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# The effects of hypochlorous acid on microflora of chicken meat

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**Abstract**
Contamination of poultry meat with foodborne pathogens as well as spoilers remains an important issue. In present study we have tested the effects of hyphochlorous acid on microflora of chicken samples stored at 2-8ºC during 7 days. Hyphochlorous acid was sprayed on 5 chicken samples and 5gr of same samples without hyphochlorous acid treatment were used as control. Total DNA was extracted from samples and 16S r RNA sequences were done by next gene sequencing. A total of 11 genus (*Acinetobacter* sp, *Arthrobacter* sp, *Brochothrix* sp, *Carnobacterium* sp, *Flavobacterium* sp, *Myroides* sp, *Pseudomonas* sp, *Psychrobacter* sp, *Janthinobacterium* sp, *Oxalobacteraceae* sp, *Vagococcus* sp) and 19 species (*Brochothrix thermospacta, Carnobacterium maltaromaticum, Carnobacterium divergens, Flavobacterium antarcticum, Oxalobacteraceae bacterium, Psychrobacter cryohalolentis, Psychrobacter urativorans, Psychrobacter glacincola, Pseudomonas fragi, Pseudomonas psychrophila, Pseudomonas chlororaphis, Pseudomonas libanensis, Pseudomonas jesseni, Pseudomonas fluorescens, Pseudomonas azotoformans, Pseudomonas weihenstephanensis, Vagococcus salmoninarum, Vagococcus fessus, Vagococcus fluvialis*) were detected from the samples. While *Oxalobacteraceae bacterium,* and *Pseudomonas azotoformans* were detected only from hyphochlorous acid applied samples, *Flavobacterium antarcticum*, and *Pseudomonas fluorescens* were detected only from non hyphochlorous acid applied samples. The results of our study showed that except minor differences, hyphochlorous acid treatment did not change the micro biome of chicken meat samples and hyphochlorous acid application did not prevent spoilage.

**Keywords:** chicken, hypochlorous acid, micro biome, spoilage

## Introduction
Proteins are highly important for human nutrition. In general meat and meat products are important source of protein. Especially chicken meat is accepted healthier because of high protein and less lipid content. Production of poultry meat in Europe during 2016 was around 14 million tons and in Turkey approximately 2 million tons [1] although chicken meat is an important protein source in the world as well as in Turkey microbial contamination and spoilage of chicken meat is more common than red meat.

Meat is one of the most perishable foods and necessary to take precaution to extent their shelf life. Meat is like a culture medium for microorganism due to its chemical composition and microbial growth leads to meat deterioration or spoilage. If the number of the microorganism reaches certain level in raw meat, human consumption is not suitable for spoiled meat [2, 3, 4]. Many factors play role for the initial number of microorganism in the meat including physiological status of the animal and, contamination into slaughterhouses and during processing, as well as temperature and other conditions of storage during distribution [4]. Many bacteria are responsible for the meat spoilage especially aerobic psychro trophic Gram-negative bacteria, yeasts, molds, hetero fermentative lactobacilli, and spore-forming bacteria [5]. Microbial contamination reduces the shelf-life of foods and increases the risk of foodborne illness. Traditional methods of preserving foods from the effect of microbial growth include thermal processing, drying, freezing, refrigeration, irradiation, modified atmosphere packaging, and adding antimicrobial agents or salts. So they use natural or synthetic antimicrobial agent's especially essential oils, plant extracts and chemical compounds. Recently, food safety became a fundamental concern for both consumers and the food industry. The main disinfectants used for carcass disinfection in chicken establishments are chlorine, chlorine compounds, hydrogen peroxide, lactic acid, acetic acid, propionic acid, formic acid, organic acids, ozone, cetylpyridinium chloride (CPC) (a quaternary ammonium compound), trisodium phosphate and other base phosphates, sodium carbonate, sodium hydroxide, sodium bisulfate, potassium sorbate, gluter aldehyde and sodium hypochlorite's [7-14]. These disinfectants are usually added to the process water used in one of the carcass processing steps such as hair removal, scalding, washing and cooling and are applied by spraying or dipping methods. In vitro, hypochlorous acid is highly active against all bacterial, viral, and fungal human pathogens and it was shown that a small amount of hypochlorous acid is able to kill spore-forming and non-spore bacteria in a short time period [15, 16, 17]. In the present study the effects of hypochlorous acid application on spoilage of chicken meat samples was evaluated.

