Prevalence Of Toxigenic Anaerobic Microorganisms And Their Toxins In Some Fish Products

Hanan, G.A. Seadawy
Animal Health Research Institute, Dept. of Food Hygiene

ABSTRACT

One hundred and eighty random samples were collected from different localities of different hygienic levels, these samples included 30 random samples each of, salted fesiekh, salted sardine, smoked herring, smoked salmon, carb stick and mussels were examined bacteriolgically for the presence of toxigenic anaerobes and their toxins. The obtained results revealed that the mean value of anaerobic microorganisms in the examined fesiekh, salted sardine, smoked herring, smoked salmon, carb stick and mussels samples were $4.3x10^3$, $2.5x10^3$, $9.3x10^3$, $3.6x10^2$, $1.4x10^2$, and $0.9x10^2$, respectively while the mean value of Clostridium perfringens were $1.1x10^3$, $0.93x10^2$, $0.3x10^2$, $0.13x10^2$, $0.01x10^2$, and $0.06x10^2$, respectively.

The identification of Clostridial species revealed the presence of *Cl. tertium, Cl. sordilli, Cl. cornis, Cl. sporogenes* and *Cl. bifermentans.*

The public health importance and hygienic significance of isolated organisms were discussed as well as the control measures to improve the quality of such products.

INTRODUCTION

Fish is regarded as being more perishable than other high protein foods because quickly attacked by bacteria and become unfit for human consumption. In order to overcome such problem different methods of food preservations were developed. Curing, smoking and drying go back to antiquality. The shelf-life of processed fish should be longer than that of fresh raw products especially when stored anaerobically *(1)*.

Clostridia are the most frequently anaerobes associated with the food. They are able to survive in such type of environment through the production of resistant spores *(2)*.

Cl. botulinum and *Cl. perfringens* are the two most important species involved with food borne illness and cause food spoilage *(3)*.

The isolation of *Cl. botulinum* from food is generally considered to be of less significance than the detection of the toxin *(4)*.

In Cairo, an outbreak of *Cl. botulinum* intoxication has been recorded due to consumption of inadequatly prepared salted fermented fish (Fesiekh) *(5)*.

Cl. perfringens remains a major cause of food-borne illness and is continuing concern to the food service industry *(6)*. Because of the ubiquitous distribution of the *Cl. perfringens* it is difficult if not impossible to exclude spores of this bacterium during the processing of various fish products and its presence must be assumed *(7)*. Strains of the organism are divided into five types A to E based on the production of extracellular lethal toxin (alpha, beta, epsilon and iotar) and virtually all food poisoning out breaks are caused by type A strains *(8)*. *Cl. perfringens* has been implicated in many food poisoning outbreaks in American foods *(9)*.

Therefore, this work was planned to evaluate some marketed processed fish products anaerobically with special reference to the toxigenicity of the isolated strains.

MATERIAL AND METHODS

Collection of samples

A total of 180 samples of different fish products (each of 30, fesiekh, salted sardine, smoked herring, smoked salmon, carb stick and mussels) were collected from different

supermarkets in Cairo and Zagazig provinces. Samples were directly transferred to the laboratory in ice box without delay and were subjected to bacteriological examination.

Preparation of samples

Samples were prepared according to the recommended technique *(10)*.

Enumeration of the anaerobic bacteria

Enumeration was carried out *(11)*, using Reinforced Clostridial Medium (RCM) incubated anaerobically at 37°C for 24 hours.

Enumeration of *Clostridium perfringens*

Tryptose Sulphate Cycloserine agar (TSC) medium anaerobically incubated at 37°C for 48 hours *(10)*.

Isolation of clostridium organisms *(12)*

By using cooked meat medium, anaerobically incubated at 37°C for 24 hours, then streaked onto 10% sheep blood agar plates and incubated anaerobically at 37°C for 24 hours.

Identification of isolates

Identification of the anaerobic micro-organisms was carried out according to preveious technique *(13, 14)*.

Biochemical reactions

Suspected isolates were identified according to the recommended scheme *(15)*.

Typing of *Clostridium perferingens*

Demonstration of *Cl. perfringens* toxins was carried out by dermonecrotic reaction test in guinea pigs *(16)* and its identification by neutralization test *(17)*.

Screening test for detection of *Clostridium botulinum* toxins

Clostridium botulinum toxins was detected by mouse challenge test *(4)* (toxin assay).

