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Some Characters of *Cl. perfringens* Isolated from Fresh and Marketed Processed Meat

Khalid A. Alkheraije
Shaqra University, Shaqra, Kingdom of Saudi Arabia
Email: dr.khalidalkheraije@yahoo.com

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ABSTRACT

This study was carried out a fresh meat immediately after slaughter and a marketed processed cattlemeat (sausage and minced meat). A total of 530 meat samples were examined for the presence of *Cl. perfringens*, 423 were from fresh meat obtained immediately after slaughtering (108 cattle meat, 101 sheep meat, 100 camel meat and 114 buffaloe meat) and 107 processed meat (57 from sausage and 50 from minced meat). *Cl. perfringens* was isolated from 204 (48.2%) of fresh meat samples, 61 (56.5%) from cattle, 53 (52.5%) from sheep meat, 45 (45%) from camel meat and 45 (39.5%) from buffaloe meat. The isolation rate of *Cl. perfringens* was higher in processed meat, it was isolated from 68 (63.6%) of which 45 (78.9%) from sausage and 23 (46%) in minced meat. The processed meat was found to harbour higher viable count ranging between 4×10^2 - 7×10^6 *Cl. perfringens* cells/gm meat than that Fresh meat in which the number ranged from 10^2:5×10^6 cells/gm meat. Typing of isolated strains revealed that the majority of it was of *Cl. perfringens* type A, 2 of type B, 3 of type C and one type D. Sixty strains of *Cl. perfringens* type A were randomized and tested for heat resistance at 100˚C and the results were recorded. Production of enterotoxin by 10 strains of *Cl. perfringens* was performed by ligated ileal loop test in rabbits. It was done by injection of whole culture in skimmed milk, cell extracts and concentrated culture filtrates of the organism in the ileal ligated loop of rabbits and the results were recorded.

Keywords: *Cl. perfringens*; Fresh Meat; Marketed Processed Meat

1. Introduction

Members of genus clostridium are widely reconized as enteric pathogenes for man, domestic animals and wildlife [1]. *Cl. perfringens*, anaerobic gram-positive bacterium is ubiquitous in the intestinal flora of human and animals, and is also commonly isolated from environmental materials such as soil and water [2]. Among animals and broilers only *Cl. perfringens* type A was responsible for disease outbreaks in broilers from 14 other bacterial isolates were studied [3]. The usage of term food "poisoning strains" as reported by [4] based on resistance to 100˚C for one hour is fallacious and should be bonded and the term restricted to those strains known to be capable of producing enterotoxin. *Cl. perfringens* is a leading cause of food-borne poisoning in the USA [5,6].

Because of *Cl. perfringens* can be found almost everywhere, the source of the strain responsible for any outbreak of food posioning have to be considered. So, this study was carried out to reveal the percentage of *Cl. perfringens* isolation from freshly slaughtered and processed meat, typing of isolates, testing the heat resistance of some isolates and detection of the ability of some different isolated strains to produce enterotoxin using the rabbitileal loop method.

2. Materials and Methods

Bacteriological examination of 423 fresh meat samples of different species of animals and 107 processed meat samples were done. The samples were firstly inoculated into prepared cooked meat media and incubated anaerobically at 37˚C for 48 hours. Then loopful form it was streaked on neomycin sulphate sheep blood agar plates and incubated anerobicaslly for further 48 hours. The growing suspected colonies were examined for their morphological and cultural characters and picked up for purification and identification on sheep blood agar plates. Isolates were preserved on cooked meat broth. The morphological, cultural and biochemical activities were recorded. Nagler reaction and toxin-antitoxin plate test were done according to [7]. Strains identified as *Cl. perfringens* by cultural and biochemical tests were typed according to [8]. Sixty samples from which *Cl. Perfrin-*

gens type A was isolated, were used for viable count. Ten sample from meat of each species (cattle, buffaloes, camel and sheep) and ten from each of processed meat (sausage and minced) were taken as random samples. The preparation of samples and cultivation on tryptose-sulfite-cycloserine (TSC) agar medium forviable count done asmentioned by [9] and the results were recorded. Preparation of spore suspension of *Cl. perfringens* was done according to [10,11]. Determination of spores heat resistance was done according to [12]. The production of enterotoxin by 10 strains of *Cl. perfringens* was detected by ligated ileal loop test in rabbits. It was done by injection of whole culture in skimmed milk, cell extracts and concentrated culture filtrates of the organism in ileal ligated loop of rabbits [13-16].

