenized in a stomacher (MPW.302 Poland) for one minute to provide a dilution of 10^{-1} Such homogenate was used for all bacterial investigations.

b) Preparation of serial dilutions:

From the prepared homogenate; 1/10 dilution; 1 ml was taken by a sterile pippete then transferred to a sterile test tube containing 9 ml sterile peptone water 0.1% and was shaken well using a test tube shaker to made a dilution of 1/100 using a new sterile pippete 1 ml was transferred from 1/100 dilution to next tube for making a dilution of 1/1000; the process repeated till dilution of 10^{-5}.

3-Total anaerobic count:

The enumeration technique was done by Using plate count technique according to ***Roberts et al. (1995)***. From each of the prepared dilution 0.1 ml was taken and inoculated into each plate of Reinforced Clostridia agar "RCA" (LAB M23). The inoculums were spread over the entire surface of the agar using a sterile bented glass rod until the inoculum was completely dried. After the agar surface had been appeared to be dried, the plates were overloaded with an additional layer (10 ml) of the melted respective medium at 50-55° C, after the solidification of the overlaid layer; all plates were incubated anaerobically in Gas-Pack anaerobic jar ***(Brewere and Allgeier, 1966)*** at 37 C for 48 hours. Counting and calculation were recorded.

4- Isolation of *Clostridium* species *(ICMSF. 1978):*

The surface of each sample was sterilized by using hot spatula and two bean size pieces were obtained from the deeper parts, then inoculated separately into two tubes of freshly prepared, previously boiled and cooled cooked meat medium.One of the two inoculated tubes was heated

at 80°c for 15 minutes in water bath with a depth of water more than the level of the material in tubes, while the second tube was left unheated. Both tubes were then incubated anaerobically at 37°c for 48 hrs. A loopful from each unheated tube was streaked onto the surface of 10% sheep blood agar with neomycin sulphate (200 mg /ml) for isolation of C. *perfringens* ***(Smith and Holdeman. 1968)*** While, the heated tubes were then streaked on 10% sheep blood agar plates for isolation of other clostridia. Inoculated plates were immediately incubated anaerobically at 37°C for 24 - 48 hrs. After incubation, the anaerobic growth on the blood agar plates was examined macroscopically and microscopically. Selected suspected colonies were transferred to tubes of freshly boiled and cooled cooked meat medium and incubated anaerobically at 37°C for 24 hours to have a pure culture of isolates for further identification.

5- Identification of *Clostridium species*:

Phenotypic characteristics of *Clostridium species*

Table (1): *Phenotypic Identification of Clostridium species:*

Biochemical test		Xylose	Mannose	Maltose	H2s	Nitrate reduction	Indol	Mannitol	Sucrose	Lactose	Glucose	Gelatine	Spores
Species	***C. perfringens***	-	+	+	+	+	-	-	+	+	+	+	**Co**
	C. subterminal	+-	+	+	-	+	-	-	+	+	+	-	**To**
	C. bifermentans	-	-	-w	+	+	+	-	-	-	+	+	**C/so**
	C. sporogens	-	-	-w	+	+	-	-	-	-	+	+	**So**

+ = Positive reaction. Co = Central oval.
+w = Weak reaction. So = Sub terminal oval.
- = Negative reaction. To = Terminal oval.
V = Variable reaction. C/so = Central- sub terminal oval.

Isolates were identified according to ***Smith and Holdeman (1968), Willis (1977), Macfaddin (1980), Smith and Williams (1984)*** as the following; Staining, cultural characteristics, Nagler's reaction, and different biochemical reactions.