Serological identification of toxin type

Clostridium botulinum toxins were identified by using specific antitoxin, mixed with the suspected tested material and injected into a pair of mice. Another pair was injected without antitoxin and observed for three days. Survival of a single pair indicated the presence of single type of toxin.

RESULTS AND DISCUSSION

Anaerobic bacterial count

Anaerobes are widely distributed in lakes coastal water, gills and intestinal tracts of fish. Fish like any other food, can be also contaminated during handling and processing with any of the well known food poisoning organisms *(18)*. Moreover, *Cl. perfringens* could be isolated from the body surface and alimentary canal of several species of fish *(8)*. Moreover, sea food are susceptible to all of common food poisoning microorganisms including *Cl. perfringenes* and *Cl. Botulinum (19)*.

Result recorded in Table 1 revealed that the mean ±SE value of total anaerobic count in salted fesiekh was $4.3x10^3 \pm 1.7x10^3$ with a minimum of $2x10^2$ and maximum of $4x10^5$ while salted sardine was $2.5x10^3 \pm 9x10^2$ with a minimum of less than 10^2 and maximum of $2x10^4$ and in smoked herring was $9.3x10^3 \pm 3x10^3$ with a minimum of less than 10^2 and maximum of $6x10^4$ anaerobes/g. Nearly similar results were recorded *(20)*. The relatively high anaerobic count in salted feseikh, salted sardine and smoked herring may be attributed to the similar anaerobic storage conditions under which salted fish were kept before marketing.

The same table revealed that the mean values of total anaerobic count in smoked salmon, carb sticks and mussels were $3.6 x10^2 \pm 1.4 x10^2$, $1.4 x10^2 \pm 0.25x10^2$ and $0.9 x 10^2 \pm 0.25 x 10^2$, respectively with a minimum of less than 10^2 for all types of examined fish products and maximum of $3x10^3$, $5x10^2$ and $6x10^2$ anaerobes/g. respectively. This may be attributed to the well adjusted and hygienic condition during all the steps of preparation of such products.

Table 1. Statistical analytical results of anaerobic count/g in examined fish products (n = 30)

Fish product	Minimum	Maximum	Mean	± S.E.
Salted Fesiekh	2×10^2	4×10^5	4.3×10^3	1.7×10^3
Salted sardine	$< 10^2$*	2×10^4	2.5×10^3	9×10^2
Smoked herring	$< 10^2$	6×10^4	9.3×10^3	3×10^3
Smoked salmon	$< 10^2$	3×10^3	3.6×10^2	1.4×10^2
Carb stick	$< 10^2$	5×10^2	1.4×10^2	0.25×10^2
Mussels	$< 10^2$	6×10^2	0.9×10^2	0.25×10^2

* $<10^2$: zero (negative plate).

Table 2 showed that at frequency range $<10^2$, the highest percentage of anaerobic count was recorded for mussels (60%), while zero percentage was recorded for fesiekh. Forty percentage for each of smoked salmon and carb sticks were recorded as the same aforementioned anaerobic count range. Sixty percent of each salted sardine, smoked herring and carb sticks samples had anaerobic count at range $10^2<10^3$ while relatively lower percentage (50%) was recorded for each of fesiekh and smoked salmon at the same anaerobic count. It was obvious that highest percentage of examined samples occupied the frequency range $10^2<10^3$. The carb sticks and mussels anaerobic count wasn't exceed frequency range $10^2<10^3$ while 3.4% of examined fesiekh was recorded at frequency range $10^5>10^6$ comply with the legal requirement *(21)*. The latter range wasn't recorded for any of other examined fish products.

Table 3 showed that the mean value of total *Clostridium perfringens* count in salted fesiekh, salted sardine, smoked herring, smoked salmon, crab stick and mussels were $1.1x\ 10^3 \pm 6.8x10^2$, $0.93x10^3 \pm 0.7x10^2$, $0.3x10^2 \pm 0.15x\ 10^2$, $0.13x\ 10^2 \pm 0.09x10^2$, $0.007\ x10^2 \pm\ 0.01x\ 10^2$ and $0.06x10^2 \pm\ 0.01\ x\ 10^2$, respectively with a minimum of less than 10^2 for all types of examined fish products and maximum of $2x10^4$, $2x10^3$, $3x10^2$, $2x10^2$, 10^2 and $2x10^2$ anaerobes/g respectively. Such results showed relatively high *Clostridium perfringens* count, which might be attributed to the low quality of raw fish and the used salt in preparation and the temperature at the time of storage.

