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MICROBIOLOGICAL EVALUATION AND IMPROVING THE SANITARY MEASURE OF FROZEN CHICKEN CARCASSES

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SUMMARY

One hundred of random frozen chicken carcasses were purchased from supermarkets in Cairo Governorate for bacteriological examination. Standard methods were used to determine anaerobic and some aerobic microorganisms.

Samples were classified into two groups (50 of each). Group I was examined for anaerobes, group II was frozen at -18°C for 1, 2, 3 months then examined for aerobic bacteria. Isolates from group I were *C. sporogenes*, *C. perfringes* and *C. sordelli* with incidences of 15%, 12% and 12%, respectively. While the mean value of total bacterial counts were $0.21x10^4 \pm 1.00$ after one month, $1.31x10^3 \pm 5.97$ after two months and $0.2x10^3 \pm 1.4$ after three months of freezing with a reduction 98.60%, while the mean value of Enterobacteriaceae

counts were $1.4x10^2 \pm 1.1$, $0.71x10^2 \pm 3.18$ and $1.92x10 \pm 8.19$ organisms/cm^2 after freezing for 1, 2 and 3 months, respectively with a reduction 98.3%.

The incidence of Staphylococcus spp. were 60%, 70%, 20% and 16% after one month, while it was 50%, 56%, 16% and 14% after 2 months and 44%, 48%, 14% and 12% after 3 months for neck skin samples, cloaca skin swabs, muscles of thigh and breast. Salmonella strains recovered from the examined samples were 10%, 12%, 2% and zero%. Antibiogram of antibiotic sensitivity of prevalent isolates were conducted. Application of decontaminant

as lactic acid 2% for 30 seconds reduced the microbial populations to 92%.

Public health importance as well as improving the quality of retailed frozen chicken carcasses were discussed .

INTRODUCTION

Poultry meat is the most popular food product worldwide. Several factors contribute to the popularity of this product, of which sensory, dietary and economic values are most important.

Poultry meat is one of the animal protein which is less expensive because of strain selection for growth potential and improve feed conversion (Richardson and Mead, 1999).

Although poultry meat constitutes an excellent source of high quality, easily prepared and digested animal protein of the first class, which contains all essential amino acids, many vitamins and minerals which are necessary for maintaining life and promoting growth yet, it is liable to harbour different types of microorganisms and constitute the largest potential source of food borne gastroenteritis (Pearson and Dutson, 1997 and Zahra, 2001).

Broilers are vulnerable to microbiological stress while they are still alive due to the intensive production methods. Furthermore, large number of birds undergoing slaughter by assembly-line procedures encourages the dissemination of microorganisms of both quality and public health concern. Therefore, deteriorative bacteria and food poisoning organisms are often associated with frozen chickens.

However, contamination of carcasses during their processing is mainly with spoilage organisms, presence of potentially pathogenic microorganisms such as salmonella spp., *E. coli*, *Staphylococcus aureus* and *C. perfringens* are commonly occur (Davis and Board, 1988; Cunningham, 1987 and Waldroup, 1996).

Therefore, during processing poultry carcasses may become contaminated with both spoilage and pathogenic microorganisms from fecal material. Additional sources of microbial contamination are the possibility tools, equipments, structural components of the facility, human contact, and carcass to carcass contact (Huffman, 2002).

Traditional meat inspection procedures cannot always assure that consumers will not be exposed to infectious doses of meat borne pathogens (Brown et al., 2000). The hygienic condition of frozen chicken meats can be assured only by the development of hazard analysis critical control point (HACCP) systems for frozen meat production processes. Bacteriological analysis of carcass surface has become an important source of information in developing and implementing HACCP system for poultry dressing operation.

So, the aim of this work to evaluate the microbiological contaminant and try to improve the quality of frozen chicken carcasses.

MATERIAL AND METHODS

Collection of samples:

A total of 100 random frozen chicken carcass samples were purchased from different markets in Cairo Governorate and transferred to the laboratory in an ice bag without delay for insection.

