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Prevalence of *Streptococcus gallolyticus ssp. Gallolyticus* in dogs in some urban areas in Kalyobia Governorate with special reference to animal-human interface

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Abstract

House hold pets are kept by people for enjoyment, guardians or psychological support, it can be infected by a wide variety of pathogenic bacteria to animals. Some of these bacteria are of zoonotic importance which can be transmitted to contact people. A total of (40) urine samples were collected from housed dogs located in Toukh city center in Kalyobia Governorate, Egypt. Twenty samples were collected from apparently healthy dogs and the other 20 samples from suspected urinary tract infected dogs manifested clinically by cystitis. The samples were represented with equal distribution between male and female dogs. On the other hand, 20 blood samples were collected from contact children, (10) children were apparently healthy and (10) of them suspected clinically as rheumatic fever patient children. All samples are subjected to bacteriological examination for detection of *Streptococcus. gallolyticus ssp. gallolyticus* .The results showed that *S. gallolyticus ssp. Gallolyticus* was isolated at a percentage of 5.8% and 10.3% from total isolates of clinically suspected male and female dogs respectively. Other isolated organisms are included as *Staphylococcus auresus, Streptococcus pyogens, Streptococcus zooepidemicus, Streptococcus bovis, Escherichia .coli, Klebseila* and *Pseudomonas aeruginosa*. On the other hand *S. gallolyticus ssp. Gallolyticus* was isolated at a percentage of (20%) from clinically suspected rheumatic fever patient children blood samples. Other samples from contact children hands, dogs buccal cavities and urine sand boxes were examined bacteriologic ally to estimate the route of transmission possibilities (licking of hands) which is not clear till now. The isolates were confirmed by using Vitek 2 compact system and PCR. In vitro sensitivity of *Streptococcus. gallolyticus ssp. gallolyticus*against 11 different antibiotics revealed its susceptibility to Ampicillin, amoxicillin/clavulanic acid, Cefotaxime, Erythromycin, Penicillin and vancomycin while there were resistant to ciprofloxacin and clindamycin.

Keywords: *S. gallolyticus ssp. gallolyticus, dogs, urinary tract infection*

Introduction

It became so clear now that the number of house hold dogs is dramatically increased. Although the fact those pets are significantly beneficial to the society, there are number of health hazards associated with owning such pets **Damborg *et al*., 2008).** The close contact between household pets and people offers favorable conditions for diseases transmission either directly by contact (e.g. petting, licking or physical injuries) or indirectly through contamination of food and environments **(Song *et al*., 2013**). E. coli, Staphylococcus spp., Enterococcus spp., Streptococcus spp., Proteus spp., Klebsiella spp., and Pseudomonas spp. are mostly isolated from the urinary tract infection of dogs. **(Ling *et al.,* 2011)**. Streptococcus spp. are opportunistic pathogens that present nor-

mallyin upper respiratory, intestinal, lower urinary, and genital tracts in dogs of all ages. It cause localized infection or septicemia, the clinical significance of isolation of streptococcal organisms should be interpreted according to clinical signs and pathologic findings (**Lamm *et al.* 2010).**

In human, acute rheumatic fever is caused by an autoimmune response to throat infection with *Streptococcus pyogenes*. Cardiac involvement during acute rheumatic fever can result in rheumatic heart disease, causing heart failure and death. The diagnosis of acute rheumatic fever is entirely clinical, without any laboratory gold standards, so accurate data and effective approaches to diagnosis, prevention, and treatment still needed **(Carapetis *et al.,* 2016).**

S. gallolyticusssp. gallolyticus is a member of group D streptococci, is an inhabitant of the animal and human gastrointestinal tract. Furthermore, it is a facultative pathogen which causes endocarditic and septicemia. *S. gallolyticus* subsp. *gallolyticus* may be transmitted either directly or indirectly between animals and humans. However, the mechanism of transmission is an unsolved issue. **(Jessika *et al.,* 2015)**.