Isolation and identification of clostridium organisms

The incidence of clostridial species in examined fish products was recorded in Table 4. *Cl. perfringenes* showed the highest incidence (20%) isolates from fesiekh samples, also *Cl. Sordilli* and *Cl. sporgenes*, while *Cl. tertium* and *Cl. bifermentans* could be isolated from fesiekh samples (10% of each). Concerning salted sardine samples *Cl. sordilli* showed the highest incidence (26.7%) followed by *Cl. tertium* (16.7%), *Cl. perfringenes* and *Cl. sporogenes* (13.3% of each) meanwhile *Cl. bifermintans* was 10%. Similar results are recorded by previous investigators *(20,22)*. While in smoked herring *Cl. perfringenes* and *Cl. sporogenes* (10% of each), *Cl. sordilli* (6.7%) and 3.3% of *Cl. tertium* and *Cl. Bifermentance* (3.3%) were recorded (Table 4). Concerning smoked salmon the incidence of *Cl. sordilli* was (10%), *Cl. perfringenes* and *Cl. sporogens* could be isolated in an equal rate (25%) while *Cl. tertium* was (3.3%). Concerning carb stick *Cl. tertium* showed the highest incidence (20%) followed by *Cl. cornis* (10%) and *Cl. perfringenes* (3.3%) on the other hand the most frequent isolates from mussels was *Cl. sporogenes* (6.7%) followed by *Cl. perferingenes* and *Cl. tertium* (3.3% of each).

Cl. botulinum and its toxin failed to be detected in all examined fish products with regard to salted fish, these results could be assigned to the high amount of salt used during processing of salted fish *(20)*.

Table 2. Frequency distribution of anaerobic count/g in examined fish products (n = 30)

Fish product	$< 10^2$		10^2- $< 10^3$		10^3- $< 10^4$		10^4- $< 10^5$		10^5- $< 10^6$	
	No.	%	No.	%	No.	%	No.	%	No.	%
Salted Fesiekh	0	0	15	50	10	33.3	4	13.3	1	3.4
Salted sardine	3	10	18	60	5	16.7	4	13.3	0	0
Smoked herring	6	20	18	60	3	10	3	10	0	0
Smoked salmon	12	40	15	50	3	10	0	0	0	0
Carb stick	12	40	18	60	0	0	0	0	0	0
Mussels	18	60	12	40	0	0	0	0	0	0

Table 3. Statistical analytical results of *Clostridium perfringens* count/g in examined fish products (n = 30)

Fish product	Minimum	Maximum	Mean	± S.E.
Salted Fesiekh	$< 10^2$	2×10^4	1.1×10^3	6.8×10^2
Salted sardine	$< 10^2$	2×10^3	0.93×10^3	0.7×10^2
Smoked herring	$< 10^2$	3×10^2	0.3×10^2	0.15×10^2
Smoked salmon	$< 10^2$	2×10^2	0.13×10^2	0.09×10^2
Carb stick	$< 10^2$	10^2	0.07×10^2	0.01×10^2
Mussels	10^2	2×10^2	0.06×10^2	0.01×10^2

Table 4. Incidence of Clostridial species in examined fish products (No. 30 of each)

Isolated organisms	Fesiekh		Salted sardine		Smoked herring		Smoked salmon		Carb stick		Mussels	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Cl. perferingens	6	20	4	13.3	3	10	2	6.7	1	3.3	1	3.3
Cl. botulinum	-	-	-	-	-	-	-	-	-	-	-	-
Cl. tertium	3	10	5	16.7	1	3.3	1	3.3	6	20	1	3.3
Cl. sordilli	6	20	8	26.7	2	6.7	3	10	-	-	-	-
Cl. cornis	-	-	-	-	-	-	-	-	3	10	-	-
Cl. sporogenes	6	20	4	13.3	3	10	2	6.7	-	-	2	6.7
Cl. bifermentans	3	10	3	10	1	3.3	-	-	-	-	-	-
Total	24	80	24	80	10	33.3	8	26.7	10	33.3	4	13.3

The same table declares that the high incidence of Clostridia was recorded in fesiekh and salted sardine samples (80%) followed by smoked herring and carb stick samples (33.3%), then smoked salmon samples (26.7%) while mussels samples showed the lowest incidence (13.3%). The high incidence of clostridia in examined samples reflected the bad hygienic status of these products. It is important to alter fish technologists in Egypt to overcome that general high incidence of anaerobes occurring in salted fish especially with the repeated outbreaks by *Cl. botulinum* intoxication .

