

ISOLATION AND IDENTIFICATION OF *STAPHYLOCOCCUS* SPECIES FROM DOG AND HUMAN

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Abstract

This study aimed to isolate and identify the *Staphylococcus* species from otitis externa samples of dogs as well as from the tonsillitis, rhino sinusitis and skin lesions of human. The study plan was divided into two parts. The first one including the collection of 100 swabs involved 50 from humans and 50 from dogs from different location in Baghdad governorate during November (2021) to March (2022). Then the sterile swab samples were inoculated on Mannitol salt agar, purified, and identified according to colony morphology, Gram stain, and biochemical tests. The bacterial isolates were confirmed by VITEK technique. The rates of prevalence of staphylococci using the traditional bacteriological methods among the tested samples of dogs and human were showed a significant variation ($P < 0.05$) in their values. Significant increases ($P < 0.05$) in prevalence of *Staphylococci* were detected in ear of dogs (60%) and nasal cavity of human (60.6%) when compared to other human samples; 25% in tonsils and 0% in skin. Using the VITEK system, different species of *Staphylococcus* species were detected in the tested samples of dogs and humans (Table 2). In dog, the predominant species in otitis samples was *S. pseudintermedius* (43.3%), which followed by *S. lentus* (16.6%), and *S. warneri* (13.3%). In human, the predominant species in tonsillitis *S. aureus* (100%) and the predominant species in rhinosinusitis samples and *S. hominis* (35%) followed by *S. epidermidis* (30%). However, no positive samples for different *Staphylococcus* species were found in skin lesions. The findings of biochemical tests revealed that the isolates of all *Staphylococcus* species were positives for Gram stain, catalase and high salt containing media; whereas, *S. aureus* and *S. pseudintermedius* were the only positive species for coagulase; and the other *Staphylococcus* species were negatives.

This study concluded that the presence of *S. pseudintermedius* is a fact in infection of areas concluded in this study but in previous studies this bacteria was misdiagnosed with *S. aureus* that appeared with low number of isolates. Furthermore studies are of great importance to demonstrate the culture and genetic characteristics of different species of *Staphylococcus* isolated from dogs or other domestic and wild animals.

Keywords: *Staphylococcus*, VITEK technique, *S. pseudintermedius*, Otitis externa,

Introduction

Staphylococcus are tiny Gram-positive cocci that got their name because they looked like bunches of grapes, currently, more than 40 known species of *Staphylococcus* exist (Krute and Bose, 2015; Al-Graibawi and Yousif, 2021). Due to their ubiquity and adaptability, it inhabits the skin, skin glands and mucous membranes of people, other mammals and bird, as well as the environment (Vrbovska *et al.*, 2020). Mammals can develop localized and systemic infections from *staphylococci* ranging from small wounds infections to potentially fatal illness including endocarditis and osteomyelitis (Shi and Zhang, 2012; Osmonov *et al.*, 2013). Currently, staphylococcal species have the potential to act as human pathogens (Savini *et al.*, 2013).

Staphylococcus pseudintermedius is well known cause of canine otitis externa and dog and cat pyoderma. It has also been linked to bacteremia, endocarditis and rhinosinusitis in humans. Dogs were their primary host when *S. cornubiensis* was identify from human skin (Carrol *et al.*, 2021).

Staphylococcus hyicus is a species that lives in donkeys and pigs and is the cause of bovine mastitis, polyarthritis, and swine exudative epidermitis. Sepsis in a farmer who was working close to piglets has also been documented (Casanova *et al.*, 2011).

Staphylococcus aureus causes many diseases in livestock and other animals such as urinary tract infection in buffaloes in Iraq (Kanaan and AL-Shammary, 2013; Al-Iraqi *et al.*, 2016). It also causes food poisoning in human because animals are considered a potential source of primary contamination (Fey *et al.*, 2003). Due to their innate characteristics, *S.aureus* is the leading cause of nosocomial and community-acquired infections (Al-Hamadany, 2012; Yassin *et al.*, 2022). Hence, this study aimed to isolate and identify the *Staphylococcus* species from otitis externa and human (tonsillitis, rhino sinusitis and skin lesions).

Methodology

Throughout the period of November 2021 to march 2022, samples of dogs and humans were collected in Baghdad governorate. A total 50 of canine samples were collected from all otitis-infected animals; in addition to 50 human samples collected from tonsillitis, rhinosinusitis and skin lesions. After growth on MSA mannitol salt agar, *Staphylococcus* colonies were purified and examined by bacteriological techniques mentioned by other studies (Quinn *et al.*, 2004; Markey *et al.*, 2015), and then identified on the colonies from each pure culture on basis of colony characteristic, gram stain, morphology and biochemical tests (catalase and coagulase) confirmation of bacterial species were done using biochemical tests and VITEK®2 system. Statistical analysis was carried out using the One- and Two-Way ANOVA in the GraphPad Prism (6.0.1) Software. Differences between the obtained values were considered significant at $P < 0.05$ (Gharban, 2022; Razooqi *et al.*, 2022).

Results

The rates of prevalence of staphylococci using the traditional bacteriological methods among the tested samples of dogs and human were showed a significant variation ($P < 0.05$) in their values. Significant increases ($P < 0.05$) in prevalence of *Staphylococci* were detected in ear of dogs (60%)

and nasal cavity of human (60.6%) when compared to other human samples; 25% in tonsils and 0% in skin (Table 1).