Formerly *S. gallolyticus ssp. gallolyticu*s was known as *S. bovis*biotype I., but it was reclassified to *S. gallolyticus ssp. gallolyticus* based on its ability to gallic acid hydrolysis **(schlegel *et al,* 2003)**. *S. gallolyticus* subsp. *Gallolyticus* was identified in several animal species **(Sekizaki *et al.,* 2008)** and was incriminated in 24% of human endocarditic **(Sillanpää *et al.,* 2008).** Several studies discussed the association between streptococcal endocarditis and cancer colo, *S. gallolyticus ssp. gallolyticus* may act as a zoonotic organism **(Dumke *et al.,* 2014).** Tell now there is no explanation for the pathomechanism, the transmission routes and thezoonotic potential of *S. gallolyticus ssp. Gallolyticus* **(Hogg and pearson 2009).** The VITEK 2 gram-positive (GP) identification card (bioMérieux, Marcy l'Etoile, France) was redesigned to achieve higher accuracy in the identification of gram positive cocci, furthermoreit provides reliable results for the identification of gram positive cocci under routine laboratory conditions (**Guido and Pascale 2005)**.

The purpose of this study is to investigate the possible causes of urinary tract infection in house hold dogs and to clear the existence of *S. gallolyticus ssp. gallolyticus* among streptococcus spp. isolates. The study also aimed to discuss the possibility of transmission of such microorganism between these dogs and contact children.

Materials and Methods

Samples collection:

A total of 40 urine samples from dogs were collected in this study from house hold dogs. Twenty samples were taken from apparently healthy dogs while another 20 samples were taken from dogs showing symptoms of urinary tract infection manifested by cystitis (hemturia, stranguria, pollakiuria and incontinence). The samples were obtained by catheterization. On the other hand 20 blood samples were taken from 10- 12 old children. Ten blood samples were taken from apparently healthy children and 10 blood samples were taken from suspected primary as rheumatic fever children (fever greater than 39°C at the onset of illness and resolved over several weeks and arthralgia). All blood samples were taken from children contacted with the same dogs under investigation. Another 60 swab samples were taken from children contact hands, buccal cavities of the same dogs and its urine sand boxes, 20 swabs from each respectively.

Bacteriological examination:

A loop full from each urine sample sediment (centrifuged 3000 rpm/ 10 min) was streaked onto sheep blood agar, nutrient agar, mac-Conkey's agar, EMB, mannitol salt agar and Edward's media. Swabs and blood samples were streaked onto sheep blood agar, brain heart infusion agar. The inoculated plates were incubated at 37ºC for 24-48 hours aerobically and examined for bacterial growth. Suspected colonies, appearing on different media were subculture, purified and preserved in semisolid agar media for further identification. Pure cultures were further examined for morphological, staining and cultural characteristics and biochemical reactions as described

by **Quinn *et al.*, (*2002)***

Identification method with VITEK2 compact system for un- identified *Streptococcus* isolates (Pincus, 2006)

A total of (18) isolates of *streptococci* which were not identified by traditional methods were identified by ViTEK2 compact system. *Streptococci* isolates were typed by ViTEK2 compact system after confirmed by PCR for its 16 RNA common gene *streptococcus*. The test panels (ID-GPS, BIOMERIEUX) were automatically filled by a vacuum device, sealed and inserted into VITEK2, reader-incubator module (BIOMERIEUX) and subjected to kinetic fluorescence measurement every 15 min. the result were interpreted by (ID-GPS) database and final results were obtained automatically.

Detection of *streptococcus* by using PCR

DNA extraction. For more confirmation that the isolates are streptococci. DNA extraction from pooled 18 isolates of *S. gallolyticus ssp. gal-lolyticus* were performed (5 from urinary tract infected dogs, 2 from contact patient, 3 from contact hands, 5 from dogs buccal cavities and 3 from urine sand boxes) pooling was done for each group separately using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. The pooled isolates are divided to 5 groups. Briefly, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56^{O}C for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

Oligonucleotide Primer: Primers used were supplied from **Metabion (Germany)** are listed in table (1)

PCR amplification: Primers were utilized in a 25- µl reaction containing 12.5 µl of EmeraldAmp Max PCR Master Mix **(Takara, Japan)**, 1 µl of each primer of 20 pmol concentration, 4.5 µl of water, and 6 µl of DNA template. The reaction was performed in an Applied biosystem 2720 thermal cycler.

Analysis of the PCR Products.