## Material and Methods
In our study, we purchased 5 different chicken meat samples from retailed markets. Hyphochlorous acid was acquired pharmacy (Crystaline® NPH Turkey). Spray form of

hyphochlorous acid is used for application of hyphochlorous acid on one piece of 5gr chicken meat samples, until all meat surface get wet by spraying.

**DNA isolation:** Bacterial genomic DNA was isolated using phenol chloroform method with some modifications [18]. Shortly on 5 gr chicken meat sample 4ml distilled water was added, vortexed and 1 ml of supernatant was transferred to a 1.5 ml sterile centrifuge tube. Samples were centrifuged 5 min at 13000 rpm. Supernatant was discarded and pellet was homogenized in 200 µl resuspension buffer (50mM Tris pH:8 10mM EDTA,%7 sucrose). Resuspended pellets were supplemented with 2 µl lysostaphin of 1mg/ml, and 5 µl lysozyme of 20mg/ml and incubated at 37°C for 30 min. After incubation 20 µl EDTA (0.5 M), 26 µl SDS (%10) and 5 µl Proteinase K of 20mg/ml were added to lysis solution and further incubated 30 min at 37°C. Equal volume of phenol: chloroform (1:1) was added to the lysis solution and after centrifugation upper phase was transferred to a 1.5 ml micro centrifugation tube. Sodium acetate 3M (1/10 volume), pH 5.2, and 300 µl isopropanol was added and incubated at -20$^0$C for 15 min. Then centrifuged at 15 000 rpm for 20 minutes at 4˚C. Supernatant discarded and 300 ml 70% alcohol was added to pellet. After centrifugation at 15,000 rpm for 5 min at 4˚C the supernatant was removed, pellet was dried and Resuspended with 50 µl distilled water and then 1 µl RN ase of 10mg/ml (Thermo Fisher USA) was added. Genomic DNA was analyzed by electrophoresis using %1 agarose gel with Safe View ™ Classic (ABM Canada) and visualized under UV [18].

**Micro biome Analysis**

Micro biome analysis was done by sequencing after amplification of 16S r RNA using V1-V8 (27 F and R1407) primers. Sequence analysis was done by GATCH Biotech Company (Constance, Germany) using PP acbiors II.

**Results**

Micro biome analysis results of hyphochlorous acid applied and non-applied samples (control samples) were compared. The most common bacteria found in control sample hyphochlorous acid applied of the first company (A) was *B. thermospacta* with a rate of 66.3%, and 65.23%, respectively. Other species detected are given in Table 1. In chicken meat samples from company B, *B. thermospacta* was also the most common species with 69.36% and 69.92%, in control and hyphochlorous acid applied samples, respectively (Table 2).

**Table 1:** Incidence of bacteria in the micro biome of hyphochlorous acid applied (AH) and non-applied (AS) chicken meat sample A

| AS | | | AH | | |
|---|---|---|---|---|---|
| **Phylum (%)** | **Species** | **% Rate** | **Phylum (%)** | **Species** | **% Rate** |
| Actinobacteria (1.66) | *Arthrobacter* sp | 1.66 | Actinobacteria (1.95) | *Arthrobacter sp.* | 1.95 |
| Firmicutes (69.82) | *Brochothrix thermospacta* | 66.27 | Firmicutes (68.02) | *Brochothrix thermospacta* | 65.23 |
| | *Brochothrix sp* | 1.29 | | *Brochothrix sp.* | 1.28 |
| | *Carnobacterium maltoromaticum* | 1.13 | | *Vagococcus salmoninarum* | 1.51 |
| | *Vagococcus salmoninarum* | 1.13 | | | |
| Proteobacteria (14.16) | *Acinetobacter* sp | 4.03 | Proteobacteria (10.71) | *Psychrobacter glacincola* | 0.67 |
| | *Psychrobacter cryohalolentis* | 1.77 | | *Acinetobacter sp* | 3.01 |
| | *Psychrobacter urativorans* | 1.34 | | *Psychrobacter sp.* | 3.18 |
| | *Psychrobacter sp* | 7.02 | | *Psychrobacter cryohalolentis* | 2.68 |
| | | | | *Psychrobacter urativorans* | 1.17 |
| Unidentified Bacteria (14.80) | | 14.80 | Unidentified Bacteria (19.32) | | 19.32 |

