

Bacteriological and molecular studies on *Coagulase-positive Staphylococci* isolated from oral cavity of dogs and cats

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Abstract

Staphylococci form part of the normal flora of humans and a wide variety of animals. Some Staphylococcal species also cause infections for human and household pets, This study was conducted to investigate *Coagulase-positive Staphylococci* in dogs and cats ,A total of 100 oral swabs were collected from 65 dogs and pet cats,of both sexes from stray and housed pets (27stray and 73 housed), which selected from different clinics and shelters in Cairo and Giza governorates.The prevalence of *S. aureus* was identified in(3/ 27) 11% cats and in (2/65) 3% dogs . *Staphylococcus pseudintermedius*was isolated from one (1%) dogs, The prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in dogs was (1/2) and in cats it was (1/3) of isolated strains The prevalence of methicillin-resistant *Staphylococcus pseudintermedius* (MRSP)was one (1.5%from dog) In conclusion, oral cavity of cats and dogs harbormethicillin-resistant *Staphylococcus*

Keywords:*Coagulase-positive Staphylococci,MRSA, Dogs, Cats, Oral cavity*

INTRODUCTION

Dogs and cats have become an important part of families in Egypt (Abdel-moein& Samir ,2011) , *Coagulase-positive Staphylococci* serve as resident members of the normal cutaneous and mucosal microflora of dogs and cats (Lee et al., 2003) .*S.pseudointermedius* and *S. aureus* are the most dominant CPS species comprised natural mucosal bacterial flora of dogs and cats (Hariharan, 2011) , colonization of

Staphylococcus aureus and *Staphylococcus pseudintermedius* in mouth of dogs and cats have been reported(Abdel-moein et al., 2012;Ivana et al ., 2011 ;Sivakami et al ., 2015 &Bierowiec et al ., 2016).

Now days *Staphylococcus aureus* and *Staphylococcus pseudintermedius* (formerly *S. intermedius*) are receiving attention in both human and veterinary medicine because of increased reports of methicillin resistance, *MRSA* was recorded in Egypt as one of the Mediterranean countries where highest proportions of *MRSA* occur and pet animal have also reported as source of *MRSA* (Borg et al.,2007 ;Loeffler& Lloyd, 2010 Abdel-moein& Samir ,2011). *Methicillin-resistant S. pseudintermedius* (MRSP) colonization has also been reported in dogs and cats (Guardabassi et al., 2004& Frank and Loeffler, 2012). Mouth constitute one of the major sites of colonization of *MRSA in pet animals* (Misic et al .,2015) meanwhile the role of pet animals in the transmission of *MRSA* in the community is not well defined. However, those who work on farms, own pets, and work in veterinary hospitals may be at greater risk for *MRSA* colonization or infection perhaps because of transmission of *MRSA* between humans and animals (Stein ,2009) A gene known as *mecA* gene is responsible for the resistance to methicillin which codes for penicillin-binding protein PBP 2A (Kaniaet al., 2004).

So this study was conducted to throw light over *Coagulase-positive Staphylococci* of the oral cavity of dogs and cats from Cairo and Giza Governorates beside the antibiogram activity of the isolated strains and identificationof resistance genes(*mecA*&*femA*) in *S.aureus*.

2. MATERIAL AND METHODS

2.1. Animal and Samples collection

Oral swabs were collected from 100 pet animals (65 dogs and 35 cats) the ages ranged from 6 months to 6 years ,weighed from 8 to 40 kg, of both sexes (46 male

dogs, 19 female dogs, 11 male cats and 24 female cat), from stray and housed pets (27stray pets and 73 housed pets) .

2.2. Bacteriological and biochemical examination

Swabs were inoculated in Carry and Blair transport media and returned back to the laboratory for culture and identification The collected samples were cultured onto sheep blood agar and mannitol salt agar. The inoculated plates were incubated for 24-48 hours at 37° C. colonies were identified as mannitol fermenting (yellow) colonies on mannitol salt agar and β hemolytic on blood agar. The suspected colonies were picked up and tested for Gram's reaction. Colonies showed Gram positive cocci that arranged in irregular clusters were tested for catalase, coagulase positive colonies were tested by VITEK2 compact (Quinn et al.,2011)

2.4. Antimicrobial Susceptibility Testing

Staphylococcus aureus and *Staphylococcus pseudinter-medi* suspension was prepared and adjusted to a McFarland standard of 0.5–0.63 used for direct AST on VITEK2 System)(Pincus, 2006).

2.5. PCR assay

PCR amplification of 23S ribosomal DNA of resistance genes of *S.aureus*isolates was carried out using the following primers (table 1). DNA was extracted and QIAamp DNA mini kit instructions, with minor modification. Only one ml of cultured colonies in BHI centrifuged then discarded the supernatant and washed the pellets and centrifuged again. After extraction of DNA of each bacterial isolates, PCR master mix was prepared according to Emerald Amp GT PCR master mix (Takara) Code No. RR310A kit mixing deoxy-nucleoside. Amplified PCR products were run on 1.2% agarose gel by Agar gel electrophoresis (Sambrook et al., 1989) with minor modification and visualized by gel documentation system (Kodak) after staining by ethidium bromide

3. RESULT

3.1 .Infection rate of Coagulase-positive staphylococci in oral cavity of dogs and cats.