Preparation of samples

Preparation of samples for anaerobic cultivation:

Frozen carcasses were left on the bench for 2 hours for defrosting and thawing before sampling. Fifty frozen chicken carcass samples were examined from skin over thigh, and breast and from neck skin and around cloaca skin collected using swab and skin excision.

A pea-sized piece from each sample was inoculated into tubes of freshly prepared cooked meat broth in duplicate after sterilization.

One tube was heated at 80°C for 10 minutes to eliminate vegetative organisms while another one left without heating. All tubes were anaerobically incubated at 37°C for 48 hours.

A loopful from each tube was cultured onto each 10% sheep blood agar plates with 200 µg/ml neomycin sulfate for heated and non-heated tubes respectively. The plates were anaerobically incubated at 37°C for 48 hours.

The growing surface colonies were picked up in a pure form and inoculated into tubes of freshly prepared cooked meat broth for physical, morphological and cultural identification according to Cruickshank et al. (1975) and Finegold and Martin (1988). Typing of *C. perfringens* isolates was done by the intradermal inoculation test in guinea pigs according to Oakley and Warrack (1953), total anaerobic bacterial count was determined by the plate count technique according to Cruickshank et al. (1975).

Preparation of samples for aerobic cultivation (ICMSF, 1978):

Preparation of dilutions:

One ml of the original dilutions was transferred to a sterile test tube containing 9 ml of sterile peptone water (0.1%) to be diluted in a sequential manner preparing ten fold sterile dilution up to 10^{-6}; to cover the expected range of samples contamination.

Bacteriological examination

Total aerobic bacterial count (APHA, 1984)

Enterobacteriaceae count (AOAC, 1990).

Staphylococcus aureus count (ICMSF, 1978).

Isolation of salmonella organisms (Koneman et al., 1994).

Chemical decontaminant and their preparation:

Lactic acid: lactic 88% L-lactic acid (Elnasr Phar. Co.) was diluted with sterile distilled water to obtain a solution containing 2% lactic acid (2.27 ml of 88% lactic acid + 97.93 ml sterile distilled water).

Antibiogram of antibiotic sensitivity test of prevalent isolates (Long, 1974).

RESULTS

Table (1) Clostridial species recovered from skin and muscles of frozen chicken carcasses.

Samples	No. of examined samples	No. of +ve samples	*C. perfringens*				*C. sporogenes*		*C. sordelli*	
			A		D					
			No.	%	No.	%	No.	%	No.	%
Neck skin	50	20	4	8	2	4	10	20	4	8
Cloaca skin swabs	50	25	6	12	2	4	12	24	5	10
Muscles of thigh	50	10	3	6	1	2	4	8	2	4
Muscles of breast	50	6	2	4	1	2	2	4	1	2
Total	200	61	15	30	6	12	28	56	12	24

Table (2) Statistical analysis results of total bacterial counts/cm^2 of frozen chicken carcasses after air freezing at -18°C (n = 50).

Duration of freezing	Aerobic bacteria			Enterobacteriaceae		
	Min.	Max.	Mean ± SE	Min.	Max.	Mean ± SI
One month	$2x10^3$	$1x10^4$	$0.21x10^4$±1.0	$3.0x10^2$	$1.3x10^3$	$1.4x10^2$±1.
2 monthes	$9.8x10^2$	$3.78x10^4$	$1.31x10^3$±5.97	$1.05x10^2$	$6.87x10^2$	$0.71x10^2$±3
3 monthes	$5.61x10^2$	$9.73x10^3$	$0.2x10^3$±1.4	5.31x10	$1.9x10^2$	1.92x10±8
Reduction %			98.60%			98.30%

Table (3) Number and percentages of frozen chicken carcasses contaminated with Staphylococcus spp. (n = 50).