Table 1: Isolation rate of staphylococci in dogs and humans identified by using the traditional bacteriological methods

Host	Site of swap	Total No. of samples	Positive	
			No.	%
Dog	Ear	50	30	60 A
Human	Tonsils	12	3	25 B
	Nasal cavity	33	20	60.6 A
	Skin	5	0	0 C

Variation in vertical large letters refer to significant differences at P<0.05

Using the VITEK system, different species of *Staphylococcus* species were detected in the tested samples of dogs and humans (Table 2). In dog, the predominant species in otitis samples was *S. pseudintermedius* (43.3%), which followed by *S. lentus* (16.6%), and *S. warneri* (13.3%). In human, the predominant species in rhinosinusitis samples were *S. hominis* (35%) followed by *S. epidermidis* (30%) and tonsillitis *S. aureus* (100%). However, no positive samples for different *Staphylococcus* species were found in skin lesions.

Table 2: *Staphylococcus* species identified by the VITEK®2 system, both in terms of number and percentage based on the host and isolation site

Species	Dog		Human					
	Otitis		Tonsillitis		Rhinosinusitis		Skin	
	No.	%	No.	%	No.	%	No.	%
<i>S. pseudintermedius</i>	13	43.3 A	0	0 B	2	10 B	0	0 B
<i>S. lentus</i>	5	16.6 B	0	0 B	3	15 B	0	0 B
<i>S. warneri</i>	4	13.3 B	0	0 B	0	0 D	0	0 B
<i>S. chromogens</i>	2	6.6 C	0	0 B	0	0 D	0	0 B
<i>S. aureus</i>	2	6.6 C	3	100 A	1	5 C	0	0 B
<i>S. hemolyticus</i>	1	3.3 C	0	0 B	1	5 C	0	0 B
<i>S. xylosus</i>	1	3.3 C	0	0 B	0	0 D	0	0 B
<i>S. saprophyticus</i>	1	3.3 C	0	0 B	0	0 D	0	0 B
<i>S. simulans</i>	1	3.3 C	0	0 B	0	0 D	0	0 B
<i>S. hominis</i>	0	0 D	0	0 B	7	35A	0	0 B
<i>S. epidermidis</i>	0	0 D	0	0 B	6	30A	0	0 B

Variation in vertical large letters refer to significant differences at P<0.05

The findings of biochemical tests revealed that the isolates of all *Staphylococcus* species were positives for Gram stain, catalase and high salt containing media; whereas, *S. aureus* and *S. pseudintermedius* were the only positive species for coagulase; and the other *Staphylococcus* species were negatives (Table 3).

Table 3: Results of biochemical tests used in identification of bacterial isolates

Species	Gram	Catalase	High salt containing media	Coagulase tube
<i>S. aureus</i>	+	+	+	+
<i>S. pseudintermedius</i>	+	+	+	+
<i>S. lentus</i>	+	+	+	-
<i>S. hominis</i>	+	+	+	-
<i>S. warneri</i>	+	+	+	-
<i>S. chromogens</i>	+	+	+	-
<i>S. hemolyticus</i>	+	+	+	-
<i>S. simulans</i>	+	+	+	-
<i>S. saprophyticus</i>	+	+	+	-
<i>S. xylosum</i>	+	+	+	-
<i>S. epidermidis</i>	+	+	+	-

Discussion

This study concluded two categories the first one is the isolation of *Staphylococcus* spp. from dog otitis and this study record isolation of this bacteria at a percentage of 60% this results considered as primary cause of otitis in dogs and also may considered that the *Staphylococcus* spp. was a normal flora of external ear canal these facts was mentioned by many research in different part of the world (Lilenbaum *et al.*, 2000; Hariharan *et al.*, 2006; Lyskova *et al.*, 2007; Yaas, 2021). The differences between this study and other similar studies; for example in Iran, Sarchahi *et al.* (2007) recorded 85%, and Zamankhan Malayeri *et al.* (2010) recorded 73.8%. This may related to the cases what are acute or chronic and the different diagnostic tools that used .the second categories was referred to isolation of *Staphylococcus* from human tonsils, nasal cavity and skin infection and the study confirmed the presence of *Staphylococcus* in these all areas with signs of infection the higher rate of isolation was in the nasal cavity and this agree with the fact that the *Staphylococcus* was a normal habitant of upper respiratory tract (Konno *et al.*, 2006). Coagulase negative *Staphylococci* (CoNS) had the higher percentage among the isolates in this study and this disagree with other studies (AL-Qurashi, 2000; Zamankhan Malayeri *et al.*, 2010); in which, the researchers were noted that the main isolate were coagulase positive *Staphylococci* (COPs) other studies in agreement with our results (Lilenbaum *et al.*, 2000; Silva, 2001). Generally, most of researchers in this field mention that the pathogenicity may accompanied with (COPs) this study showed that (CoNs) is the highest this differences may also related to the activity and the level of bacterial enzymes which may be differ with the stage of the disease. Among species of staphylococci our study indicate the highest species identified was *S. pseudintermedius* this result confirmed the importance of this bacteria as a pathogenic *Staphylococci* in human and dogs in another word this study confirmed the zoonotic behavior of this bacteria (Börjesson *et al.*, 2015).

Conclusion

We concluded that the presence of *S. pseudintermedius* is a fact in infection of areas concluded in this study but in previous studies this bacteria was misdiagnosed with *S. aureus* that appeared with

low number of isolates. Furthermore studies are of great importance to demonstrate the culture and genetic characteristics of different species of *Staphylococcus* isolated from dogs or other domestic and wild animals.

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