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Effects of Different Storage Period and Temperature on Microbiological Quality Parameters of Chicken Meats

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Abstract

Purpose: In this study, it is purposed that microbiological analysis of 30 pieces of chicken meat samples which obtained from different retail outlets of Canakkale province with regard to hygiene and sanitation at different times cold and frozen storage and presence of some food pathogens and antimicrobial resistant profiles of different isolated bacteria groups were determined.

Methods: 30 chicken parts (15 legs, 5 wings and 10 breasts) were used in the study. Samples brought to the laboratory via cold chain. Microbial enumeration was enumerated by means of most probable number method. All of the isolated bacteria were identified on species-basis by utilizing tests.

Results: All analyzed chicken samples were exposed to microbial contaminations. Furthermore, it is determined that there has been a significant increase of microbial hygiene and sanitation indicator bacteria groups in 3. and especially 6. day. However, *E.coli*, *C.freundii*, *Proteus* sp. and important food pathogens bacteria *Listeria* sp., *Pseudomonas* sp., *Salmonella* sp. have been isolated from all chicken samples. And isolated bacteria were seen to have Multiple Antibiotic Resistant against different 10 antibiotics values ranging from 0.21 to 0.38.

Conclusion: High levels of pathogens and indicator microorganisms prove that the production process from the slaughter to the storage is unsanitary, and that hygiene regulations are not observed in spite of technological advancements.

Key words: Antibiotic resistant, chicken meat, microbiological quality

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Introduction

Chicken meat is among the most consumed food in the world, because it is low in fat and high in protein. But epidemiological records suggest that it is among the major causes of the food poisoning, primary reason of which is

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known to be microbial contamination due to the unsanitary production and storing conditions (1). Therefore, it is of utmost importance to monitor the length of storage and the storage temperature. Recently, the use of growth promoters and of antibiotics to eradicate infections has been the focus of a growing concern. This results in the development of the genes of resistance to antibiotics and accumulation of antibiotics, which is very hazardous to human health (2). Hence, it is crucial to reveal the antibiotic resistance profiles of the bacteria isolated from chicken meat and to determine the appropriate antibiotics for the treatment of infections.

The present study aims to monitor the microbial change occurring in chicken meats supplied from the market at varying storage temperatures and periods and to lay bare the antibiotic resistance profiles of the bacteria isolated from these samples.

Materials and Methods

Supply of Chicken Meat

30 chicken parts (15 legs, 5 wings and 10 breasts) were used in the study. Samples brought to the laboratory via cold chain were put in temperature-controlled condition on the 1st day, and stored at + 4 ºC for 3 and 6 days and at – 20 ºC for 1 and 3 months.

Microbiological Analyses

Microbial enumeration [Total Mesophilic Aerobic Bacteria (TMAB) and Total Psychrophilic Aerobic Bacteria (TPAB)] and isolations [*E.coli, Citrobacter freundii, Proteus* sp., *Listeria* sp., *Salmonella* sp. and *Pseudomonas* sp.] were carried out by using standard microbiological methods. Total Coliform (TC), Faecal Coliform (FC) and Faecal Enterococcus (FE) were enumerated by means of most probable number method (3). All of the isolated bacteria were identified on species-basis by utilizing tests

specified in Bergey's Manual of Determinative Bacteriology (4).

Antibiotic Resistance Profiles and Multiple Antibiotic Resistances (MAR)

For the determination of antibiotic resistance profiles of isolated bacteria against to the antibiotics Erythromycin (E15 µg/mL), Furazolidone (FR50 µg/mL), Chloramphenicol (C30 µg/mL), Ampicillin (A10 µg/mL), Oxytetracycline (O30 µg/mL), Kanamycin (K30 µg/mL), Gentamicin (G10 µg/mL), Cefoxitin (CN30 µg/mL), Cefmetazole (CMZ30 µg/mL) and Cefotaxime (CE30 µg/mL) were examined by using Disc Diffusion method (5). MAR indices were determined via Krumperman (6).