**Table 2:** Incidence of bacteria in the micro biome of hyphochlorous acid applied (BH) and non-applied (BS) chicken meat sample B

| BS | | | BH | | |
|---|---|---|---|---|---|
| **Phylum** | **Species** | **% Rate** | **Phylum** | **Species** | **% Rate** |
| Bacteroidetes (3.13) | *Myroides sp.* | 3.13 | Bacteroidetes(2.91) | *Myroides sp* | 2.91 |
| Firmicutes (83.88) | *Brochothrix thermospacta* | 69.36 | Firmicutes (81.28) | *Brochothrix thermospacta* | 69.92 |
| | *Brochothrix sp.* | 1.71 | | *Brochothrix_sp* | 1.13 |
| | *Carnobacterium maltoromaticum* | 3,98 | | *Carnobacterium maltoromaticum* | 2.26 |
| | *Vagococcus salmoninarum* | 7.63 | | *Vagococcus fessus* | 0.71 |
| | *Vagococcus fessus* | 0,63 | | *Vagococcus fluvialis* | 0.54 |
| | *Vagococcus sp* | 0.57 | | *Vagococcus salmoninarum* | 5.65 |
| | | | | *Vagococcus sp.* | 1.07 |
| Proteobacteria (3.02) | *Pseudomonas chlororaphis* | 1.71 | Proteobacteria (3.45) | *Pseudomonas chlororaphis* | 1.72 |
| | *Pseudomonas sp* | 1.31 | | *Pseudomonas psychrophila* | 1.19 |
| | | | | *Janthinobacterium sp* | 0.54 |
| Unidentified Bacteria | | 9.97 | Unidentified Bacteria | | 12.36 |

Hyphochlorous acid applied and non-applied samples, company (C) samples also had high rates of *B. thermospacta*, 73.7% and 64.68%, respectively (Table 3).

**Table 3:** Incidence of bacteria in the microbiome of hyphochlorous acid applied (CH) and non-applied (CS) chicken meat sample C

| CS | | | CH | | |
|---|---|---|---|---|---|
| **Phylum** | **Species** | **% Rate** | **Phylum** | **Species** | **% Rate** |
| Actinobacteria(0.75) | *Arthrobacter sp* | 0.75 | | | |
| Firmicutes (78.21) | *Brochothrix thermospacta* | 73.7 | Firmicutes(67.87) | *Brochothrix thermospacta* | 64.68 |
| | *Brochothrix sp.* | 0.95 | | *Brochothrix sp* | 1.18 |
| | *Carnobacterium maltoromaticum* | 2.36 | | *Carnobacterium maltoromaticum* | 2.01 |
| | *Vagococcus salmoninarum* | 1.20 | | | |
| Proteobacteria (11.1) | *Pseudomonas chlororaphis* | 5.75 | Proteobacteria (42.34) | *Pseudomonas chlororaphis* | 12.08 |
| | *Pseudomonas sp* | 2.60 | | *Pseudomonas sp* | 4.90 |
| | *Pseudomonas fragi* | 1.10 | | *Pseudomonas fragi* | 1.30 |
| | *Pseudomonas psychrophila* | 1.65 | | *Pseudomonas psychrophila* | 1.71 |
| Unidentified Bacteria | | 9.94 | Unidentified Bacteria | | 10.96 |

When we compared results for hyphochlorous acid applied and control samples of the fourth company (D) *B. thermospacta* were the most detected bacteria with 23.04%, and 32.17%, respectively (Table 4).

