

## **Bacteriological examination of normal upper respiratory tract of puppies with particular reference to staphylococci**

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### **ABSTRACT**

The nasal bacterial flora of 60 apparently normal puppies were examined, with special reference to *Staphylococcus* species. A total of 106 bacterial isolates were encountered, out of which the coagulase-negative *Staphylococcus epidermidis* was the most common microbe (58.5%). Other bacteria encountered were *Staphylococcus intermedius* and *Bacillus* species with an incidence of 9.4%, respectively. *Corynebacterium xerosis* showed an incidence of 4.7%, while the lowest incidence of 2.8% was recorded for *Escherichia coli*. The incidence of *Staphylococcus intermedius* recorded in this study is of public health importance because the nasal carriage has been implicated as an important factor in introducing *Staphylococcus* species onto sites of skin of humans and dogs. Hence, there is an urgent need to educate pet owners on the zoonotic potentials of staphylococcal organisms. The need for regular check-ups cannot be overemphasized.

**Key words:** puppies, staphylococci, coagulase test, zoonosis

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### **Introduction**

*Staphylococcus* species often form part of the normal bacterial flora of the skin and mucosal surfaces of the respiratory, upper alimentary and urogenital tract of mammals and birds. Hence, Staphylococci are easily spread between animals and man through direct contact or contact with excretions which contain staphylococci (NOBEL, 1992a).

*Staphylococcus aureus*, *Staphylococcus intermedius* and *Staphylococcus hyicus* are well-known pathogens which affect mainly humans, dogs and pigs, respectively (NOBEL,

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1992a; PATEL et al., 2002). Although, the coagulase-positive *Staphylococcus intermedius* is a pathogen of dogs, this pathogen has been cultured from feline skin lesions (MEDLEAU and BLUE, 1988; WHITE, 1991). The nasal carriage has been implicated as an important factor in introducing *Staphylococcus* species onto the skin or other sites of humans and dogs; (HARVEY and LLOYD, 1994; PATEL et al., 2002)

In Nigeria, as of now for security reasons and due to lack of adequate knowledge of implications of zoonosis, there is an increasing population of dog owners, with dogs living freely among people, especially children. This close relationship of pet animals to their owners may constitute a potential public health hazard. ADEKEYE (1981) reported *Staphylococcus aureus* of canine biotypes from man and animals.

As a result of an increasing number of dogs kept as security guards and pets in many homes, this investigation was carried out to determine the potential bacterial pathogens of upper respiratory tracts in clinically normal puppies.

### **Materials and methods**

*Sample collection.* Total of 60 puppies were sampled during this study as they were presented for routine check-up at the Veterinary Clinic Mokola and Veterinary Teaching Hospital, University of Ibadan. The sampling began in November 1999 and continued until late February, 2000. Deep nasal swabs were collected from clinical normal puppies between the ages of 6 months and under. Samples were transported immediately on ice (Coleman® Flask) to the Department of Veterinary Microbiology and Parasitology, University of Ibadan, Ibadan.

The swabs were inoculated onto 7 percent human blood agar (Oxoid Columbia blood agar®) and MacConkey agar No. 2 (Oxoid CM 109®) plates and were incubated aerobically at 37 °C for 24-72 hours. Gram-positive, cluster forming, catalase-positive and oxidase-negative cocci were characterized as staphylococci according to standard methods (LANGLOIS et al., 1990; BARROW and FELTHAM, 1993). Other organisms, were examined for their morphological, staining, cultural characteristics and biochemical characteristics according to standard methods (BARROW and FELTHAM, 1993)

*Coagulase activity of staphylococcal isolates.* Using human, rabbit, cattle and goat plasmas colonies yielding Gram-positive cocci with catalase-positive and oxidase-negative reaction were subjected to coagulase test by tube method as follows. Two drops of overnight cultures in TSB (Trypticase Soy broth, Merck®, Germany) were added to test tubes containing 1.0 mL of freshly prepared 1:10 dilution of citrated human plasma in saline. The mixture was incubated at 37 °C and examined every two hours for clot formation over a period of 24 hours (LANGLOIS et al., 1990).

## Results

All 60 puppies examined were positive for staphylococci and a total of 72 staphylococci isolates encountered. The coagulation of cattle plasma in the current study was lower than that obtained for human, rabbit and goat. Overall, 10 (14%) of the staphylococci isolates were coagulase-positive, while 62 (86%) were coagulase-negative (Table 1).

Table 1. Coagulase activities of staphylococci isolated from puppies in Ibadan.

