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Isolation and identification of motile *Aeromonas* spp. in turkey meat*

Cetin KOCA, Belgin SARIMEHMETOGLU

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

Summary: In this study a total of 80 packaged turkey meat samples purchased from different supermarkets in Ankara including 40 leg and 40 breast meat were analysed for the presence of motile *Aeromonas* species. Totally 43 of 80 turkey meat samples (53.75 %) were determined as positive for motile *Aeromonas* spp. The distribution of the isolat were 62.5 %, 45 % in leg and breast samples, respectively. Also it is found that two different motile *Aeromonas* spp. were isolated in same breast samples; in 2 of the samples both *A. hydrophila* and *A. sobria* (11.1 %), in 1 of the samples *A. hydrophila* and *A. caviae* (5.6 %) and in 1 of the samples *A. caviae* and *A. sobria* were isolated (5.6 %). *A. hydrophila* was isolated as the most prevalent species from all of the positive samples. As a result, turkey meat that analyzed in this study were found to be contaminated with a high level of motile *Aeromonas* spp., and this is a potential risk for public health.

Key words: *Aeromonas hydrophila*, motile *Aeromonas* spp., turkey meat

Hindi etlerinden hareketli *Aeromonas* türlerinin izolasyon ve identifikasyonu

Özet: Bu çalışmada Ankara'nın değişik semtlerindeki marketlerden alınan paketlenmiş 40 göğüs kuşbaşı ve 40 but kuşbaşı örneklerinden oluşan toplam 80 hindi eti *Aeromonas* spp. varlığı yönünden incelenmiştir. Çalışma kapsamında incelenen, 80 hindi eti örneğinin 43'ünden (% 53.75), göğüs ve but kuşbaşı örneklerinden sırasıyla % 45 ve % 62.5 düzeyinde hareketli *Aeromonas* türleri izole edilmiştir. Ayrıca aynı göğüs eti örneklerinden iki farklı hareketli *Aeromonas* spp. izole edilmiştir. Buna göre iki (% 11.1) örnekten *A. hydrophila* ve *A. sobria*, bir (% 5.6) örnekten *A. hydrophila* ve *A. caviae* ve bir (% 5.6) örnekten *A. caviae* ve *A. sobria* izole edilmiştir. Örneklerden izole edilen en yaygın tür *A. hydrophila* olarak saptanmıştır. Sonuç olarak, çalışma kapsamında incelenen hindi etlerinin büyük bölümünün hareketli *Aeromonas*'larla kontamine olduğu saptanmış olup, bu durum halk sağlığı açısından potansiyel bir risk oluşturmaktadır.

Anahtar sözcükler: *Aeromonas hydrophila*, hareketli *Aeromonas* spp., hindi eti.

Introduction

Motile *Aeromonas* spp. are pathogens that cause foodborne gastroenteritis in human and extraintestinal symptoms such as; septicemia, wound infections, menengitis, endocarditis and osteomyelitis (13, 38) with a high mortality rate in immunocompromised person. As published before, the main virulance factors that have an effect on pathogenity are; extracellular toxins (enteretoxin, hemolysin and protease), structural features (pilli, S-layer, lipopolysaccharide), adhesion and invasion (6, 9, 20, 27). *Aeromonas* spp. can grow and produce toxins in refrigerated conditions (11) indicating that refrigeration can not be effective enough to control the pathogens (23). As *Aeromonas* spp. are frequently isolated from food due to their psycrotrophy and the existence of the pathogens in water, feces of humans and animals, the risks of foodborne *Aeromonas* infections are increased. Pathogens are mostly isolated from; rivers, lakes, sewers, clorinated drinking water (1, 5, 12, 17, 24, 25, 31), retail fresh vegetables (33), sea foods (1, 2, 17), red and minced meat (10, 22, 26, 29, 37), raw and pasteurized milk (21, 35), unpasteurized cheese (34). It is also widespread in fresh water fishes (14, 28, 40). There are only limited studies on determination of motile *Aeromonas* spp. in turkey meat in different countries whereas none in Turkey. The aim of this study was to determine the motile *Aeromonas* spp. in packeged turkey leg and breast meat that were offered for sale in supermarkets in Ankara.