Duration of freezing	Neck skin samples	Cloaca skin swabs	Muscles of thigh	Muscles of breast
One month	30 (60%)	35 (70%)	10 (20%)	8 (16%)
2 months	25 (50%)	28 (56%)	8 (16%)	7 (14%)
3 months	22 (44%)	24 (48%)	7 (14%)	6 (12%)

Table (4) Number and percentages of frozen chicken carcasses contaminated with Salmonella spp. (n = 50).

Sampling part	Neck skin		Cloaca swabs		Thigh muscles			Breast muscles
	No.	%	No.	%	No.	%	No.	%
Salmonella	5	10%	6	12%	1	2%	-	-

Table (5) Reduction percentages of bacterial count on the examined chicken carcasses using lactic acid spray 2%.

Microorganisms	Swabs	Skin excision
Aerobic bacterial count	80.0%	75.0%
Enterobacteriacea	75%	69%
Staph. spp.	90%	85%

Table (6): Antibiogram of antibiotic sensitivity of prevalent isolates of aerobic and anaerobic organisms

Antimicrobial disc	*C. perfringens* (15)			Staphylococcus spp. (25)			Enterobacteriaceae (20)			Salmonella spp. (12)		
	R	I	S	R	I	S	R	I	S	R	I	S
Chloramphenicol	0	0	15	20	5	-	3	2	15	2	-	10
	0.0	0.0	100	80.0	20.0	-	15.0	10.0	75.0	17.0	-	83.0
Tetracycline	0	0	15	3	2	20	-	2	18	5	2	5
	0.0	0.0	100	12	8	80	-	10	90	41	18	41
Penicillin-G	0	0	15	4	3	18	5	5	10	2	2	8
	0.0	0.0	100	16	12	72	25	25	50	18	18	64
Nitrofurantoin	7	3	5	15	5	5	10	2	8	3	2	7
	46	20.7	33.3	60	20	20	50	10	40	25	16	59
Oxytetracycline	6	4	5	5	5	15	3	2	15	5	5	2
	40	26	34	20	20	60	15	10	75	41	41	18
Neomycin	91	3	3	10	5	10	6	2	12	4	3	5
	60	20	20	40	20	40	30	10	60	33	26	41
Erythromycin	12	3	0	3	1	21	10	4	6	2	7	3
	80	20	0	12	4	84	50	20	30	16	59	25
Ampicillin	10	3	2	18	2	5	4	-	16	2	2	8
	66	20	14	72	8	20	20	-	80	16	16	68

R.: resistant. I.: Intermediate. S.sensitive

DISCUSSION

Nowadays, poultry meat including frozen chicken carcasses are considered to be the most popular and wholesome for human consumption than meat of other animal species which affected with various diseases.

The results in table (1) illustrates frozen chicken carcasses samples harbourd three species of colostridial organisms which are *C. perfringenes*, *C. sporogenes* and *C. sordelli*. Thus, *C. sporogenes* represent the highest rate of isolation followed by *C. perfringenes* and then *C. sordelli*. Typing of *C. perfringenes* revealed that type "A" was the most predominant than type "D" and non toxic variants and lastly *C. sordelli*. It is of interest to note the recovery rate of *C. sporogenes* which was detected in 10, 12, 4 and 2 samples out of 20, 25, 10 and 6 positive isolates of neck skin, cloaca skin swabs, muscles of thigh and muscles of breast respectively, because this organism is highly proteolytic and considered to be the main cause of spoilage of poultry products. These findings agree with those obtained by McKay and Kumchy (1969). *C. sordelli* was recorded in somewhat lower incidence than *C. sporogenes*. On the other hand, *C. perfringens* type "A" superior to type D (Table 1) which act as the main cause of food poisoning in man.