Results

1-The total anaerobic count:

Table (2): *Total anaerobic plate count of examined meat samples*

Sheep-meat samples N=30	**Count C.F.U./g**		
	Min.	Max.	Mean ± SE
	$5.1x10^3$	$8.9x10^3$	$6x10^3 \pm 2.9x10^3$

2-Prevalence of different *Clostridium specie*s in the examined sheep meat samples:

Table (3): *Prevalence of Clostridium species in the examined meat samples.*

Sheep-meat samples	***C. bifermentans***		***C. sporogens***		***C. subterminal***		***C. perferingens***	
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
No =30	3	10	13	43.3	9	30	9	30

Discussion

Meat may be contaminated with clostridial spores during the slaughtering process and the following handling. Since *C. perfringens* present in the normal flora of intestinal tract of animals. Contamination of the carcass from the intestinal contents, as well as, dust, soil or from workers is virtually unavoidable. In the present work, as illustrated in Table (1), the total anaerobic plate count in the examined meat was $6x10^3 \pm 2.9x10^3$. This result disagrees with the result obtained by ***Hedia et al., (2009)*** who detected the total anaerobic plate count in cattle meat samples by higher ratio with a mean value of $2.17x10^3$ and $8.7x10^3$ for fore and hind quarter respectively. Furthermore, ***Hassan (1994)*** detected the *C. perfringens* count of cattle meat samples with a mean value of $1.17x10^3$ +_ 0.35 and $1.90x10^3$+_0.74 in basateen and moneeb abattoir, respectively. ***Hedia (2009)*** mentioned lower prevalence of *C. perfringens* in cattle meat samples for fore quarter with an average of $1.7x10^2$ and higher prevalence for hind quarter with an average $2.1x10^3$. In camel meat samples, *clostridium perfringens* were varied from 1x10 to $2x10^3$ with an average $5.2x10^2 \pm 3.8x10^2$ resulted from five (25%) positive meat samples for fore quarter, $1.5x10^2$ to $3.1x10^3$ with an

average of 1.2 $x10^3$ ± $3.6x10^2$ obtained from eight (40%) positive meat samples for hind quarter. Higher ratio were obtained by ***Hedia et al., (2009)*** with an average of $2.2x10^3$ and $2.4x10^3$ for fore and hind quarter respectively. Concerning sheep meat samples, *C. perfringens* count were varied from 2.9x10 to 2.1 $x10^3$ with an average of $3.9x10^2$ ± $1.9x10^2$ obtained from four (20%) positive meat samples for fore quarter, $1.4x10^2$ to $1x10^3$ with an average of $7x10^2$ ± $3x10^2$ resulted from seven (35%) positive meat samples for hind quarter. ***Hassan (1994)*** reported higher clostridium perfringens count with a mean value of $2.84x10^3$+_0.80 and $2.50x10^3$+_0.49 in basateen and moneeb abattoir, respectively.

The recorded data in table (3) demonstrated the prevalence of *Clostridium species*; the prevalence of *C. bifermentans, C. sporogens, C. subterminal,* and *C. perfringens* was10%, 43.3%, 30%, 30% sheep meat samples, respectively. Most of them have deteriorative effect rather than being disease linked bacteria ***(Gibbs, 1971). El-Ged and Saad (1985)*** isolated *C.follax, C.sphenoid, C.tertium, C.sporogenes* and *C.butyrcium* from muscle, liver and intestinal content samples while isolated *C.perfringens* only from intestinal content samples. ***El-Seedy et al. (1989)*** only isolated *C.novyi*. Nearly similar results were obtained by ***Hedia et al., (2009)*** who isolated *C.bifermentans, C.sporogens, C.subterminal* and *C.tertium* from cattle and camel meat. Much higher results were recorded by ***El-Naenaeey (1989)*** who isolated *C. perfringens* from cattle meat with an incidence of 56.5%. Lower results were obtained by ***Cohen et al., (2006)*** who isolated the organism from beef meat with an incidence of 4.5%. While ***Phillips et al., (2008)*** failed to detect C.perfringens from retail ground beef samples. These results were in harmony with the results detected by ***Hedia (2009)*** who isolated *C.perfringens* by percentage 20% & 33.4% from examined cattle raw meat (fore and hind quarter). Much higher result were obtained by ***Khalid (2013)*** who isolated *C.perfringens* from cattle meat samples by percentage 20%. Very high incidence of *C.perfringens* in ground beef samples (96%) were detected by ***Guran et al., (2014)***. ***Fahim et al., (2017)*** detected lower incidence of *C.perfringens* in raw beef samples (16%). Concerning the percentage of isolation of *C. perfringens* from camel meat samples were 30% & 45% for fore and hind quarter, respectively. ***El-Ged and Saad***