Materials and Methods

Material

In this study, (at least 200 g of each) a total 80 samples of 40 packaged turkey leg and 40 turkey breast meat belonging to different companies which were offered for sale in different supermarkets in Ankara were used.

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

Test strain; For this aim *Aeromonas hydrophila* ATCC 7966 (Oxoid C1020 L) test strain was used.

pH meter; In order to evaluate the pH of the samples, Ignold LOT 406-MG-DXK-57/25, Nel Electronic was used.

Method

All of at least 200 g of packaged turkey leg meat in small pieces and turkey breast meat in small pieces samples, which were taken the same day that analysed for *Aeromonas* spp., were collected aseptically and carried under cold chain to labarotory. Turkey meat samples were plated on specific agars after the enrichment according to the method and the suspected colonies tested biochemically for identification (7, 30).

Isolation of Motile *Aeromonas* spp.

Enrichment; 25 g of each piece of turkey meat samples were taken, placed in sterile plastic bags, added 225 ml of 0.1 % alkaline peptone water (pH 8.4 – 8.6; Oxoid CM 9), homogenized in stomacher for 2 mins and incubated for 24 h in 28ºC incubator.

Plating and the evaluation of the suspected colonies; After the incubation, enrichment fluid streak plated to *Aeromonas* Agar (Oxoid CM 833, supplement Oxoid SR 155) which contains 5 mg/l ampicillin (Oxoid SR 136) and plates were incubated for 24 h in 30ºC incubator.

Following the incubation, dark green centered green opac colonies were accepted as suspected. From the typical colonies at least 5 were chosen and incubated on Tryptone Soy Agar (TSA, Oxoid CM 131) for 24 h in 30ºC incubator. The colonies which grew on TSA were tested for; Gram stain, oxidase, catalase, motility, resistance to a vibriostatic agent O/129 (2-4-diamino-6, 7-diisopropylpteridine), growth in Nutrient broth whether containing 5 % of NaCl or none. Identification was done from the colonies grew.

Identification of motile Aeromonas spp.; From the colonies detected as *Aeromonas*, esculin hydrolysis, growth on KCN broth, H_2S formation from cystein, gas formation from d-glycose, acid formation from arabinose, d-mannitol and salisin fermentation, metil red-voges proskauer and indol tests were done for the identification (30). The biochemical reactions of motile *Aeromonas* species were given in Table 1.

Table 1. Identification tests applied for motile *Aeromonas* species (30).
Tablo 1. Hareketli *Aeromonas* türlerinin identifikasyon testleri.

Biochemical tests	*A. hydrophila*	*A. caviae*	*A. sobria*
Esculin hydrolysis	+	+	–
Growth in KCN broth	+	+	–
H_2S from cysteine	+	–	+
L-arabinose utilization	+	+	–
Fermentation of salicin	+	+	–
Fermentation of mannitol	+	+	+
Gas from D-glycose	+	–	+
Metil red test	+	+	–
Voges-proskauer test	+	–	V
Indol production	+	+	+

V: Variable

Results

According to the analysis, 43 (53.75 %) of the total 80 samples are found positive for *Aeromonas* spp. From the 25 (62.5 %) of the 40 turkey leg and 18 (45 %) of the 40 turkey breast meat samples motile *Aeromonas* spp. were isolated (Table 2).

Table 2. Motile *Aeromonas* spp. rates isolated from turkey meat samples.
Tablo 2. Hindi etlerinin hareketli *Aeromonas* türleri ile kontaminasyon düzeyi.

Samples	No. of samples	No. of positive samples	% of positive samples
Turkey leg	40	25	62.5
Turkey breast	40	18	45.0
Total	80	43	53.75

From the 25 of the turkey leg meat which were detected as contaminated with motile *Aeromonas* spp., 14 (56.0 %) *A. hydrophila*, 8 (32.0 %) *A. sobria* and 3 (12.0 %) *A. caviae* were isolated. From the 18 of the turkey breast meat which were detected as contaminated with motile *Aeromonas* spp., 11 (61.1 %) only *A. hydrophila*, 2 (11.1 %) only *A. sobria* and 1 (5.6 %) only *A. caviae* identified while the turkey breast meat samples which identified two species together, 2 (11.1 %) *A. hydrophila*

Table 3. Distribution of motile *Aeromonas* spp. in positive samples.
Tablo 3. Pozitif örneklerde hareketli *Aeromonas* türlerinin dağılımı.