As shown in table (2) it was noticed that destructive effect of home freezing (-18°C) for 3 months on the surface flora of examined chicken carcasses. Table (2) showed the average and range of total aerobic bacterial counts per cm^2 of surveyed carcasses skin $0.21x10^4 \pm 1.0$ and $2x10^3$ to $1x10^4$ CFU after one month freezing; $1.31x10^3 \pm 5.97$ and $9.8x10^2$ to $3.78x10^4$ CFU after 2 month-freezing and $0.2x10^3 \pm 1.4$ and $5.61x10^2$ to $9.73x10^3$ CFU after 3 months freezing. From the aforementioned results, it is evident that the reduction in bacterial counts after freezing were significant as well as during storage, total aerobic bacteria counted to decrease, and the major proportion of destroyed microorganism on examined skin, caused by lethal effect of freezing was recorded in the first reading (after one month freezing) as the initial bacterial counts (before freezing) were nearly reduced to tenth, while the other two readings revealed a least successive reductions of contaminating aerobes. Therefore, a percentage of destroyed

bacteria by slow freezing, applied in this study were commonly higher than that induced by fast freezing. Also the estimated decrease 98.60%), were in agreement with that recorded by Abu-Ruwaida et al. (1996) and Mahmoud et al. (2007).

Concerning the change occurred in the counts of Enterobacteriaceae organisms on the skin surface by freezing as recorded in table (2) values were sharply and gradually decrease to be averaged by $1.4x10^2 \pm 1.10$, $0.71x10^2 \pm 3.18$ and $1.92x10 \pm 8.19$ with the range of $3x10^2$ to $1.3x10^3$, $1.05x10^2$ to $6.87x10^2$ and $5.31x10$ to $1.9x10^2$ organisms per cm^2 of chicken carcasses skin and muscles after freezing for 1, 2, 3 months respectively. At the end of frozen storage period (3 months), the initial contaminants of Enterobacteriaceae on fresh skin were reduced by 98.3% approximately. A significant decrease in Enterobacteriaceae counts on surveyed chicken skins by freezing was agree with that reported by Bolder et al. (1981).

Concerning the numbers and percentages of skin samples of neck and cloacal swabs and muscles of thigh and breast with Staphylococcus spp. In table (3) were Staphylococcus spss. Can recognized in 30 (60%), 35 (70%), 10 (20%) and 8 (16%) in neck skin samples, cloacal skin swabs, muscle of thigh and muscle of breast respectively in first month frozen and 25 (50%), 28 (56%), 8 (16% and 7 (14%) after frozen storage for 2 months and 22 (44%), 24 (48%), 7 (14%) and 6 (12%) after frozen storage for 3 months.

The results in table (4) showed that salmonella spp. were isolated from neck skin in 5 samples (10%), 6 (12%), 1 (2%) and 0 (0.0%) from neck skin, cloaca swabs, thigh and breast muscles, respectively.

Spraying of chicken carcasses with 2% lactic acid for 30 seconds (Table 5) reduce count and percentage to 80% in swabs, 75% skin excision in aerobic plate counts, 75% and 69% in Enterobacteriaceae, 90% and 85% reduced count percentage in Staphylococcus spp. and 92%, 86% reduction in count of coliforms bacteria respectively.

This may be attributed to the difference in methodology of sample collection with subsanitation which has been reported by Greer and Dilts (1992) and Mansour (2006).

Antibiogram of aerobic and anaerobic pathogens could be variable from one place and

from case to another. This may be explained to the wide use of chemotherapeutic drugs and the variation in its use which may produce new resistant forms. For this reason, one of the steps in the treatment of aerobic and anaerobic infection is the use of the propriate chemotherapeutic agent.

The kind of antibiotic should better be selected on the basis of its sensitivity which could be detected by laboratory examinations.

As shown in table (6) all tested isolates of *C. perfringens* were highly sensitive to chloramphenicol, tetracycline and penicillin G.