Results

Microbiological variation of chicken samples, isolated bacteria groups and their antibiotic resistant profiles are presented in Table 1, 2, and 3, respectively.

The microbiological enumeration results indicated that as the cold storing period increases (1st, 3rd, and 6th day at +4 ºC), so do the number of the microorganisms as indicators of hygiene and sanitation. On the other side, it was discovered that the number decreased in the deep freezing storage (1 and 3 months at -20 ºC). In consideration of the length of storage, the lowest bacteria

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load was detected at - 20 °C for 1 months, while the highest was found to grow at + 4 °C for 6 days (Table 1).

Table 1. Periodically isolated microorganisms rate (log cfu/g)

Microorganisms	Samples	Storage period (log_{10} kob/g) (X±Sx)				
		1. day	3. day	6. day	1. month	3. month
TPAB	Leg (n=15)	3.95 ± 0.77	4.78 ± 0.77	5.09 ± 0.44	4.74 ± 0.53	3.89 ± 0.48
	Wing (n=5)	5.14 ± 0.55	4.92 ± 0.85	5.03 ± 0.80	4.74 ± 0.94	4.08 ± 0.71
	Breast (n=10)	4.77±1.24	4.94 ± 0.59	4.79± 0.69	4.43 ± 0.52	4.08 ± 0.46
TMAB	Leg (n=15)	3.52 ± 0.87	4.94 ± 0.44	5.20 ± 0.53	4.68 ± 0.52	3.14 ± 1.05
	Wing (n=5)	3.44 ± 0.63	4.15 ±0.54	5.27 ±0.19	2.16 ± 1.97	3.21 ±0.25
	Breast (n=10)	3.57 ±0.65	4.53 ±0.87	5.33 ±0.57	2.12 ± 0.74	3.03 ± 0.48
TC	Leg (n=15)	3.51 ± 1.14	3.66 ± 0.98	4.02 ± 0.79	1.97 ± 2.43	4.48 ± 0.58
	Wing (n=5)	3.79 ± 0.79	3.98 ± 0.64	4.69 ±0.58	3.55 ± 2.07	3.31 ± 0.66
	Breast (n=10)	3.97 ± 0.49	4.00 ± 0.76	4.45 ± 0.89	3.33 ± 2.33	3.05 ± 1.86
FC	Leg (n=15)	3.59 ± 1.13	2.89 ± 1.66	3.52 ± 1.63	2.02 ± 2.25	3.91 ± 1.33
	Wing (n=5)	3.82 ± 0.79	3.83 ± 0.50	3.13 ± 1.96	3.89 ± 2.19	3.81 ± 0.86
	Breast (n=10)	4.19 ± 0.52	4.14 ± 0.95	3.59 ± 1.44	2.63 ± 2.28	2.85± 2.08
FE	Leg (n=15)	3.09 ± 1.46	4.11 ± 0.61	3.80 ± 0.44	1.67 ± 2.14	2.42 ± 1.60
	Wing (n=5)	3.60 ± 0.78	3.58 ± 0.86	4.73 ± 0.42	2.89 ± 2.65	2.43 ± 1.43
	Breast (n=10)	2.97 ± 1.17	3.70 ±0.49	4.25 ±0.69	3.01 ± 2.18	1.74 ±1.53

n: Sample number

179 bacteria were isolated along with significant food pathogens from the samples as hygiene and sanitation indicators. Table 2 reveals that the isolated bacteria increase in number in cool storing conditions, while the numbers decrease in deep freezing.