The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 20 µl of each PCR product were loaded in each gel slot. A 100 bp DNA Ladder (Fermentas, Germany) were used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

Table (1). Oligonucleotide primers sequences of primers used in PCR amplification assays and their respective PCR products.

Gene	Primer sequence	Length of amplified product	Primary denaturation	Amplification (35 cycles)			Final extension	reference
				Secondary denaturation	Annealing	Extension		
16S rRNA	CGGGGGATAAC-TATTGGAAACGATA ACCTGTCACCCGATGTAC-CGAAGTA	912 bp	912 bp	94˚C 30 sec.	55˚C 40 sec.	72˚C 50 sec.	72˚C 10 min	**Osakabe *et al.*, (2006)**

Antibiotics susceptibility test:

The sensitivity of isolated *S. gallolyticus ssp. gallolyticus* strains were carried up using **Mueller Hinton agar (Oxoid)** plates and the standard disc diffusion method according to **Quinn *et al*. (2002)** using 11 different antibiotic discs. The results were interpreted according to the **NCCLS (2002).**

Results (the results illustrated in tables 2-4, sheet 1, photo 1 and 2)

Table (2). Prevalence of isolated micro- organisms among dogs under investigation by using traditional methods

Isolated micro-organisms	Apparently healthy dogs				Urinary tract infected dogs			
	Male n= 10		Female n=10		Male n= 10		female n= 10	
	No.	%*	No.	%*	No.	%*	No.	%*
Staphylococcus aureus								
Coagulase +ve	-		1	12.5	5	14.7	4	13.7
Coagulase -ve	2	40	1	12.5	-	-	-	-
Streptococci								
S.pyogenes	-		1		4	11.7	5	17.2
S.zooepidemicus	1		1	12.5	3	8.8	4	13.7
S.bovis	-	20	-	12.5	3	8.8	2	6.9
N.I.**	-		-		2	5.8	3	10.3
E.coli	1	20	2	25	8	23.5	6	20.7
Klebsiella	-	-	-	-	3	8.8	2	6.9
Pseudomonas								
P. aeruginosa	1	20	2	25	6	17.6	3	10.3
Total	5		8		34		29	

* the percentage calculated according to total number of isolates in each item.
** N.I. (not identified) the isolates were typed by using Vitek 2 compact system after confirmation by PCR streptococcus common gene

Table (3). Prevalence of isolated micro- organisms among contact children patients' blood sample by using traditional methods

Isolated micro-organisms	Contact patients (n=10)
	No.
***Staph. aureus* coagulase +ve**	6/10
Streptococci	
S. pyogens	2/10
N.I. **	2/10

** N.I. (not identified) the isolates were typed by using Vitek 2 compact system and confirmed by PCR streptococcus common gene.

Table (4). Prevalence of *S. gallolyticus ssp. Gallolyticus* in different swab samples (n=20)

Swab sample	*S. gallolyticus ssp. gallolyticus*	
	No.	%*
Contact hands	3	15
Dogs buccal cavities	5	25
Urine sand boxes	3	15

* The percentage calculated according to total number of samples

Sheet (1). Biochemical details for *Streptococcus gallolyticus ssp. gallolyticus* by using Vitek 2 compact system

BiochemicalDetails																	
2	AMY	+	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	-	11	AGLU	+
13	APPA	+	14	CDEX	-	15	AspA	(-)	16	BGAR	-	17	AMAN	-	19	PHOS	-
20	LeUA	+	23	ProA	-	24	BGURr	-	25	AGAL	+	26	pyrA	-	27	BGUR	-
28	AlaA	+	29	TYrA	-	30	dSOR	-	31	URE	-	32	POLYB	+	37	dGAL	+
38	dRIB	-	39	iLATk	-	42	LAC	+	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	+	50	NC6.5	+	52	dMAN	+	53	dMNE	+	54	MBdG	+	56	PUL	+
57	dRAF	+	58	O129R	+	59	SAL	+	60	SAC	+	62	dTRE	+	63	ADH2s	-
64	OPTO	+															

18 strains were identified as *S. gallolyticus ssp. gallolyticus* by using Vitek 2 compact system. Two strains were isolated from infected children(2/10), 2 strains from urinary tract infected male dogs and 3 from urinary tract infected female dogs. Finally 11strains isolated from different swabs as mentioned in table (4)