The results of bacteriological examination of oral swabs recorded in (table 2) revealed that 6 of the 100 oral swabs of pet animals were positive for *Coagulase-positive Staphylococci* . with infection rate of 3 (4.6%) in dogs and 3(8.5%) in cats,represented as 5 isolates *Staphylococcus aureus* (2 (4%) from housed dogs , (4%) from housed cats) and 1(18%) from stray cats and 1 isolate *Staphylococcus pseudointermeidus* (1 (1.5%) from dogs)

3.2. The results of in vitro sensitivity test:

The results of *Staphylococcus spp* in vitro sensitivity test (table 3) showed that of the *Staphylococcus aureus* and *Staphylococcus pseudointermeidus* tested strains were sensitive for the most tested antibiotic agents. Meanwhile, *staphylococcus aureus* was resistant for methicillin; oxacillin appeared in 2(40%) of tested isolates

3.3 PCR results:

PCR results showed that *mecA* were detected in 2 studied *S.aureus* Strains isolated from dogs and cats Meanwhile, *femA* was not detected in 2 studied *S.aureus* Strains Fig (1)

4. DISCUSSION

Staphylococcus is a group of bacteria that can cause a number of diseases as a result of infection (Devriese *et al.*, 2005). In addition to *S. aureus*, other CPS species can cause severe infections compared with those caused by *coagulase-negative Staphylococci* (Sasaki *et al.*, 2007). The pathogenicity of *coagulase-positive staphylococci* are related to the production of many virulence factors including toxins, and enzymes from which coagulase enzyme was consider as the

most important one. Coagulase production was described as one of the most reliable criteria for the identification of pathogenic *Staphylococcus* species. *Staphylococci* producing coagulase are usually pathogenic (Quinn *et. al.*, 2002). In Egypt, the association between pet animals and humans has been changed throughout the last few years. The number of owned dogs and cats was dramatically increased in the Egyptian society that render dogs and cats in close contact with humans. Moreover, dogs and cats are kept inside houses to be considered as common households that permit a close physical contact with such animals at high-frequency basis which facilitates the transmission of pathogens between pets and human contacts (Abdel-moein and Samir, 2011) In this study, the isolated rate of *Staphylococcus aureus* strains from dogs is 2% (Table, 2). In contrary, several studies recorded higher rate ranged from 5 % to 7% as mentioned previously by Rubin & Chirino-Trejo, (2010) and Abdel-moein & Samir ,(2011), respectively

Staphylococcus aureus strains detected in cats was 8.5% ,The obtained result was lower than that obtained by Ivana *et al .*,(2011) who recorded the rate of 20% , On the other hand, the result was higher than that reported by Abdel-moein & Samir ,(2011) who detected the rate of *Staphylococcus aureus* was 2.1%.

The isolated rate of *Staphylococcus aureus* strains from stray cats was 18 % and from pet cat was 4% , this value was in contrast to result obtained by Bierowiec *et al .*, (2016)who recorded it's prevalence of *S. aureus* in domestic cats as 19.17%, while it's prevalence in the feral cat population was only 8.3%.

Also the isolated rate of *Staphylococcus pseudointermedius* strains from dogs was 6 % (Table 2) which nearly similar to result obtained by Awoyomi & Ojo (2014) & Rubin & Chirino-Trejo, (2011) who found that it

represents as 5.9%, 10% respectively. and this disagree with result obtained by Sleiniute&Siugzdaite,(2015) who found that it represents 45.9%.

Staphylococcus pseudintermedius is a veterinary pathogen that has seldom been described as an agent of human disease (Sykes, 2013). And *S. pseudintermedius* isolation depends on the dog's health. (Sleiniute&Siugzdaite, 2015).

In present study methicillin resistance in *S. pseudintermedius* reported (table 3) ,methicillin resistance in *S. pseudintermedius* is emerging as an important clinical problem in veterinary medicine in many countries, including the United States and parts of Europe (Weese and van Duijkeren, 2010).and have reported in oral cavity of dogs (Laarhoven et al., 2011; van Duijkeren et al., 2011 ; Bannoehr and Guardabassi, 2012)

MRSA Infection in small animals, principally in dogs, has been recorded in many countries, with wound infections, surgical site infections, pyoderma, otitis and urinary tract infections most commonly reported (Weese and van Duijkeren, 2010).In the present study, only 2% *Staphylococcus aureus*(MRSA) was isolated from dogs nearly similar to previous work (Abbott et al ., 2010&Loeffler et al ., 2011).

We concluded that *Staphylococci* are spread among cats and dogs. Two species of coagulase-positive *Staphylococci* –*Staphylococcus aureus* and *Staphylococcus pseudintermedius* were identified by using vitek 2 compact .MRSA considered as an emerging zoonosis with a public health burden.