Table 2. Bacterial species isolated from chicken samples

Period	1. day			3. day			6. day			1. month			3. month		
	L	W	B	L	W	B	L	W	B	L	W	B	L	W	B
Listeria sp.	1	-	-	2	-	-	4	1	-	-	-	-	-	-	-
Pseudomonas sp.	3	-	1	4	1	1	5	2	2	2	-	1	2	-	2
E.coli	14	4	5	5	1	9	11	2	6	2	5	5	6	1	1
C. freundii	3	2	3	3	1	2	3	1	-	1	2	3	5	3	3
Proteus sp.	2	-	1	2	-	2	4	1	3	3	3	-	-	-	-
Salmonella sp.	2	-	-	3	1	1	1	1	1	-	-	2	-	-	-
Sample total	25	6	10	19	4	15	28	8	12	8	10	11	13	4	6
Total	41			38			48			29			23		

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The resistance profiles of the isolated bacteria were tested against 10 different antibiotics; it was found out that they exhibited the highest resistance against E15, and the lowest against G10 antibiotics. MAR indices obtained from the entire sampling were found to oscillate between 0.21 and 0.38. The highest MAR indices were obtained from *C.freundii* (0.38) and *Salmonella* sp. (0.38), and the lowest from *E.coli* (0.21) and *Proteus* sp. (0.21) strains (Table 3).

Table 3. Antibiotic resistant percent of isolated bacteria and MAR index

	Antibiotic resistance rate (%)					
Antibiotics	*E.coli* (n =77)	*C.freundii* (n= 35)	*Proteus* sp. (n= 21)	*Pseudomonas* sp. (n= 26)	*Salmonella* sp. (n=12)	*Listeria* sp. (n=8)
E15	28 (36.36)	17 (48.57)	0 (0)	19 (73.07)	6 (50)	2 (25)
FR50	25 (32.46)	0 (0)	0 (0)	9 (34.61)	2 (16.66)	3 (37.5)
C30	12 (15.58)	12 (34.28)	0 (0)	11 (42.30)	7 (58.33)	1 (12.5)
A10	15 (19.48)	26 (74.28)	12 (57.14)	10 (38.46)	9 (75)	8 (100)
O30	39 (50.64)	16 (45.71)	12 (57.14)	18 (69.23)	3 (25)	4 (50)
K30	14 (18.18)	24 (68.57)	11 (52.38)	7 (26.92)	1 (8.33)	2 (25)
G10	0 (0)	0 (0)	0 (0)	0 (0)	2 (16.66)	2 (25)
CN 30	29 (37.66)	26 (74.28)	11 (52.38)	11 (42.30)	10 (83.33)	1 (12.5)
CMZ30	0 (0)	15 (42.85)	0 (0)	5 (19.23)	3 (25)	1 (12.5)
CE30	0 (0)	0 (0)	0 (0)	1 (3.84)	3 (25)	1 (12.5)
MAR index	0.21	0.38	0.21	0.35	0.38	0.28

n:Number of isolated bacteria

Discussion

In monitoring the hygiene and sanitation of the chicken meats, a high TMAB number refers to a high risk of spoilage, while a low level of TMAB indicates inefficient storing conditions (1). In a study carried out in Van (7), TMAB numbers in legs and breasts were found to be $1.4x10^6$ cfu/g and $1.0x10^7$ cfu/g, respectively. Álvarez-Astorga et al., (8) found that the numbers of psychrophilic bacteria range from 5.96 to 7.87 log10 cfu/g. Moreover, Chaiba et al. (9) discovered that the number of Mesophilic bacteria in breast was between 4.74 ± 0.34 and 6.18 ± 0.55 log cfu/g, and that of psychrophilic bacteria between 4.02 ± 0.35 and 4.48 ± 0.27 log cfu/g.

As per the Turkish Food Codex Regulation (10), the maximum number allowed is 4.69 – 5.69 log cfu/g. The values obtained in the present study were found to fall in this range. Besides, while the results are similar to those of Chaiba et al. (9), it was found out that the rates of the microorganisms were lower than those obtained by the other researchers. On the other side, the presence of faecal coliform and enterococcus is the indicator of faecal contamination during the slaughter and unsanitary storing conditions (11). Chaiba et al. (9), found the coliform and faecal coliform load to

be 4.64 log kob/g and 3.89 log cfu/g, while Pipova et al. (12) enumerated coliform and enterococcus to be 10^5- 10^6 log cfu/g and 10^3- 10^5 log cfu/g. The results obtained in this study are similar to those of Chaiba et al. (9), but lower than those of Pipova et al. (12).