**Table 4:** Incidence of bacteria in the microbiome of hyphochlorous acid applied (DH) and non-applied (DS) chicken meat sample D

| DS | | | DH | | |
|---|---|---|---|---|---|
| **Phylum** | **Species** | **% Rate** | **Phylum** | **Species** | **% Rate** |
| Firmicutes (42.38) | *Brochothrix thermospacta* | 32.17 | Firmicutes(37.18) | *Brochothrix thermospacta* | 23.04 |
| | *Carnobacterium divergens* | 1.38 | | *Carnobacterium divergens* | 1.51 |
| | *Carnobacterium maltoromaticum* | 4.88 | | *Carnobacterium maltoromaticum* | 7.90 |
| | *Vagococcus salmoninarum* | 4.15 | | *Vagococcus salmoninarum* | 4.18 |
| | | | | *Vagococcus fessus* | 0.55 |
| Proteobacteria (36.57) | *Pseudomonas chlororaphis* | 5.37 | Proteobacteria (44.78) | *Pseudomonas chlororaphis* | 10.98 |
| | *Pseudomonas sp* | 4.25 | | *Pseudomonas sp* | 4.83 |
| | *Pseudomonas fragi* | 2,50 | | *Pseudomonas fragi* | 5.54 |
| | *Pseudomonas psychrophila* | 2.18 | | *Pseudomonas psychrophila* | 1.91 |
| | *Pseudomonas jesseni* | 1.97 | | *Pseudomonas jesseni* | 1.66 |
| | *Psychrobacter sp.* | 2.55 | | *Psychrobacter sp.* | 3.72 |
| | *Acinetobacter sp* | 14.83 | | *Acinetobacter sp* | 12.38 |
| | *Pseudomonas fluorescens* | 1.70 | | *Janthinobacterium sp* | 2.31 |
| | *Pseudomonas libanensis* | 1.22 | | *Oxalobacteraceae bact..* | 0.75 |
| | | | | *Pseudomonas azotoformans* | 0.70 |
| Unidentified Bacteria | | 20.85 | Unidentified Bacteria | | 18.04 |

Hyphochlorous acid applied and control samples of the fifth company (E) *B. thermospacta* were also the most detected bacteria with 92.09%, and 58.87%, respectively (Table 5).

**Table 5:** Incidence of bacteria in the micro biome of hyphochlorous acid applied (EH) and non-applied (ES) chicken meat sample E

| ES | | | EH | | |
|---|---|---|---|---|---|
| **Phylum** | **Species** | **% Rate** | **Phylum** | **Species** | **% Rate** |
| Firmicutes (63.76) | *Brochothrix thermospacta* | 58.87 | Firmicutes(95.28) | *Brochothrix thermospacta* | 92.09 |
| | *Brochothrix sp* | 0.88 | | *Brochothrix sp* | 2.23 |
| | *Carnobacterium maltoromaticum* | 0.51 | | *Vagococcus salmoninarum* | 0.80 |
| | *Vagococcus salmoninarum* | 3.50 | | | |
| Proteobacteria (19.87) | *Pseudomonas chlororaphis* | 0.60 | Proteobacteria (0.99) | *Pseudomonas sp* | 0.99 |
| | *Pseudomonas fragi* | 2,58 | | | |
| | *Psychrobacter sp.* | 3.18 | | | |
| | *Acinetobacter sp* | 5.39 | | | |
| | *Psychrobacter cryohalolentis* | 0.55 | | | |
| | *Janthinobacterium sp* | 7.57 | | | |
| Unidentified Bacteria | | 13.69 | Unidentified Bacteria | | 3.89 |

A total of 19 species, *Brochothrix thermospacta, Carnobacterium maltaromaticum, Carnobacterium divergens, Flavobacterium antarcticum, Oxalobacteraceae bacterium, Psychrobacter cryohalolentis, Psychrobacter urativorans, Psychrobacter glacincola, Pseudomonas fragi, Pseudomonas psych rophila, Pseudomonas chlororaphis, Pseudomonas libanensis, Pseudomonas jesseni, Pseudomonas fluorescens, Pseudomonas azotoformans, Pseudomonas weihenstephanensis, Vagococcus salmoninarum, Vagococcus fessus, Vagococcus fluvialis* were detected from all samples. While *Oxalobacteraceae bacterium,* and *Pseudomonas azotoformans* were detected only from hyphochlorous acid applied samples, *Flavobacterium antarcticum,* and *Pseudomonas fluorescens* were detected only from non hyphochlorous acid applied samples.

## Discussion

Spoilage of chicken meat is a great concern of food industry as well as public health. Many studies were reported to

determine the bacteria involved in spoilage and the efficacy of the treatments to avoid spoilage.