Turkey samples	Positive samples	Single species						More than one species					
		A. hydrophila		*A. sobria*		*A. caviae*		*A. hydrophila* and *A. sobria*		*A. hydrophila* and *A. caviae*		*A. sobria* and *A. caviae*	
		n	%	n	%	n	%	n	%	n	%	n	%
Leg	25	14	56.0	8	32.0	3	12.0	–	–	–	–	–	–
Breast	18	11	61.1	2	11.1	1	5.6	2	11.1	1	5.6	1	5.6
Total	43	25	58.1	10	23.3	4	9.3	2	4.7	1	2.3	1	2.3

and *A. sobria*, 1 (5.6 %) *A. hydrophila* and *A. caviae*, 1 (5.6 %) *A. sobria* and *A.caviae* were identificated . From the positive samples the most identificated species was *A. hydrophila* (Table 3).

Furthermore, average pH value of turkey leg samples found 6.0 whereas 5.7 for turkey breast samples.

Discussion and Conclusion

Even though *Aeromonas* spp. are psycrotrophic, toxin productive, isolated from most foods and cause of gastroenteritis and extraintestinal infections, limited numbers of studies have been done on determination of *Aeromonas* spp. in turkey meat.

In this concept; Singh (37) isolated motile *Aeromonas* spp. in ground meat samples from different animal species (19 ground beef, 4 ground chicken, 4 ground pork and 4 ground turkey) and he reported that all of ground turkey meat samples were contaminated with *Aeromonas* spp. and he found that 56 % (14/25) of isolates from ground turkey meat samples were *A. hydrophila*, 16 % (4/25) *A. caviae*, 8 % (2/25) *A. sobria* and 24 % (6/25) *Aeromonas* spp. The isolation ratio was higher than our study but their results were similar with ours that all three of the species were isolated. Hudson et al. (19) identified 5 (83 %) *A. hydrophila* in 6 samples of ready-to-eat turkey meat. In their study the isolation ratio of motile *Aeromonas* spp. was higher than our study. Hudson and Lacy (18) in their study which involved 396 food samples that including 3 ready to eat foods that produced from turkey meat was not found any *Aeromonas* spp. However in our study 53.75 % of *Aeromonas* spp. was isolated. The main reason of difference between the studies is used to be less samples in other studies. In this case according to the compared studies it is fairly hard to make an evaluation. Other reasons of the differences are thought to be as; the hygiene conditions of sample production system, the preservation conditions and the recontamination after the production.

Reported mean pH values for turkey leg and breast meat samples is 6.0 and 5.7 respectively. *Aeromonas* spp. are very sensitive to pH below 5.5 and optimum pH for growth is 7.2 (23, 32). Similarity between the pH of the breast meat samples that analyzed in this study and the pH in which *Aeromonas* spp. are sensitive, may explain why the contamination levels of the leg samples are higher than the breast samples.

Some studies on chicken samples done by Ternström and Molin (39), Bernhart *et al*. (8), Hanninen (16), Akan (3), Akan *et al*. (4), Sarımehmetoglu and Kuplulu (36), Yucel and Erdem (41) have a significant contamination levels of 53.3 %, 98 %, 93 %, 56.3 %, 90.5 %, 82.9 %, 86.95 % with *Aeromonas* spp. respectively, showing similarity with the high contamination levels of turkey leg and breast meat samples.

As a result of this study, done for the determination of *Aeromonas* spp. in packaged turkey meats, it is found that samples are considerably contaminated with *Aeromonas* spp. causing risks for public health especially for immunocompromised person, children and aged thus precautions should be taken. In this concept, the contamination of turkey meat and the products should be decreased, necessery controls should be done in every step of the production. HACCP and GMP systems should be established for food related enterprises including; personal hygiene and education and disinfection of tools and equipments in order to prevent cross and secondary contamination. As the pathogens are able to survive and grow in refrigerated conditions the preservation times should be shorted in markets and houses.

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Address for correspondance
Dr. Belgin Sarımehmetoğlu
Ankara Üniversitesi Veteriner Fakültesi
Besin Hijyeni ve Teknolojisi Anabilim Dalı
Ankara,Turkey
e-mail: bsarimeh@veterinary.ankara.edu.tr