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## **STAPHYLOCOCCI IN ANIMALS. CHARACTERISTICS, DISTRIBUTION AND ITS PUBLIC HEALTH SIGNIFICANCE.**

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### **Hayvan Staphylococ'larının özellikleri, Dağılımı ve Halk Sağlığı yönünde Önemi**

**Summary:** 133 staphylococci isolated from various specimens of different species of domestic animals were studied from the stand point of their properties and distribution. In addition, a brief discussion of the public health significance of staphylococcal infection which has demonstrated zoonotic association was presented.

Majority of the isolates were recovered from pus abscesses from horse, cattle, dog and cat. Limited number of staphylococci were obtained from samples of vaginal discharge, synovial fluid and milk. All of these specimens were received from the clinics of Veterinary Faculty. 27 strains out of 133 staphylococci were isolated from livers, heart blood, kidney and affected joints of chicken's.

More strains of *Staphylococcus aureus* were recovered than of *Staphylococcus albus* from these specimens. It was found that 51 isolates produced golden-coloured, Alpha or Beta haemolytic colonies on sheep blood agar and coagulated rabbit plasma. 27 strains also produced golden-coloured colonies and were coagulase positive, but did not produce haemolysis. 20 strains produced white and Beta-haemolytic colonies, but they were coagulase-positive. Coagulase negative 35 strains produced white and nonhaemolytic colonies.

Although fermentation of mannitol and gelatin liquefaction is recognised as a character of pathogenic strains, results of the present study did not serve to distinguish pathogenic strains from non-pathogenic varieties.

It is clear from the results of different workers including the author that the coagulase test is the most reliable criterion of pathogenicity.

**Özet:** Çeşitli evcil hayvan türlerine ait marazi maddelerden izole edilen 133 staphylococ şuşunun dağılımı ve özellikleri üzerinde çalışıldı. Buna ilaveten zoonotik ilişki gösteren ve halk sağlığı yönünden önemli staphylococ enfeksiyonu hususunda kısa bir tartışma sunuldu.

Suşların büyük bir çoğunluğu at, sığır, köpek, kedi apse içeriğinden ve belirli adette staphylococ şuşu ise vagina akıntısı, sinovya sıvısı ve süt numunelerinden izole edildi. Bu

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marazi maddeler bakteriyolojik muayene için Fakültemiz kliniklerinden gönderilmiştir. 133 staphylococ suşundan 27'si muayene için laboratuvarımıza gönderilen civcivlerin karcığı, kalp kanı, böbrek veya arızalı eklemlerinden izole edildi.

Bu marazi maddelerden *Staphylococcus albus*'tan daha çok *Staphylococcus aureus* suşu elde edildi. 51 suş koyun kanlı agarı üzerinde altın sarısı renginde Alpha-veya Beta hemolitik koloniler oluşturdu ve tavşan plasmasını koagüle ettiler. 27 suş ise altın sarısı koloni oluşturmalarına ve koagülaz-pozitif olmalarına rağmen hemoliz meydana getirmemişlerdir. Beyaz ve hemolitik koloni oluşturan 20 suş koagülaz testi ile pozitif sonuç vermiştir. Koagülaz-negatif olan 35 suşta beyaz ve hemolitik olmayan koloniler halinde üremişlerdir. Mannit fermentasyonu ve gelatini eritme patogen suşların bir karakteri olarak tanımlarsada, bizim yapmış olduğumuz çalışmalar bu sonuçları teyit etmemiştir.

Fakat bizimki dahil bir çok araştırmacıların çalışma sonuçları, koagülaz testinin en güvenilir bir patojenite testi olduğunu göstermektedir.

## Introduction

Staphylococci are wide spread in many places in nature and most of them live a purely saprophytic existance. Some are pathogenic and capable of causing disease in man and animale (6, 23).

Most of the lesions produced by organisms of these species are superficial inflammation with pus formation in all domestic animals. Once pathogenic strains gain a foothold in the deeper tissues of the exposed animal, their multiplication cause necrosis and abscess formation. In some instances, the organisms may invade the lymphatics, blood stream and metastatic foci usually develop (6, 9).