Types of *Cl. perfringenes*

Tables 5 and 6 revealed that out of 6 strains (20%) isolated from fesiekh samples, 4 strains (13.3%) were toxigenic and typed as 2 strains (6.7%) of type A and 2 strains (6.7%) of mixed type A and D, while 2 strains (6.7%)

were non toxigenic. Four strains of *Cl. Perfringenes* could be isolated (13.4%) from salted sardine (two type A toxigenic and two non toxigenic strains). With regard to smoked herring out of 3 strains (10%), 2 strains (6.7%) were non toxigenic and one strain (3.3%) was toxigenic of type D. In smoked salmon 2 strains (6.7%) were non toxigenic while carb stick and mussels one strains of each (3.3%) was non toxigenic. This result indicated that *Cl. perfringenes* type A was the most prevalent toxigenic strain in examined salted fish samples.

Recent record *(23)* on food borne outbreaks in Austrerlia , indicated that 14% of resturants and commerical caters food poisoning was due to *Cl. Perfringenes* in toxications. Out breaks was constitute fish (16%) and sea food (6%).

Table 5. Prevalence of non toxigenic and toxigenic strain of *Cl. perfringens* in examined fish products (n = 30)

Fish product	Positive cases			
	Non toxigenic		Toxigenic	
	No.	%*	No.	%*
Salted Fesiekh	2	6.7%	4	13.3%
Salted sardine	2	6.7%	2	6.7%
Smoked herring	2	6.7%	1	3.3%
Smoked salmon	2	6.7%	0	0%
Carb stick	1	3.3%	0	0%
Mussels	1	3.3%	0	0%

* In relation to the total number of the total examined samples.

Table 6. Typing of toxigenic *Cl. perfringens* toxins from examined fish products (n = 30)

Fish product	Type of toxigenic isolates					
	Type A		Type D		Type A&D	
	No.	%	No.	%	No.	%
Salted Fesiekh	2	6.7%	0	0	2	6.7%
Salted sardine	2	6.7%	0	0	0	0
Smoked herring	0	0	1	3.3%	0	0

REFERENCES

1-Sofas, J.N. (1994): Microbial growth and its control in meat, poultry and fish. pp. 359. Advances in meat research. 1st Ed. Blackie Academic and Professional Champion and Hall. London, New York.

2-Barnes, E.M. (1985): Isolation methods for anaerobes in food. Int. J. Food Microbiol., 2: 81.

3-Mead, G.C. (1992): Principles involved in the detection and enumeration of clostridia in foods. Int. J. Food Microbiol., 17: 135.

4-Hobbs, G., Growther, G.S. and Neaves, P. (1982): Detection and isolation of Clostridium botulinium. In: Roberts, J.E.L. and Skinner, F.A. (ed): Isolation and identification method for food society for applied bacteriology, technical series No. M, Academic press, London 151-164.

5-Rakha, M.A. (1992): An outbreak of botulism intoxication due to salted fermented fish (Fesiekh). Paper presented at the expert consultation on fish technology, Alexandria, Egypt.

6-Stringer, M.F.; Turnbull, P.C.B. and Gilbert, R.J. (1980): Application of serological typing to the investigation of outbreaks of C. perfringens food poisoning 1970-1978. J. Hyg. Camb., 84: 44.

7-Bean, N.H. and Griffin, P.M. (1990): Food borne disease outbreak in the United States, 1973-1987: Pathogens, vehicles and trends. J. Food Part., 53: 804.

8-Labble R.G. (1988): Clostridium perfringens. Food Technol., 42: 195.

9-Wen, Q. and Mcclane, B.A. (2004): Detection of enterotoxigenic clostridium perfringens type A isolates in American retail foods. Appl. Environm. Microbial., 70 (5): 268-291.

10-ICMSF (International Commission on Microbiological Specification for Food) (1978): Microorganisms in foods University of Toronto press, Toronto, Ontairo, Canada.