Statistical analysis for data of loop fluid volume/length ratio of heat resistant and heat sensitive strains when whole cultues, cell extracts and cell filtrates injected, was carried out according to [17].

3. Results

The results of bacteriological examination of 423 fresh meat samples of different species of animals and 107 marketed processed meat sample were illustrated in **Table 1**. *Cl. perfringens* was isolated from 204 samples of fresh meat (48.23%) and from 68 samples of processed meat (63.6%) Total viable count of *Cl. perfringens* carried out by plate count dilution method on tryptose-sulphitecycloserine agar (TSC) medium where *Cl. perfringens* developed the characteristic black colonies.

The statistical analysis of **Table 1** revealed that:

In fresh meat:

Correlation coefficient = −0.764 (strong reverse correlation);

P-value = 0.685 *i.e.* P > 0.05 (significant difference).

In processed meat:

Correlation coefficient = −1 (strong reverse correlation).

P-value = 0.577 *i.e.* P > 0.05 (significant difference).

The results of total viable count of *Cl. perfringens* were recorded in **Table 2** which showed that considerable variation between fresh and processed meat samples in the viable count.

Heat reisstant strains: sixty strains of *Cl. perfringens* type A were taken as random samples from all the isolates, 40 isolates from fresh meat consisted of 10 from each species and 20 from processed meat, 10 from sausage and 10 from minced meat. Each strain was inoculated into D.S. medium for largest number of spore crops and highest resistance of spores [10]. The results were recorded in **Table 3**.

Table1. The results of *Cl. perfringens* isolation in fresh and processed meat.

Meat samples	Number of samples	Positive		Negative	
		No.	Percentage %	No.	Percentage %
1) Fresh meat:					
Cattle	108	61	56.5%	47	43.5%
Sheep	101	53	52.5%	48	47.5%
Camel	100	45	45%	55	55%
Buffaloe	114	45	39.5%	69	60.5%
Total of fresh meat	423	204	48.2%	219	51.8%
2) Processed meat:					
Sausage	57	45	78.9%	12	21.1%
Minced meat	50	23	46.0%	27	54.0%
Total of processed meat	107	68	63.6%	39	36.4%
To total of fresh and processed meat	530	272	51.3%	258	48.7%

Table 2. Viable count of *Cl. perfringens* in fresh and processed meat samples.

Serial No. of sample	No. bact. cells/gm meat in different types of meat					
	Cattle	Camel	Buffalo	Sheep	Sausage	Minced meat
1	4×10^3	3×10^3	11×10^2	1×10^2	4×10^2	2×10^3
2	5×10^3	4×10^3	3×10^3	3×10^2	15×10^3	13×10^3
3	1×10^4	1×10^4	1×10^4	2×10^3	24×10^3	15×10^3
4	13×10^3	13×0^3	25×10^3	3×10^3	32×10^3	17×10^3
5	13×10^3	4×10^4	21×10^3	3×10^3	7×10^4	2×10^4
6	18×10^3	5×10^4	28×10^3	3×10^3	1×10^5	28×10^3
7	28×10^3	51×10^3	11×10^4	4×10^3	14×10^4	3×10^4
8	4×10^4	12×10^4	18×10^4	8×10^3	1×10^6	5×10^4
9	18×10^4	3×10^6	3×10^5	1×10^4	11×10^5	2×10^5
10	1×10^6	5×10^6	3×10^5	2×10^5	7×10^6	1×10^6

Table 3. The incidence of heat (100˚C) resistant strains of *Cl. perfringens* type A in fresh and processed meat.