The microbial populations associated with the meat environment are known as belonging to the groups of *Enterobacteriaceae*, lactic acid bacteria (LAB), *Brochothrix thermosphacta, Pseudomonads, Acinetobacter, Pseudomonas, Brochothrix, Flavobacterium, Psychrobacter, Moraxella, Staphylococcus,* and *Micrococcus* [19-24]. In our study, *Acinetobacter, Pseudomonas, Brochothrix, Flavobacterium, Psychrobacter* and lactic acid bacteria were detected, but *Moraxella, Staphylococcus, Micrococcus* and *Enterobacteriaceae* were not detected.

Psychrophilic *Brochothrix thermospacta* is responsible for spoilage of meat and meat products and usually associated with the deterioration of fresh meat [13-18]. Previous studies reported that, *Brochothrix thermospacta* is constitute a significant part of the spoilage microbiota in aerobic and in vacuum stored meat [31-34]. In aerobic storage at low temperatures several *Pseudomonas* species are often isolated from spoiled meat including *Pseudomonas fragi*, *Pseudomonas lundensis*, *Pseudomonas fluorescens* and *P. putida* [25, 33, 35, 36]. In our study, *P. fragi* and *P. putida* were detected from spoilage microbiota, but *P. lundensis* were not detected. In previous studies *P. fragi* was found to be lower in meat stored under modified atmosfer as well as in vacuum packaged meat [7, 8, 9].

Two members of Carno bacteria, *C. divergens* and *C. maltaromaticum* were reported to be common in spoiled meat [25]. *C. maltaromaticum* reported to be a responsible for spoilage of fresh chicken [26-28]. In our study *C. maltaromaticum* was also found in both hyphochlorous acid treated and non-treated samples.

*Janthino bacteria* are Gram-negative, motile, aerobic bacteria that are commonly isolated from soil and aquatic samples [41].

In our study, phylum of Actinobacteria, Firmicutes, Proteobacteria and Bacteriodetes were detected in chicken samples. Percentage of Firmicutes in hyphochlorous acid applied and non-applied samples were between 37.18% - 81.28% and 42.38% - 83.88%, respectively (Table 4 and 2). Among the phylums, members of Firmicutes were higher than other phylums in all samples except the sample D. Percentages of Proteobacteria in hyphochlorous acid applied and non-applied samples were between 3.45% - 44.78% and 3.02% - 36.57%, respectively (Table 4 and 2). In previous studies it was reported that, as the result of our study, members of Firmicutes were common in deterioration microbiota [41, 42, 43, 44]. It was reported that members of Firmicutes were proportion of 50-90% studies in cecum microbiota [42, 43]. In our study, members of Firmicutes were more common except hyphochlorous acid applied sample D in which Proteobacteria percentage was slightly higher. In another study, *Salmonella* spp. was detected from 32(8%) out of the 400 collected samples chicken carcass in Turkey [45].

In our study a total of 11 genus (*Acinetobacter* sp, *Arthrobacter* sp, *Brochothrix* sp, *Carnobacterium* sp, *Flavobacterium* sp, *Myroides* sp, *Pseudomonas* sp, *Psychrobacter* sp, *Janthinobacterium* sp, *Oxalobacteraceae* sp, *Vagococcus* sp) and 19 species (*Brochothrix thermospacta, Carnobacterium maltaromaticum, Carnobacterium divergens, Flavobacterium antarcticum, Oxalobacteraceae bacterium, Psychrobacter cryohalolentis, Psychrobacter urativorans, Psychrobacter glacincola, Pseudomonas fragi, Pseudomonas psych rophila, Pseudomonas chlororaphis, Pseudomonas libanensis, Pseudomonas jesseni, Pseudomonas fluorescens, Pseudomonas azotoformans, Pseudomonas weihenstephanensis, Vagococcus salmoninarum, Vagococcus fessus, Vagococcus fluvialis*) were detected from the samples. While *Oxalobacteraceae bacterium, Janthinobacterium* sp, and *Pseudomonas azotoformans*, were detected only from hyphochlorous acid applied samples, *Flavobacterium antarcticum*, and *Pseudomonas fluorescens* were detected only from non hyphochlorous acid applied samples. In conclusion although minor differences were found between hyphochlorous acid applied and non-applied samples, hyphochlorous acid application did not prevent the spoilage of chicken meat.

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