Staphylococci are as well the causative agent of some serious infections such as mastitis in most species of domestic animals; pyaemia in lambs; septicemia and arthritis in poultry; acne, furunculosis and batryomycosis in horse and cows; dermatitis and septicaemia in dog and cat (2, 4, 10, 12, 14, 15, 22, 28).

In man staphylococci are usually found in pyogenic lesions and infections such as osteomyelitis, renal carbuncle, peri-renal abscess, bronchopneumonia. Some cases pyaemia, septicaemia and malignant endocarditis may result from spread from a primary localised abscesses (9). Cases of food poisoning are frequently due to the enterotoxin produced by certain staphylococci growing in cooked meats, milk and milk-products, fish and gravies (19, 21).

Many strains show a marked degree of variation to antibiotic and chemotherapeutic agents. These antibiotic resistance strains has become of a great epidemiological and therapeutic importance (3, 17, 25).

Some zoonotic infections of staphylococci may be maintained in nature either by animals or man and transmission may occur in either or both directions (8, 20, 21, 24).

Staphylococci are now classified in separate two species according to several laboratory tests which are based on their biological features. Organisms of this genus are the only bacteria that produce coagulase which causes citrated or oxalated plasma to coagulate. Due to this fact, the coagulase test has been accepted as an important criterion of pathogenicity. Coagulase-positive pathogenic strains most of which produce Alpha-Beta-or Delta-haemolysins and golden coloured colonies are called *Staph. aureus*. Coagulase-negative less virulent strains which produce white and non-haemolytic colonies are designated as *Staph. epidermidis* (1, 6, 13, 16).

The purpose of the present study was to describe some of the properties and the distribution of the strains isolated from various species of animals. In addition we discussed the public health significance of staphylococcal infection which has demonstrated zoonotic associations and opportunities for reciprocal transmissions between domestic animals and man.

### **Materials and Methods**

A total of 150 specimens received from the clinics were examined during the period of 4 years. 60 per cent of these samples were pus from abscesses of various species of domestic animals such as horse (19), Cattle (21), dog (27), cat (17) and sheep (6). Of the 150 specimens 40 percent were individual milk samples from known cases of mastitis (36), vaginal discharge (14), synovial fluid (10). 106 strains of staphylococci were isolated from the specimens mentioned above. In addition, in the course of carrying out diagnostic examination of poultry, 27 strains were recovered from livers, heart blood, kidneys and affected joints.

#### **Isolation**

The media used for isolation and subculture of staphylococci was sheep blood agar plates. This medium contained 2percent tryptose (Difco), 0.1 percent glucose, 0.5 percent sodium chloride and 2 percent agar (pH 7.0); sheep blood was added to 5 percent. All specimens were streaked upon this medium which was then inoculated at 37 °C. for overnight and left at room temperature for an additional 24 hours to observe any further development of pigment. Colonies

resembling those of staphylococci were studied microscopically for the characteristic Gram reaction and grapelike formation of cocci. Gram staining was conducted according to standard procedure. These typical isolates regardless of pigmentation were inoculated into nutrient broth for carrying out of their biological features.

#### Coagulase production test

Citrated 0.5 ml of rabbit plasma, previously diluted 1:4 with saline was used for the tube test. Five drops of an overnight broth culture were added to this plasma. The tubes were incubated in a waterbath at 37 °C. for one hour and at intervals up to 4 hours. If the strains did not show coagulation at the end of this period, the tubes were kept in incubator for overnight and final reading was then made.

#### Haemolysin production

The basic medium used for this purpose was the same as they employed for preliminary isolation. An overnight broth culture of a colony from a strain was spread over a sheep erythrocyte agar plate and incubated at 37 °C. for 24 hours. Each colony was inspected for a surrounding haemolysin effect.

#### Biochemical reactions

Glucose, lactose, maltose, manntitol, salicin, xylose and arabinose were used. Readings were made after incubation for 3 days at 37 °C.

#### Gelatin liquefaction

Tubes of nutrient gelatin were stab inoculated, and incubated at 37 °C. for 6 days. The tubes were kept for 20 minutes at 4 °C. before reading each day.

