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Detection the enterotoxin producing capacity of coagulase positive *Staphylococcus* by EIA (Enzyme Immuno Assay) isolated from turkey meat*

Serhat KILIÇ[1], Ozlem KUPLULU[1]

[1] Ankara University, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology, 06110, Diskapi, Ankara, Turkey.

Summary: A total of 52 turkey meat samples (including 39 legs and 13 wings), of three national companies were obtained from different markets in Ankara and were examined for the presence of enterotoxigenic coagulase positive staphylococci by EIA (Enzyme immuno assay). Average micrococci/staphylococci count of the samples was 6.3 x 10^2 cfu/g with a minimum of 1.0x10^2 cfu/g and a maximum of 4.3x10^6 cfu/g. Five of the samples (9.61 %) had coagulase positive staphylococci with the following counts; minimum 1.0x10^2 cfu/g, maximum 1.3x10^4 cfu/g, and average 9.3x10^2 cfu/g. Thirteen out of 241 (5.3 %) isolates were found coagulase positive. Four of the coagulase positive isolates were enterotoxigenic and two of these had only type C, one isolate had only type B and one isolete had both B and C type enterotoxin production ability. In conclusion, the examined turkey meats were found to be partially contaminated with enterotoxin producing coagulase positive staphylococci. Elimination of enterotoxigenic staphylococci from turkey meat is very important to protect public health. This can be substantially achieved by the establishment and management of poultry slaughterhouses, which apply systems such as GMP, HACCP and have general hygienic practices

Key words: Coagulase positive staphylococci, EIA, enterotoxin, turkey meat,

Hindi etlerinden izole edilen koagulaz pozitif stafilokoklarda enterotoksin oluşturma yeteneklerinin EIA (Enzyme Immuno Assay) ile belirlenmesi

Özet: Çalışmada 3 farklı ulusal firma tarafından üretilen ve Ankara'da çeşitli marketlerden alınan 39 but ve 13 kanat olmak üzere toplam 52 hindi etinden izole edilen koagulaz pozitif stafilokokların enterotoksin oluşturma kabiliyeti EIA (Enzyme Immuno Assay) yöntemi ile araştırılmıştır. Analizler neticesinde 52 hindi etinin minimum 1.0 x 10^2 kob/g, maksimum 4.3 x 10^6 kob/g ve ortalama 6.3 x 10^2 kob/g düzeyinde mikrokok/stafilokok ile kontamine olduğu belirlenmiştir. Örneklerden 5'inde (% 9.61) minimum 1.0 x 10^2 kob/g, maksimum 1.3 x 10^4 kob/g ve ortalama 9.3 x 10^2 kob/g düzeyinde koagulaz pozitif stafilokok tespit edilmiştir. Örneklerden izole edilen 251 mikrokok/stafilokok izolatının 13'ünün (% 5.3) koagulaz pozitif olduğu saptanmıştır. Koagulaz pozitif stafilokoklardan 4'ünün enterotoksin oluşturduğu, bunlardan 2'sinin tip C, 1'inin tip B ve 1'inin hem tip B hemde tip C toksin üretebildiği belirlenmiştir. Sonuç olarak analiz edilen hindi etlerinin koagulaz pozitif stafilokoklar ile kısmen kontamine olduğu bulunmuştur. Halk sağlığının korunması açısından hindi etlerinden koagulaz positive stafilokokların elimine edilmesi önem taşımaktadır. Bu da temel olarak HACCP ve GMP sistemlerini uygulayan ve genel hijyen kurallarına uyan kanatlı kesimhanelerinin kurulması ve işletilmesi ile gerçekleştirilebilir.

Anahtar sözcükler: EIA, enterotoksin, hindi eti, Koagulaz pozitif stafilokok.

Introduction

Staphylococci are important bacteria in human and animal diseases. Coagulase positive and/or coagulase negative staphylococci can be also etiological agents of diseases. Additionally, *Staphylococcus aureus* is the predominant cause of staphylococcal food poisoning, resulting from ingestion of a group of preformed staphylococcal enterotoxins (3).