On the contrary, most of these isolates were highly resistant to neomycin, erythromycin and ampicillin, nearly similar observation have been reported by Nessbakken and Varoden (1975), Staphylococcus spp. were highly sensitive to tetracycline, penicillin G, erythromycin and oxytetracycline while most of these isolated were highly resistant to chloramphenicol, ampicillin and nitrofurantoin. Enterobacteriaceae spp. were highly sensitive to chloramphenicol, tetracycline, ampicillin and oxytetracycline while highly resistant to erythromycin, nitrofurantoin and moderate resistant to neomycin. Salmonella spp. were highly sensitive to chloramphenicol, penicillin G and ampicillin while moderate resistant to tetracycline, oxytetracycline and neomycin.

Most of isolated bacteria could induce spoilage of poultry meat while some of them responsible for some cases of food poisoning, whereas, the others were pathogenic for human and animals (Buss, 1980 and ICMSF, 1980).

It could be concluded that microbial colonization on frozen chicken carcasses decontaminated by lactic acid 2% spray for 30 seconds and the number of aerobic and anaerobic microorganism reduced by freezing.

REFERENCES

Abu-Ruwaida A.S., Sawaya W.N., Dashti B.H., Mourad M. and Al-Othman H.A. (1996): Microbiological quality of broilers during processing in a modern commercial slaughter house in Kuwait. J. Food Prot., 57: 887-892.

AOAC (1990): Official Methods of Analysis, Association of Official Agricultural Chemist, Inc., 15th Ed., Virginia, USA.

APHA (1984): Compendium of methods of the microbiological examination of foods. 2nd Ed. M.L. Speck, ed, Washington, D.C.; American Public Health Association.

Bolder N.M., Germs A.C. and Mulder R.W. (1981): Shelf life of frozen broilers parts. Inst. For Poultry Res., Beekbergen Netherlands. Cited after FSTA, 1982) 14: 2052.

Brown M.H., Gill C.O. and Hollingsworth J. (2000): The role of microbiological testing in systems for assuring the safety of beef. Int. J. Food Microbiol., 62: 7-16.

Buss M. (1980): Ecology of enterobacteriaceae on slaughter house meat. Fleischwirtschaft, 60 (8): 1529.

Cruickshank R.; Duguid J.P.; Marmion B. and Swain R.H. (1975): Medical Microbiology. 12th Ed., Liveingstone, Iowa, New York.

Cunningham F.C. (1987): Types of microorganisms associated with poultry carcasses in F.E. Cunningham and N.A. Cox Eds. The Microbiology of poultry meat products. Pp. 29-42 New York, Academic press.

Davis A. and Board R. (1988): Microbiology of meat and poultry. 1st Ed. Blackie Academic and professional, London.

Finegold and Martin (1988): Diagnostic Microbiology. 6th Ed. C.V. Mosby Company, Toronto, London.

Greer G.G. and Dilts B.D. (1992): Factors affecting the susceptibility of meat borne pathogens and spoilage bacteria to organic acid. Food Res. Inter., 25: 335-364.

Huffman R.D. (2002): Current and future technologies for the decontamination of carcasses and fresh meat. Meat Sci., 62: 285-294.

ICMSF (International Committee of Microbiological specification for Foods) (1978): Microorganisms in food, their significant and method of enumeration. 2nd Ed. University of Toronto press, Toronto and Buffalo's, Canada.

ICMSF (International Committee of Microbiological Specifications for Foods) (1980): Microbial ecology of food. Vol. 1. Univ., Toronto press. Toronto, Canada.

Koneman E.E., Alien S.D., Janda M.W.M., Screcken Berger P.C. and Winn W.C. (1994): Introduction to diagnostic microbiology. 4th Ed., J.B. Lippincot Co.. p. 41.

Long J.R. (1974): Studies on necrotic enteritis in broiler chickens. Dissertion Abst. Int., 35 (5): 2503.

Mahmoud A.R., Mahmoud A.H. and Zakary E.M. (2007): Evaluation of quality control of frozen chicken. The second scientific conference. 25-28 January, Benha Univ.

Mansour M.E. (2006): Improving the sanitary status of chicken carcasses. M.V.Sc., Thesis, Fac. Vet. Med.. Zagazig Univ., Egypt.

