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ISOLATION OF MOTILE AEROMONAS SPECIES FROM CHICKEN FAECES

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Tavuk Dışkılarından Hareketli Aeromonas Türlerinin İzolasyonu

Özet: *Hareketli Aeromonas türleri, 21 kümesten alınan 254 ishalli ve 254 normal dışkı örneğinde direkt ve zenginleştirme metodları ile araştırıldı. Direkt metotta 15 (%2.9), zenginleştirme metodunda ise 89 (%17.5) örnekte hareketli Aeromonas türleri saptandı. Hareketli Aeromonaslar, incelenen 254 ishalli örneğin 48'inden (%18.8) ve 254 normal dışkı örneğinin 41'inden (%16.1) izole edildi. Bu izolatların 53'ü (%59.6) A.hydrophila, 14'ü (%15.7) A.sobria ve 22'si (%24.7) A.caviae olarak identifiye edildi. A.hydrophila hem ishalli hem de normal örneklerde daha yüksek oranda izole edildi.*

Anahtar kelimeler: *Hareketli Aeromonas türleri, izolasyon, tavuk, dışkı*

Summary: *Motile Aeromonas species from 21 different poultry flocks were investigated in 254 diarrhoeic and 254 apparently normal faeces samples by direct plating and enrichment methods. Aeromonas spp. were detected in 15 (2.9%) samples by the direct plating method and found in 89 (17.5%) samples by enrichment method. Motile aeromonads were isolated from 48 (18.8%) of 254 diarrhoeic faeces and 41 (16.1%) of 254 apparently normal faecal samples, tested. Among these isolates, 53 (59.6%), 14 (15.7%) and 22 (24.7%) were identified as A.hydrophila, A.sobria and A.caviae, respectively. A.hydrophila was more prevalent either in diarrhoeic or normal chickens.*

Key words: *Motile Aeromonas species, isolation, chicken, faeces*

Introduction

Bacteria of the motile Aeromonas group (A.hydrophila, A.sobria, A.caviae) occur widely in fresh, estuarine waters, chlorinated drinking water, and bottled water (12,23) and are recognized as pathogens of fish, amphibians and reptiles (1,14). Motile Aeromonas species have become increasingly implicated as the causative agents of diarrhoea, wound infections and septicemia in humans (7,8,10,22). They have also been recovered from the faecal material of pigs and cattle, and found to be common contaminants in foods of animal origin (2,6,9,11,17). The studies related with the motile aeromonads of poultry are so limited. Isolation of motile aeromonads from the faeces of turkey, pet and aviary birds has been reported in a few occasion(16,21,24).dditionally, pathologic conditions in birds due to these organisms have been defined only in a few reports (4,18). Recently, A.hydrophila has been isolated from an outbreak of diarrhoea in a flock and this agent has been implicated as a cause of infectious enteritis in poultry (3).

The aim of this study was to determine the role of motile Aeormonas spp. in naturally occuring diarrhoeal diseases of chickens.

Material and Methods

Animals: Six week old chickens showing signs of watery, mucoid and bloody diarrhoea were determined in 21 flocks. Duplicate rectal samples were collected from 254 diarrhoeic chickens. As non-diarrhoeic controls, duplicate rectal samples were also obtained from 254 healthy chickens of same

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flocks in equal numbers. Healthy chickens were observed along three days for a subsequent diarrhoeal condition. Chickens had not been received antimicrobial agents as feed additives or theurepatics along 2 weeks prior to sampling.

Isolation: All rectal contents were taken into sterile containers and examined within two hours of sampling. For the isolation of motile aeromonads, direct and enrichment methods were used. In direct plating, faecal samples were streaked on blood-ampicillin agar (BAA) containing 5% sheep blood and 10 mg/l ampicillin, using sterile swabs. In enrichment procedure, 1g of faecal sample was inoculated into 10 ml of alkaline peptone water (APW, pH 8.4) and incubated at 28 °C for 24 h. APW was further diluted (1:10) with phosphate buffered saline and samples were plated on BAA with an inoculating loop. All plates were incubated at 28 °C for 24 h.

Identification: Hemolytic colonies from BAA were examined for motility and Gram's reaction and were transferred to nutrient agar slants. After an overnight incubation at 28 °C, a few drops of a 1% solution of N,N-dimethyl-p-phenylenediamine monohydro-chloride were added to the growth to determine the oxidase activity. All Gram-negative, oxidase-positive and motile organisms were screened with the following tests: oxidation/fermentation of glucose (O/F), sensitivity to 2,4-diamino-6,7-diisopropyl-pteridine (vibriostatic agent, O/129), fermentation of mannitol and salicin, utilisation of arabinose, gas production from glucose, H_2S production from cysteine hydrochloride and aesculin hydrolysis. Aeromonas spp. were differentiated according to criteria described by Popoff (19).