(1985) failed to detect *C. perfringens* from fresh camel meat samples. Similar results were reported by ***El-Naenaeey (1989)*** who isolated *C. perfringens* from fresh camel meat samples in an incidence of 45%. The result obtained by ***Kairy (1998)*** were in harmony with this result who isolated *C. perfringens* from fresh camel meat samples in an incidence of 35%. ***Shalaby and El-Mahrouk (2006)*** obtained nearly similar result , they isolated *C.perfringens* type A in an incidence of 33.7%. Higher results were reported by ***Hedia (2009)*** who isolated *C. perfringens* by percentage of 46.7% & 60% from examined camel meat (fore & hind quarter). Much lower incidence of *C. perfringens* in camel meat swab (2.7%) were detected by ***Mohamed, et al. (2010)***. Similar result obtained by ***Khalid (2013)*** who isolated *C.perfringens* from camel meat samples by percentage of 45%. In sheep meat samples, the percentage of isolation of *C.perfringens* were 25% and 40% for fore and hind quarter, respectively. Higher result obtained by ***Khalid (2013)*** who isolated *C.perfringens* from sheep meat samples in an incidence of 52.5%. ***Guran et al., (2014)*** isolated *C. perfringens* from ground sheep meat by very high ratio (88%). The incidence of *C.perfringens* in camel meat samples was higher than other examined meat samples (cattle and sheep). Moreover, the recorded results showing that the incidence in hind quarters was higher than that of four quarters. This may be attributed to more contamination and excreta near the hind quarters.

In conclusion, *C. perfringens* is considered one of the main spoilage organisms of meat leading to contaminations of great amount of decomposed meat, which ending with economic losses. Poor hygienic procedures in slaughterhouses are the main cause for meat contamination with *Clostridium species*. Since the natural habitat of *C. perfringens* in the gastrointestinal tract of slaughtered animals, the abattoir processing represents a great source for meat surface contamination by *C. perfringens* organisms.

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الملخص العربي

يعتبر وجود ميكروب الكلوستريديم في لحوم الأغنام ذو اهميه بالنسبه للصحة العامة للإنسان, وهذه الدراسة تهدف إلي التعرف علي وجود الأنواع المختلفة من ميكروب الكلوستريديم في لحوم الأغنام. لقد تم تجميع ثلاثين عينة لحوم من محلات الجزارة في محافظة الأسماعيلية وتم فحصهم بكتريولوجيا بالإضافة إلي عد الميكروبات اللا هوائيه في هذه العينات حيث وجد أن العد الكلي للميكروبات اللاهوائيه كان بمعدل 6×10^3. ولقد تم عزل ميكروبات

بنسب *C. bifermentans, C. sporogens, C. subterminal, C. perfringens* 43.3,'%10 %, 30%, 30% بالتتابع في عينات لحوم الأغنام. ويعتبر ميكروب الكلوستريديم برفرينجينز من الميكروبات الأساسية المسؤلة عن فساد اللحوم مؤديا إلي خسارة إقتصادية كبيرة. وتعد الظروف الصحية السيئه في المجازر من أهم الأسباب الرئيسيه المسؤلة عن تلوث اللحوم بميكروب الكلوستريديم حيث أن القناة الهضميه للحيوان المذبوح هي الموطن الطبيعي لميكروب الكلوستريديم وتعد خطوات التجهيز في المجازر خلال عملية الذبح من المصادر الرئيسيه المسببة لتلوث اللحوم بهذه الميكروبات .