McKay K.A. and Kumchy P.I. (1969): The incidence and significance of clostridial isolated from ready to eat meat and rendered products. Animal pathology division, Health of Animals branch, Canada. Animal research Institute, 33: 307-309.

Nessbakken T. and Varoden S.A. (1975): Acute mastitis caused by clostridium perfringens type "A". Nord. Vet. Med., 27 (3): 150-152.

Oakley C.L. and Warrack G.H. (1953): Routine typing of Clostridium welchii. J. Hyg. Comb., 51: 103-107.

Pearson A.M. and Dutson T.R. (1997): Production and processing of healthy meat, poultry and fish products. 1st Ed. Blackie Academic and Professional London.

Richardson and Mead G.C. (1999): Poultry Meat Science. P. 3 CABI publishing, New York, USA.

Waldroup A.I. (1996): Contamination of raw poultry with pathogens. World Poultry Sci. J., 52: 7-25, 84, 87, 90, 93.

Zahra A. (2001): Microbial association in cool stored poultry. M.V.Sc., Thesis (Meat hygiene) Zagazig Uni.

التقييم الميكروبيولوجى وتحسين الحالة الصحية لذبائح الدجاج المجمدة

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تم تجميع مائة عينة عشوائية من الدجاج المجمد المعروض للبيع بمحافظة القاهرة وذلك لتقييمها من الناحية الصحية. تم تقسيمها الى مجموعتين (50 لكل منها)

تم اجراء الفحص البكتيرى اللاهوائى لعدد خمسون عينة والمجموعة الثانية تم حفظها فى درجة -18°م لمدة 1، 2 ، 3 شهور وفحصها لكل من العدد الكلى للبكتريا ، الميكروبات المعوية ، ميكروب المكور العنقودى الذهبى وميكروب السالمونيلا. وكانت المعزولات من المجموعة الأولى كلوستريديا سبوروجينز 2 بيرفرينجيز سورديللى بنسب 15٪ ، 12٪ و 12٪ على التوالى. بينما فى المجموعة الثانية كان متوسط العدد الكلى للبكتيريا/سم^2 من لحوم الدواجن المجمدة (-18°م) بعد شهر $0.21\times10^4 \pm 1.0$، بعد شهرين كانت $1.31\times10^3 \pm 5.97$ وبعد ثلاثة أشهر $0.2\times10^3 \pm 1.4$ وانخفض العدد الكلى بنسبة 98.6٪ بعد نهاية فترة التجميد

وتراوح متوسط العدد الكلى للميكروبات المعوية $1.4\times10^2 \pm 1.1$، $0.71\times10^2 \pm 3.18$، $1.92\times10 \pm 8.19$/سم^2 بعد 1، 2 ، 3 شهور من التجميد. وحدث نقص فى العدد الكلى بنسبة 98.3٪ . وكانت نسب تواجد الميكروب العنقودى بعد التجميد لمدة شهر فى العينات المفحوصة هى 60٪ ، 70٪ ،20٪ و 16٪ ولمدة شهرين كانت 50٪ ، 56٪

، 16٪ و14٪ وبعد ثلاثة شهور كانت 44٪ ، 48٪ ، 14٪ و12٪ على التوالى. كما وجدت السالمونيلا فى العينات المفحوصة بنسبة 10٪ ، 12٪ ، 2٪ ولم يتم عزلها من عينات الصدر. كما أجرى اختبار حساسية للمعزولات لثمانية من المضادات البكتيرية وكانت الحساسية بنسب متفاوتة. وبتطبيق حامض اللاكتيك بنسبة 2٪ لمدة 30 ثانية على العينات المفحوصة حدث انخفاض للتجمعات البكتيرية بنسبة وصلت الى 92٪.

وقد تم مناقشة الأهمية الصحية لهذه المعزولات وتحسين جودة الدواجن المجمدة المعروضة للبيع بالأسواق