Strains origin	No. of strains tested	Heat resistance strains after boiling						Total of positive	
		30 min	60 min	90 min	120 min	180 min	240 min	No.	%
Cattle meat	10	-	4	-	-	-	-	4	40%
Camel meat	10	-	-	2	-	2	-	4	40%
Buffaloes meat	10	2*	-	-	-	-	-	-	0%
Sheep meat	10	2*	-	-	-	-	-	-	0%
Total of fresh	40	4*	4	2	-	2	-	8	20%
Sausage	10	-	4	2	-	2	-	8	80%
Minced meat	10	-	2	2	-	2	-	6	60%
Total of processed	20	-	6	4	-	4	-	14	70%
Total of fresh and processed meat	60	4*	10	6	-	6	-	22	36.7%

*Not heat resistant strain (heat sensitive strains).

The average value for the data in **Table 2** is:
4×10^3:10^6 cells/gm in cattle meat.
3×10^3 - 5×10^6 cells/gm in camel meat.
11×10^2 - 3×10^5 cells/gm in buffaloe meat.
10^2 - 2×10^5 cells/gm in sheep meat.
4×10^2 - 7×10^6 cells/gm in sausage samples.
2×10^3 - 10^6 cells/gm in minced meat samples.

Detection of enterotoxin of *Cl. perfringens*by ligated ileal loop test: Six heat resistant strains of *Cl. perfringens* type A isolated from cattle and camel meat and four heat sensitive isolated from sheep and buffaloe meat were tested for enterotoxin production in ligated ileal loop of 10 New-Zealand white rabbits. Each rabbit was used for testing three strains for a particular test sample ***i.e.*** Rabbit No. 1 for whole culture skimmed milk of 3 strains, no. 2 for culture filtrate of the same strains, No. 3 for cell extract of the same strains and soon. The tenth rabbit was used for the 3 different test sample of one strain. Intraluminal injections of 2 ml test sample were done, animals were sacrificed 20 - 24 hours later. The length, dilatation, and fluid volume of the loop were measured to calculate the loop volume (ml)/length (cm) and their mean value ratio. The loop volume/length ratio for 6 heat resistant strains of *Cl. perfringens* enterotoxin in rabbit ileal loops inoculated with whole cultures skimmed milk were 0.8, 0.9, 0.6, 0.9, 0.7, 0.5 respectively and their mean value were 0.73. The loop volume/length ratio for 4 heat sensitive strains of the same cultures were 0.7, 0.9, 0.7, 0.6 and their mean value were 0.73. The loop volume/length ratio for 6 heat resistant strains of cell extracts were 2.8, 2.1, 2.5, 2.2, 2.6, 2.3 respectively and their mean value were 2.4. The loop volume/length ratio for four sensitive strains of the same cultures were one positive (2.4) and the other three strains negative.

The loop volume/length ratio for 6 heat resistant strains of culture filtrates were 2.2, 1.3, 2.4, 1.4, 1.9, 1.4 respectively and their mean value were 1.8. The loop volume/length ratio for 4 heat sensitive strains of the same cultures were 1.1, 1.7, 0.75, 1.3 and their mean value were 1.2, and significant of these results were recorded in **Table 4**.

The gross appearance of positive loops revealed congestion, petechae, haemrrhagic inflammation and swelling of the loops due to accumulation of light brown to bloody fluids.

4. Discussion

Meat items, irrespective of species may be contaminated with spores of clostridia during the slaughtering process and the subsquent handling. Since *Cl. perfringens* is the normal flora of the intestinal tract of animals, contanimation of the carcass from the intestinal contents, as well as, soil, dust or from worker is virtually unavoidable as supported by [2].

In this work, the incidence of *Cl. perfringens* in fresh meat of different species revealed that the cattle meat was the mostly contaminated 56.5% followed by sheep 52.5%, camel 45% and buffaloe was 39.5% as in **Table 1**. These results vary too much between authers who reported different percentage isolation. [12] reported that healthy cattle contained 29% *Cl. perfringens* while [18] proved that beef and lamb carcass 29% and 85% respectively. [19] recorded that the incidence of *Cl. perfringens* in meat cattle samples was 38.4% while [18] the percentage of *Cl. perfringens* was 50%. However, although comparison could not be possible due to the fact that there is variation in hygienic standards in slaughtering and preparation of the carcass in every country.