11-Roberts, D.; Hoopper, W. and Greenwood, M. (1995): Practical Food Microbiology. Puteler and Tanar, London.

12-Carter, G.R. and Cole, J.R. (1990): Diagnostic procedures in veterinary bacteriology and Mycology. 5th Ed., Academic press, Harcout, Booace. Jou., publishers New York, Boston, Tokyo, Toronto.

13-Macfaddin, (1980): Biochem-ical tests for identification of medical bacteria (2nd Ed.) Williams and Wilkins Baltimore, London.

14-Smith, L.D.S. and Williams, B.L. (1984): The pathogenic anaerobic bacteria. Charles Thomas Publisher. USA.

15-Koneman, E. W.; Allen, S. D.; Do-Well, V.R. and Summers, W.W. (1992): Color Atlas and Textbook of Diagnostic Microbiology. 4th ed. t.B. Lip. Co., New York, London.

16-Nickleson, I.L.R. and Finne, G. (1992): Fish, Crustaceans and precooked seafood. Compendium of metrhods for the microbiological examination of foods. (3rd Ed.) APHA, Washington D.C., USA.

16-Oakly, C.L. and Warrack, G.H. (1953): Routine typing of C. Welchii. J. Hyg. Caomb., 51: 102.

17-Stern, M. and Batty, I. (1975): Pathogenic clostridia. Butterworth, London, Boston.

18-Hobbs, G. (1983): Food poisoning and fish. R.Soc. Hlth. J., 103: 144.

20-Abu-Zid S.M.A. (1998): Anaerobes in meat and fish products and their ability to toxin production. Ph.D, Thesis, Fac. Vet. Med., Cairo Univ.

21-Egyptian Organization for Standardization and Quality Control "E.O.S.Q.C." (1989): Egyptian Standard for requirements of salted fish. No. 1725.

22-Kassem, G.M.A. (1996): Health hazard due to marketed salted fishes. M.V.Sc., Thesis, Fac. Vet. Med., Cairo Univ.

23-Dalton, V.B.; Gregory, J.; Kirk, M.D.; Stafford, R.J.; Givney, R.; Kraa, E. and Gould, D. (2004): Food borne disease outbreaks in Australia, 1995-2000. Commun. Dis. Intell., 28 (2): 211-224.

الملخـص العربى

تواجد الميكروبات اللاهوائية السامة وسمومها فى بعض منتجات الأسماك

حنان جوده عبد الجواد سعداوى
معهد بحوث صحة الحيوان – مركز البحوث الزراعية

تم فى هذه الدراسة تقدير تواجد الميكروبات اللاهوائية السامة وسمومها فى مائة وثمانون عينة من منتجات الأسماك (٣٠ فسيخ ، ٣٠ سردين مملح ، ٣٠رنجة، ٣٠ سالمون مدخن، ٣٠ أصابع كابوريا، ٣٠ بلح البحر) والمشتراه عشوائيا من متاجر مختلفة فى محافظتى القاهرة والشرقية للوقوف على مدى صلاحيتها للاستهلاك الآدمى.

وكان متوسط العد الكلى للميكروبات الهوائية فى كل الأنواع السابقة هى ٤,٣×١٠^{٣}، ٢,٥×١٠^{٣}، ٩,٣ ×١٠^{٣}، ٣,٦×١٠^{٢}، ١,٤×١٠^{٢}، ٠,٩×١٠^{٢} بينما متوسط العد الكلى لميكروب الكلوستريديم بيرفرنجينز هى ١,١×١٠^{٣}، ٠,٩٣×١٠^{٢}، ٠,٣×١٠^{٢}، ٠,١٣×١٠^{٢}، ٠,٠٧×١٠^{٢}، ٠,٠٦×١٠^{٢} على التوالى.

وقد تم أيضا عزل الكلوستريديم تيرتيم، الكلوستريديم سورديلى، الكلوستريديم كورنس، الكلوستريديم سبوروجينس، الكلوستريديم باى فيرمنتنس.

وقد تم مناقشة الأهمية الصحية للعترات المعزولة وكذلك الإجراءات الصحية الواجب إتباعها لتفادى تلوث منتجات الأسماك بالميكروبات اللاهوائية وذلك لدرء خطرها والنهوض بصناعة هذه المنتجات ليتم إنتاجها بالطريقة الصحية السليمة والآمنة للمستهلك.