A number of different zoonotic and pathogenic agents are present in poultry. Also poultry meat can get cross contaminated with these agents especially during slaughter, preparation, cooking and storage processes, and food poisoning and intoxication can occur in humans by the ingestion of these contaminated foods (4). Result of epidemiological studies show that staphylococcal food poisoning is among the most prevalent causes of gastro enteritis worldwide (7, 18).

Especially in defeathering process, rubber fingers are the important contamination source of *S. aureus* and they are in contact with poultry carcass. *S. aureus* may

* This assay was summarized from master thesis.
* Bu çalışma yüksek lisans tezinden özetlenmiştir.

survive in these machines for a long time, and effective cleaning and sanitation of these rubbers after every processing period is difficult. Also these rubbers may be abraded and cracked after long using periods. Therefore, *S. aureus* may penetrate through these defaults and protect themselves from outer sanitation agents (11).

This study aims to detect enterotoxin producing coagulase positive staphylococci by EIA from turkey meat

Materials and Methods

Samples: A total of 52 packaged turkey meat samples, comprised of 39 fresh drumsticks and 13 frozen wings, which were produced by 3 main national retail turkey meat producers, were collected from supermarkets in Ankara between September and December 2006

All turkey meat samples were collected from different supermarkets in Ankara in their original packages and transferred to the laboratory in cooler bags.

Bacterial strain: S. aureus ATCC 25923 was used as positive control for confirming the appearance of colonies in plate and coagulase test.

Isolation of micrococci / staphylococci: The technique described by Baumgart (2) was used to isolate *Micrococcus* and *Staphylococcus*. Samples were taken onto agar plates and then coagulase test was used for detecting coagulase positive *Staphylococcus*.

For drumstick samples 10 g portion of each sample (skin and meat) was analyzed. Frozen wing samples were stored at 4°C 24 h to defrost, and their pH values were determined. Isolates from each positive agar plate were streaked onto Tryptone Soya Agar (Oxoid CM0131). In order the confirm coagulase positive *Staphylococcus* colonies from each positive agar were taken and identified by using coagulase test, and EIA (Enzyme Immuno Assay) was used for determining toxin production.

Detecting the capability of toxin production by coagulase positive isolates: After 28 h incubation at 37°C, coagulase positive isolates on Tryptone Soy Agar plates were inoculated into Brain Hearth Infusion Broth (Oxoid CM0225), and incubated at 42°C for 52 h (9, 13, 14, 16). Ridascreen SET A, B, C, D, E (R-Biopharm AG, Darmstadt Germany) was used to detect the toxin production.

Results

Fiftyone out of 52 samples (98 %) were found to be contaminated with micrococci and staphylococci with $1.0x10^2$ cfu/g, $4.3x10^6$ cfu/g and $6.3x10^3$ cfu/g for minimum, maximum and mean levels, respectively. Coagulase positive staphylococci were determined in 5 samples (9.61 %), with a minimum $1.0x10^2$ cfu/g, maximum $1.3x10^4$ cfu/g and mean $9.3x10^2$ cfu/g. Mean micrococci and staphylococci contamination levels in drumstick and wing samples were $8.4x10^2$ cfu/g and $2.0x10^3$ cfu/g, respectively (Table 1). All coagulase positive staphylococci were isolated only from drumstick samples.

EIA results showed that 4 of 13 isolates (30.76 %) in 5 coagulase positive samples produced enterotoxin. These 4 enterotoxin positive isolates were determined from 3 of the 52 turkey meat samples (5.76 %).

It is observed that 2 (50 %) of the enterotoxin positive isolates only produce enterotoxin C, one (25 %) isolate only produced enterotoxin B and one (25 %) isolate produce both enterotoxin B and C. All toxin producing isolates were determined in 42°C incubated for 28 and 52 hour group, and no toxin producing isolates were determined in 37°C incubated group.

The pH values of turkey meat samples were determined between 5.70 and 6.80. pH values were between 5.70 - 6.57 and 5.70-6.80 in wing and drumstick samples, respectively.