5. References

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Table 1: Primers used for the detection of resistant associated genes in isolates
F: Forward(3`-5`) ,R: Reverse (5`-3`)

Target gene	Primers sequences	Amplified segment (bp)	Reference
<i>FemA</i>	<i>F:AAAAAAGCACATAACAAGCG</i> <i>R:GATAAAGAAGAAACCAGCAG</i>	132	<i>Mehrotra et al., 2000</i>
<i>MecA</i>	<i>F:GTAGAA ATG ACT GAA CGT CCG ATA A</i> <i>R:CCA ATT CCA CAT TGT TTC GGT CTA A</i>	310	<i>McClure et al., 2006</i>

Table (2) Incidence of *Coagulase-positive staphylococci* isolated from oral cavity of cat and dog

Microorganism	No of Dog isolates				No of Cat isolates				Total no of isolate			
	Stray		Housed		Stray		Housed		dog		cats	
	No	%*	No	%*	No	%*	No	%*	No	%*	No	%*
<i>Staphylococcus aureus</i>	-	-	2	4	2	18	1	4	2	3	3	8.5
<i>Staphylococci pseudintermedius</i>	1	6	-	-	-	-	-	-	1	1.5	-	-

%*percentage in relation to No. of each examined sample

Table (3): Antibigram sensitivity for *Staph .aureus* and *Staph. pseudintermedius* bacteria by using Vitek-2 Compact system

Antimicrobial agent	Interpretation			<i>Staph .aureus</i>						<i>Staph. pseudintermedius</i>					
	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
				No	%*	No	%*	No	%*	No	%*	No	%*	No	%*
Oxacillin	≤ 2		≥ 4	2	40	-	-	3	60	1	100	-	-	-	-
Gentamicin	≤ 4	8	≥ 16	-	-	-	-	5	100	-	-	-	-	1	100
Ciprofloxacin	≤ 1	2	≥ 4	-	-	-	-	5	100	-	-	-	-	1	100
Levofloxacin	≤ 2	4	≥ 8	-	-	-	-	5	100	-	-	-	-	1	100
Moxifloxacin	≤ 2	4	≥ 8	-	-	-	-	5	100	-	-	-	-	1	100
Erythromycin	≤ 0.5	4	≥ 8	-	-	2	40	3	60	-	-	1	100	-	-
Clindamycin	≤ 0.5	1	≥ 4	2	40	-	-	3	60	-	-	1	100	-	-
Linezolid	≤ 4			-	-	-	-	5	100	-	-	-	-	1	100
Daptomycin	≤ 1			-	-	-	-	5	100	1	100	-	-	-	-
Vancomycin	≤ 4	16	≥ 32	1	20	-	-	4	80	-	-	-	-	1	100
Doxycycline	≤ 4	8	≥ 16	-	-	-	-	5	100	-	-	1	100	-	-
Tetracycline	≤ 4	8	≥ 16	-	-	-	-	5	100	-	-	-	-	1	100
Tigecycline	≤ 4	8	≥ 16	-	-	-	-	5	100	-	-	-	-	1	100
Nitrofurantion	≤ 32		≥ 12	-	-	-	-	5	100	1	100	-	-	-	-
Rifampicin	≤ 1	2	≥ 4	-	-	-	-	5	100	1	100	-	-	-	-

R: Resistant, I: intermediate, S: sensitive, No.: Number of isolates. %: Percentage in relation to no of isolated strain (*Staph .aureus*(5) and *Staph. pseudintermedius*(1))

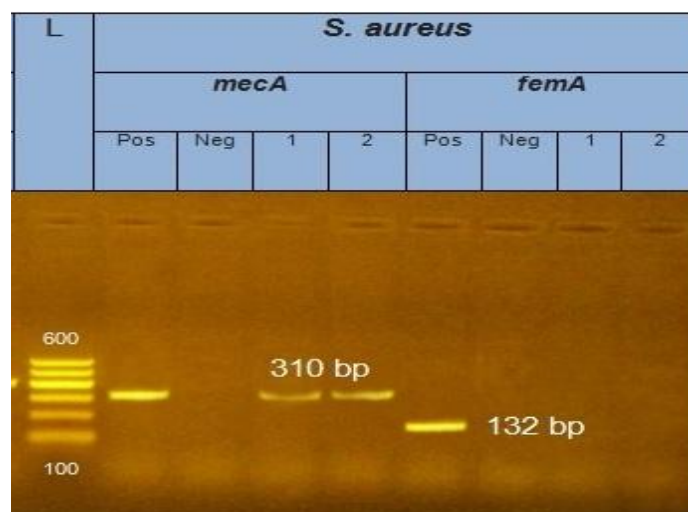


Figure (1) Agarose gel electrophoresis of PCR products after amplification of *mecA* gene at 310bp amplified product. Lane (L): 100-600bp DNA Ladder "Marker" (100 Pharmacia). lanes (1:2) positive isolates at 310 bp. . Lane Pos: Positive control (reference strain deposited to gene bank with *MRSA* ATCC 43300 methicillin-susceptible *S.aureus* ATCC 25923) . Lane Neg: Negative control and amplification of *femA* gene at 132bp amplified product. Lane (L): 100-600bp DNA Ladder "Marker" lanes (1,2) positive isolates extracted DNA