### **Results and discussion**

In the course of about more than 4 year, studies were conducted on 133 strains of staphylococci which were isolated from various specimens of domestic animals. 51 isolates produced golden-coloured, Alpha or Beta-haemolytic colonies on 5 per cent sheep blood agar and were coagulase-positive in tube test using rabbit plasma. 27 strains also produced golden-coloured colonies and were coagulase-positive, but did not produce haemlysis. 20 coagulase-positive strains of staphylococci formed white and Beta haemolytic colonies on primary isolation. 35 strains produced white, non-haemolytic colonies and were coagulase-negative. The amount of haemolysin produced was judged

by the diameter of the zone around the colony. All of the strains isolated from milk samples produced Beta-haemolysin. Strains from poultry specimens produced Alfa-and Beta-haemolysin. Isolates from pus of abscesses, vaginal discharge and synovial fluid showed wide Alpha haemolysis, and little Beta-haemolysis.

All coagulase-positive strains were the same in that they fermented glucose and mannitol. 18 of the coagulase-negative strains examined were active biochemically. These strains formed acid from glucose, lactose, salicin and xylose. Mannitol was also fermented by 5 of these strains.

Isolates obtained from specimens of dogs and cats differed from ether animal strains in that they did not form a golden pigment.

Strains of animal origin have been investigated by a number of investigators employeng tests such as coagulase production, formation of toxin, pathogenicity for laboratory animals and fermentation reactions (1, 11, 12, 13, 16, 30). The sensitivity of staphylococci to lysis by various bacteriophages has been accepted as a method of studying the epidemiology of staphylococcal infections in man and animals (5, 8, 27, 29). Most of the workers (6, 3, 18, 19) has criticised the acceptance of host pathogenicity as a criterion for *Staph. aureus*. In the present study, therefore, certain laboratory tests were employed to classify staphylococci as pathogenic or not. The limited number of fermentable substances employed, as well as the property of gelatin liquefaction and haemolysis production did not serve to distinguish the pathogenic strains from non-pathogenic ones. Likewise, production of golden yellow pigmented colonies were not an adequate indicator of pathogenicity. Because coagulase-positive strains devoid of golden pigment were frequently encountered, even on primary isolation. The property that correlates best with pathogenicity was the elaboration of coagulase. All coagulase positive strains from clinical cases, therefore were designated as *Staph. aureus*.

Koenig et. al. (16) have reported some important factors that influence the virulence of *Staph. aureus* as follows: antiphagocytic surface components of the strains; the production alpha-toxin which may promote necrosis, interfere with inflammation and injure leucocytes; the production of delayed hypersensitivity which enhances tissue necrosis and increases the susceptibility to infection. Despite the combined effects of these several factors mentioned above, the overall virulence of *Staph. aureus* for animal and laboratory animals is

comparatively low. Serious staphylococcal disease occurs only when the local or the general antibacterial defences of the host have been depressed (26).

A great deal of research has resulted, some of which has demonstrated zoonotic associations and opportunities for reciprocal transmission between domestic animals and man. Smith and Crabb (25) reported, for example, that antibiotic-resistant staphylococci were much more prevalent among human attendants of swine which were fed antibiotic supplements than among attendants of swine not fed antibiotics.

Meat-processing plants provide particularly favorable opportunities for interspecific transmission to occur. Rabenholt et. al. (21) found evidence that an outbreak of type 80/81 staphylococcal pyoderma in a poultry processing plant was related to a period in which chlortetracycline was used in the plant for ice-water bath immersion of eviscerated poultry.

Domestic animals may also acquire their staphylococcal infections from man. Smith et. al. (26) and Moeller et. al. (20) studied 295 human patients with coagulase-positive *Staphylococcus aureus* infections from rural areas and have observed that a number of their patients had transmitted infections to their cattle. Similarly, Wallace and his colleagues (27) have reported the association of type 80/81 infection in man with mastitis in cattle.

Veterinary clinics may constitute heavily contaminated environments in which reciprocal infections are possible, Live and Nichols (17) reported that 50 per cent of 4th year clinical veterinary students but only 3 per cent of preclinical veterinary students at the university of Pennsylvania carried antibiotic resistant staphylococci of phage type 80/81. Silberg et al. (24) studied the human and animal populations in another veterinary hospital environment but found little or no evidence for interspecies transmission.

In summary, the results of many investigators indicate that inter-host transmission does occur and heavily contaminated environments may be important sources of outbreaks of infection in both animals and man.

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