Discussion and Conclusion

There is no published data up to our knowledge in literature on the level of micrococci and staphylococci in turkey meat samples. Although there are some studies on contamination level of *S. aureus* in poultry meat samples. In these studies micrococci and staphylococci counts of poultry meat were 10^2 – 10^3 cfu/g with a ratio of 35 – 92.7 % (11, 17). Similarly, Adams and Mead (1) reported contamination level of *S. aureus* in turkey carcasses in 3 different slaughterhouses were between $<10^2$ cfu/g and $>10^5$ cfu/g and also they indicated that in two slaughterhouse contamination level was 10^2 cfu/g after slaughtering, and was not higher than 10^3 cfu/g after chilling processes. Götze and Schröder (8) reported that *S. aureus* contamination ratio was 43 % and 32 % in chicken and turkey carcasses, respectively. On the other

Table 1. Micrococci /staphylococci levels and cougulase positive isolate counts in turkey meat.
Tablo 1. Hindi eti örnklerinde *Staphylococcus* düzeyinin dağılımı.

Samples	Number of samples	Micrococci /staphylococci count (cfu/g)	Number of isolates	Number of coagulase positive samples (%)	Number of coagulase positive isolates (%)	Enterotoxin producing coagulase positive isolates (%)
Drumstick	39	$8.4x10^2$	176	5 (12.82)	13 (7.38)	4 (30.76)
Wing	13	$2.0x10^3$	65	-	-	-
TOTAL	52	$6.3x10^3$	241	5 (9.61)	13 (5.3)	4 (30.76)

hand Yang et al. (15) affirmed that fresh turkey muscular tissue is not an optimal environment for *S. aureus*.

In this study, 9.61 % of the samples were positive for coagulase positive staphylococci. Mead et al. (10), reported 54 % and 31 % of 35 staphylococci which were collected from turkey and 2 broiler slaughterhouses, respectively. Similarly Bystron et al. (5) determined 11 (48 %) coagulase positive *Staphylococcus* from 23 turkey minced meat samples by API technique. Contamination level differences between these studies may be related to differences in type and hygienic condition of slaughterhouses.

Some typical colonies in BP agar plates were determined as coagulase negative staphylococci. Also, Mead et al. (10) showed that 3 of 35 isolates from turkey meat and broiler samples were coagulase negative although their isolates had typical characteristics. Evans et al. (6) detected isolates that showed typical characteristics in poultry animals.

In this study, 4 out of 13 (30.76 %) coagulase positive *Staphylococcus* isolates were determined as enterotoxigenic. Interestingly, all these enterotoxigenic isolates were isolated from the same national producer, and no eneterotoxigenic isolates were detected from other two producers. Previously, Bystron et al. (5) determined 4 out of 11 (36.3 %) isolates from turkey minced meat samples, sold in different markets were enterotoxigenic. They found that within these 4 isolates, 3 of them had B type enterotoxin genes and one of them had C type enterotoxin genes. Adams and Mead (1) found enterotoxigenic staphylococci only in 2 out of 3 turkey slaughterhouses. They reported that in slaughterhouse A, 60 % of 55 isolates only produced enterotoxin C, in slaughterhouse B 4 % of 41 isolates produced enterotoxin D and 2 % produced only enterotoxin F, and no enterotoxigenic isolates were detected from 50 isolates in the third slaughterhouse. In an experimental study Yang et al. (15) inoculated different isolates to turkey meat and found that only one of them was enterotoxigenic. Our results have similarities with these studies about toxin types of entreotoxigenic *Staphylococcus*.

In this study pH of wing meat samples was between 5.70 and 6.57 and 5.70 and 6.80 in drumstick samples. Yang et al. (15) reported that pH values in fresh turkey meat were 6.00-6.58 (mean value 6.35), in fresh breast meat pH values were 5.45-6.00 (mean value 5.91). In another study Nychas and Board (12) remarked that pH values in fresh drumstick turkey meat ranged between 6.00 and 6.60, also in breast meat values were 5.60-6.00.

These results indicate that contamination of poultry meat in slaughtering process especially occurs in defeathering section and other parts also have a great importance. Additionally, equipment and working staff have a role in contamination of poultry meat. It is concluded that HACCP and GMP systems have to be established in every part of production line. In addition, effective control, taking preventive measures and adhering to hygienic requirements on equipment and staff are crucial in controlling risk factors.

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Address for correspondence:
Özlem Küplülü
Ankara Üniversitesi Veteriner Fakültesi
Besin Hijyeni ve Teknolojisi Anabilim Dalı
Ankara.
e-mail: kuplulu@veterinary.ankara.edu.tr