	N <sup>o</sup> staphylococci tested	Catalasetest		Oxidase test		Tube coagulase test		*Rabbit plasma		Cattle plasma		Human plasma		*Goat plasma	
		+	-	+	-	+	-	+	-	+	-	+	-	+	-
Vet. Clinic Mokola	58	58	0	0	58	7	51	2	56	2	56	3	55	2	56
	%	100	0	0	100	12.1	87.9	3.4	96.6	3.4	96.6	5.2	94.8	3.4	96.6
Vet. Teaching Hospital, Univ. Ibadan.	14	14	0	0	14	3	11	2	12	0	14	1	13	2	12
	%	100	0	0	100	21.4	78.6	14.3	85.7	0	100	7.1	92.9	14.3	85.7
Total	72	72	0	0	72	10	62	4	68	2	70	4	68	4	68
	%	100	0	0	100	14	86	5.6	94.4	2.8	97.2	5.6	98.6	5.6	94.4

\*Rabbit and goat plasmas were coagulated by same strains of staphylococci

A total of 106 bacterial isolates were encountered, out of which *Staphylococcus epidermidis* was the most common microbe (58.5%) followed by Streptococci species, with an incidence of 15.1%. However, *Staphylococcus intermedius* and *Bacillus* species showed an incidence of 9.4%, respectively. *Corynebacterium xerosis* showed an incidence of 4.7%, while the lowest incidence of 2.8% was recorded for *Escherichia coli* (Table 2).

Table 2. Bacteria isolated from upper respiratory tract of puppies in Ibadan.

*Bacteria	Frequency	Incidence (%)
<i>Staphylococcus epidermidis</i>	62	58.5
<i>Streptococci</i> species	16	15.1
<i>Staphylococcus intermedius</i>	10	9.4
<i>Bacillus</i> species	10	9.4
<i>Corynebacterium xerosis</i>	5	4.7
<i>Escherichia coli</i>	3	2.8

\*Some bacteria occurred as multiple infections in the puppies

## Discussion

Coagulase test is one of the tests used to identify the *Staphylococcus* species that may infect man, BARROW and FELTHAM (1993). The human, rabbit and goat plasmas used for testing the coagulase activities of the staphylococci isolated in this study showed equal sensitivity. The rabbit and goat plasmas were coagulated by same staphylococci isolates. Similar good sensitivity was earlier reported for each of the plasmas in the study of staphylococci of rabbits AJUWAPE and AREGBESOLA (2001). The coagulation of cattle plasma in the current study was lower than that obtained for human, rabbit and goat plasmas. Similar low coagulation of cattle plasma by staphylococci isolated from cattle and rabbit has been recorded (AJUWAPE and AREGBESOLA, 2001; AJUWAPE and AKINYEDE, 2001). The coagulation of human and cattle plasma continues to be used for biotyping *Staphylococcus aureus* strains DEVRIESSE (1984).

The *Staphylococcus intermedius* (9.4%) recorded in this study is of public health importance because the nasal carriage has been implicated as an important factor in introducing *Staphylococcus* species onto skin of sites of humans and dogs, PATEL et al. (2002). In Nigeria it is observed that many dog owners, for economic reasons and lack of adequate knowledge of implications of zoonosis, are not providing adequate veterinary care for their pets. However, there is increasing population of dog owners with dogs living freely among people, especially children with tender skin. TANNER et al. (2000) reported a case of human otitis in which the strain was isolated from the patient's dog. This pathogen has also been involved in outbreaks of food poisoning (KHAMBATY et al., 1994; BECKER et al., 2001). These puppies constitute a potential source of infection to other animals such as goats and cats, as previously observed by MEDLEAU and BLUE (1988), WHITE (1991) and BIBERSTEIN et al. (1984). On the other hand, HARVEY (1996) recovered *Staphylococcus intermedius* from 70% of the cases of canine superficial pyoderma. The incidence of coagulase-negative *Staphylococcus epidermidis* (86%) in this investigation is higher than the approximately 10% recorded by HARVEY (1996) in the case of canine superficial pyoderma. It is relevant to note that coagulase-negative staphylococci have been associated with virulence factors and infections are being increasingly reported, especially in immunosuppressed people (NOBEL, 1992b).

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**AJUWAPE, A. T. P., M. O. OYEBANJI, A. I. ADETOSOYE: Bakteriološki nalaz gornjih dišnih putova u zdrave štenadi s posebnim osvrtom na stafilokoke. Vet. arhiv 76, 179-184, 2006.**

**SAŽETAK**

Istraživana je bakteriološka flora sluznice nosa u šezdesetero normalne štenadi s posebnim osvrtom na vrste roda *Staphylococcus*. Sakupljeno je ukupno 106 bakterijskih izolata, od kojih je 58,5% pripadalo koagulaza negativnoj vrsti *Staphylococcus epidermidis*. Ostale izdvojene vrste bile su *Staphylococcus intermedius* i *Bacillus* sp. s učestalošću od 9.4%. *Corynebacterium xerosis* je pronađen u 4.7%, a *Escherichia coli* u 2.8% pretraženih uzoraka. Rasprostranjenost vrste *Staphylococcus intermedius* ima veliko značenje za javno zdravstvo, jer se stafilokoki s nosne sluznice mogu prenijeti na kožu ljudi i pasa. Stoga je hitno potrebno educirati vlasnike ljubimaca o mogućem zoonoznom značenju stafilokoka. Nameće se potreba redovnog pregleda pasa.

**Ključne riječi:** štenad, stafilokoki, koagulaza, zoonoza

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