Statistical analysis: The significance of difference between groups was tested by chi-square analysis.

Results and Discussion

Motile aeromonads were isolated from 48 (18.8%) of 254 diarrhoeic and 41 (16.1%) of 254 apparently normal chickens (Table.1). Difference between carriage rates of two groups was not significant ($p>0.05$). Motile aeromonads were found in all 21 flocks investigated. The isolation rate ranged from 12.3 to 24.6 % from flock to flock. Additionally, Aeromonas carriage rate was not significant in any of the flocks ($p>0.05$). Any specific clinical sign was not detected in Aeromonas harbouring animals; all three types of diarrhoea (watery, mucoid and bloody) were present. The findings of this study suggested that motile aeromonads were not the cause of diarrhoea of chickens investigated. This finding is in contrast to that reported by Efuntoye (3) who isolated A.hydrophila from 56.0% of diarrhoeic and 15.4% of healthy chicken faeces and suggested that specifically A.hydrophila was closely assocciated with the outbreaks of diarrhoea in the poultry. Since the findings of Efuntoye (3) have been obtained from only one flock and an experimental infection has not been performed, the suggestion of researcher is not convincing.

Table 1. Motile Aeromonas species in diarrheic and normal faeces.
Tablo 1. Normal ve ishalli dışkılarda hareketli Aeromonas türleri.

Type of samples	No. of isolates (%)	A.hydrophila (%)	A.sobria (%)	A.caviae (%)
diarrhoeic	48(18.8)	27(56.3)	9(18.7)	12(25.0)
normal	41(16.1)	26(63.4)	5(12.2)	10(24.4)
total	89(17.5)	53(59.6)	14(15.7)	22(24.7)

On the other hand, when the results of present study was evaluated by means of a single species, A.hydrophila, the difference between groups was not significant. Some workers (9,24) have reported the low incidence of motile aeromonads in poultry faeces. Jindal et al.(9) reported that Aeromonas spp. were isolated from 2 of 10 poultry faeces. Stern et al.(24) found Aeromonas spp. from 3 of 21 turkey faeces. These workers, however have not indicated the clinical condition of animals. A further comparison with other studies was not possible as a detailed study on the isolation of motile aeromonads species in normal and diarrhoeic faeces has not been done before.

When the isolation methods were compared, a significant difference was found ($p<0.01$). Motile aeromonads were detected in 15 (2.9%) of 508 samples by direct plating method and 89 (17.5%) by enrichment method (Table.2). All direct-plating samples were also positive in enrichment method. These results have showed that enrichment step is necessary for the primary isolation of motile aeromonads from faeces. The similar results reported in some studies (6,13). Gray ve Stickler (6) reported that an enrichment technique with APW from feaces increased the total number of isolates by 77.1% for isolation of A.hydrophila. Majeed et al.(13) who detected in motile aeromonads 11% of faecal samples by enrichment methods were unable to isolate any motile aeromonads by direct plating methods.

Table 2. Methods of isolation: Comparison in numbers (%) of positive cultures.

Tablo 2. İzolasyon metotları: Pozitif kültürlerin (%) karşılaştırılması.

No. of samples examined	positive samples on direct plating (isolation %)	positive samples after enrichment (isolation %)
508	15 (2.9)	89 (17.5)

All of the 89 strains selected for identification were found to be motile Aeromonas spp. These strains comprised 48 from diarrhoeic and 41 from normal faecal samples. All strains were motile and oxidase-positive. Based on their reactions in aesculin hydrolysis, L-arabinose utilisation, fermentation of salisin, production of gas from glucose and H_2S from cysteine, the strains were identified as A.hydrophila, A.sobria and A.caviae (Table 3). Of these 89 isolates, 53 (59.6%) were identified as A.hydrophila, 14 (15.7%) as A.sobria and 22 (24.7%) as A.caviae. It was clearly demonstrated that A.hydrophila predominated in both diarrhoeic and normal chicken faeces. It has also been generally accepted by other researchers that A.hydrophila is the most common motile Aeromonas species in either environmental samples or animal hosts (4,5,6,15, 20).

Table 3. Differential characteristics of motile Aeromonas species

Tablo 3. Hareketli Aeromonas türlerinin ayırıcı özellikleri.

Characteristics	A.hydrophila	A.sobria	A.cavine
Catalase	+	+	+
Oxidase	+	+	+
Motility	+	+	+
Resistant to 0/129 *	+	+	+
Oxidation- fermentation	Fermentative	Fermentative	Fermentative
Fermentation of manuitol	+	+	+
Aesculin hydrolysis	+	-	+
Arabinose utilization	+	-	+
Fermentation of salicin	+	-	+
Gas from glucose	+	+	-
H_2S from cysteine	+	+	-

* 2-4 diamino 6-7 diisoprophyl pteridine

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