E.coli, *C. freundii*, and *P. vulgaris* which are the basic indicators of hygiene, were intensively isolated from the samples, which indicate the lack of sanitary conditions during the slaughter, production and storage. The most isolated bacteria in the study are *E.coli*. This overlaps the findings by Jimenez et al. (11), who found that the predominant species in the slaughtering process is *E.coli*. Besides, sporadic isolation of *Pseudomonas* sp., causing most of the spoilage cases observed in poultry meat stored in refrigerator and *Salmonella* sp. and *Listeria* sp., main cause of food poisoning, increases the likelihood of cross contamination with chicken meat (1).

Literature review yielded that existing studies substantially focus on microbial load of chicken meat, and that there is exiguous research on the storage of such products in house refrigerators and deep-freezers and on the associated microbiologic load (7-9).

Therefore, the main goal of the current study is to determine the effects of storage period and temperature on microbial load. In contrast to the related literature, the obtained data showed that the number of the microorganisms increased when chicken meat was stored at + 4 ^oC for 3 and 6 days. As Bhoyar et al. (13) expressed, the bacterial load decreased when the samples were kept at -20 ^oC for 1 and 3 months. Likewise, the results of the present study showed that freezing is more efficient in reducing the number of microorganisms than the cold storage.

In the modern chicken meat industry, antibiotics are used to prevent and treat infectious diseases in animals and as antimicrobial growth regulator. When those products are treated with antibiotics in low doses for a long period of time, some bacteria species develop resistance to and subsequently become immune to those antibiotics (14).

Bacteria have grown more resistant over the last decade; therefore today multi-resistant bacteria have become a global issue. In the current study, the antibiotics used in the current study were chosen out of the most commonly used antibiotics in Turkey against the human and animal diseases induced by *Salmonella* sp., *Listeria* sp., *E.coli*, and *Pseudomonas* sp.

High resistance of the isolated bacteria to multiple antibiotics points out that poultries have overly been exposed to antibiotics with different components via feed and water. The MAR indices obtained in the study are significant to show the treatment frequency of the antibiotic. MAR index value of 0.2 and over signifies the high doses of antibiotics or exposition to such agents or media. MAR index value of less than 0.2 refers to less or no antibiotic use (15). The present research revealed high levels of MAR indices, namely 0.21 – 0.38, which is substantiated by Suresh et al.'s (2) finding of the use of high doses of antibiotics in feeding poultry. Besides, similarities between MAR indices of *E.coli* (0.21) and *Proteus* sp. (0.21), and *Salmonella* sp. (0.38) and *C.freundii* (0.38) show that both strain groups have

originated from the same contamination source (2).

The results obtained both in the present study and other studies in the related literature support the idea that chicken meat serves as an intermediate host for antibiotic-resistant bacteria between animals and humans, and that food-induced bacteria are among the sources of resistance genes.

As a conclusion, high levels of pathogens and indicator microorganisms prove that the production process from the slaughter to the storage is unsanitary, and that hygiene regulations are not observed in spite of technological advancements. Higher amount of *E. coli* defined as the indicator of faecal contamination and index microorganism is a proof of the failure to provide sanitary conditions. Moreover, it is crucial to inform the retailers and the end users of the importance of the proper storing conditions and the inhibitive effect of different storage conditions and lengths on bacterial growth. It is also important to encourage them to break their habits and to consume the products right after the purchase. On the other hand, high levels of antibiotic resistance in the isolated bacteria require the implementation of some regulatory rules to control the use of antibiotics and the like in animal husbandry.

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