Cl. perfringens was isolated from sausage in 78.9% and from minced meat in 46% (**Table 1**). This high incidence was previously recorded by [20,21]. Lower incidence was reported by [22]. The difference between the percentage of isolation between fresh 48.2% and processed meat (sausage and minced meat) 63.6%, would attributed to higher contamination during processing, due to a number of factors such as handling, addition of spices and use of trimming and poor cuts of meat.

The results obtained (**Table 2**) showed that total viable count of *Cl. perfringens* type A in sheep meat samples was the lowest, its total viable count ranged between (10^2 - 2×10^5) organism/gm while the highest number was

Table 4. Mean value of loop fluid volume (ml) and length ratio of *Cl. perfringens* enterotoxin in rabbit injected with different materials.

Strains	Challenge material		
	Cultures skimmed milk	Cell extracts	Culture filtrates
Heat resistant	0.73	2.4	1.8
Heat sensitive	0.73	0.6	1.2
Statistical significant	Non-significant	Signficant difference	Significant difference

found in camel meat samples (3×10^3 - 5×10^6) organism/gm. On the other hand, the sausage showed that the highest level of contamination (4×10^2 - 7×10^6) organism/gm and minced meat was (2×10^3 - 10^6) organism/gm. This high incidence of contamination was obtained in spite of the strict consideration rules taken during sampling and forwarding samples to the laboratory. These counts were much higher than those reported by [23,24]. This may be due to the use of highly selective media of [25]. However, the high percent of contamination of processed meat (sausage and minced meat) have been previously attributed to unhygienic excessive handling, additives and preservation. Also the high level of contamination in camel fresh meat may be due to that these animals are always driven to slaughter houses from long distance with rough treatment, subjected to starving condition and exertion which make the clostridia invade their tissue. Results obtained revealed that between 272 strains of *Cl. perfringens* isolated from fresh and processed meat only 2 were type B, 3 were type C and one type D, and the rest were of Type A. This agree with [2] who mentioned that in a commonly used classification scheme, *Cl. perfringens* is divided into five toxin types (A to E) based on the production of four toxins (alpha, beta, epsilon and iota); however, this bacterium also produces ten other toxins such as *Cl. perfringens* enterotoxin (CPE), beta2 toxins, and theta toxin (also known at perfringolysin O or PFO). The other authors were studies more characterization of type A enterotoxigenic *Cl. perfringens* strains [3,26,27].

During this investigation 60 strains of the isolates were randomized for testing for heat resistance. From the isolates of fresh meat 20% were heat resistant while from the isolates of processed meat it was 70% (**Table 3**). Similar Findings in the meat were reported by [23,28].

The total difference between the heat sensitive strains either the challenge was with whole culture, cell extract or cell filtrates (**Table 4**) was statistically significant, calculated F test = 11.152. These findings leads to a conclusion that heat resistant strains were able to produce exudation of fluid and consequent dilatation of the ileal segment more than that sensitive strains which means the ability to produce enterotoxin and this agree with [29], and not agree with that reported by [30]. The ability of some strains of *Cl. perfringens* isolated from the intestinal contents of cattle, sheep and chicken with enteritis was examined by [31], of 114 strains examined, 24% were considered significant enterotoxin producer. Also, **Table 3** concluded that only *Cl. perfringens* type A was responsible for necrotic enteritis outbreaks in broiler, where 14 bacterial isolates were studied.

5. Conclusion

This study showed that the isolation rate of *Cl. perfringens* was higher in processed meat (63.6%) than that fresh meat (48.2%). Also, the processed meat was found to harbour higher viable count than that fresh meat. Typing of isolated strains revealed that the majority of it was of *Cl. perfringens* type A. Production of enterotoxin by *Cl. perfringens* was performed by ligated ileal loop test in rabbits with different inoculations. The results revealed that the tested strains of *Cl. perfringens* type A were enterotoxin producer in rabbits.

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