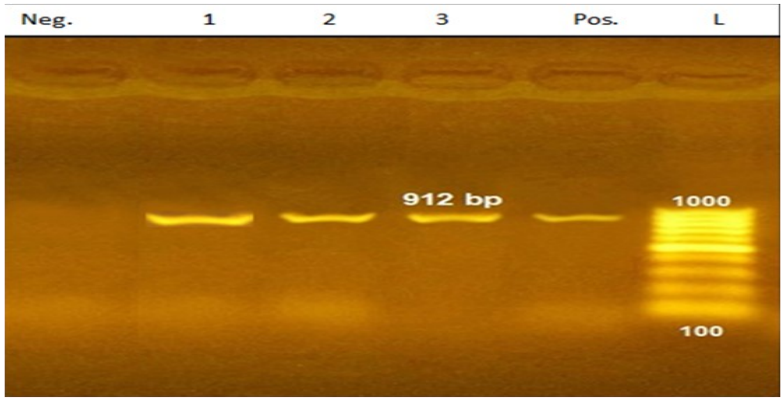

Photo. (1): Positive gene amplification at 912bp of *Streptococcus* isolates form swab samples
L. ladder Pos.: control positive Neg.: control negative
1: positive *Streptococcus* gene amplification at 912bp for contact hands swab isolates
2: positive *Streptococcus* gene amplification at 912b for buccal cavities swab isolates
3: positive *Streptococcus* gene amplification at 912bp
For urine sand boxes swab isolates

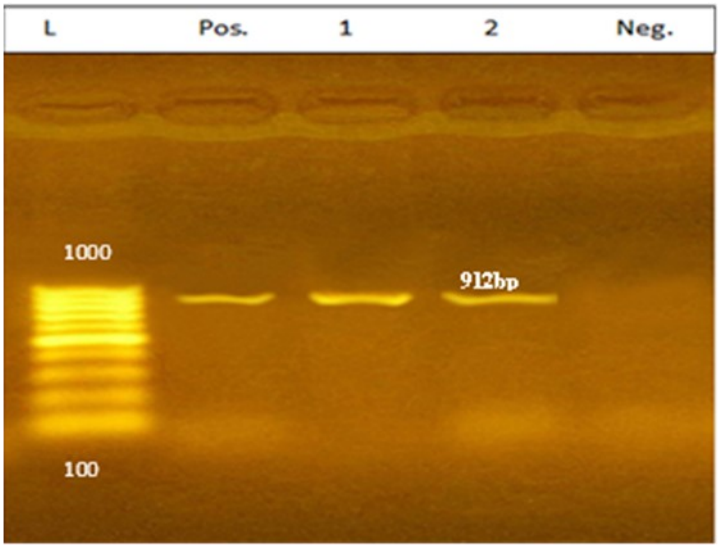

Photo. (2): Positive gene amplification at 912bp of *Streptococcus* isolates form children blood and dog urine samples
L. ladder Pos.: control positive Neg.: control negative
1: positive streptococcus gene amplification at 912bp for children blood isolates
2: positive *Streptococcus* gene amplification at 912bp for dog urine isolates

Table (5). Antibiotic sensitivity test of *S. gallolyticus ssp. gallolyticus*

Antibiotics	Symbol	Concentration /µg	sensitive		resistant	
			No.	%	No.	%
Ampicillin	AMP	10	16	88.9	2	11.1
amoxicillin/clavulanic acid	AMC	30	18	100	0	0
Cefotaxime	C TX	30	15	83.3	3	16.7
Ciprofloxacin	CIP	10	3	16.6	15	83.3
Clindamycin	DA	10	0	0	18	100
Doxycycline	DO	30	9	50	9	50
Erythromycin	E	15	15	83.3	3	16.7
Lincomycin	MY	15	10	55.5	8	44.5
Penicillin G	P	10	17	94.4	1	5.6
Tetracyclin	TE	30	2	11.1	16	88.9
Vancomycin	VA	30	18	100	0	0

% calculated according to the number of total tested*S. gallolyticus ssp. gallolyticus*isolates (n=18)

Discussion

Pet animals as dogs considered as important source for zoonotic bacterial pathogens transmission to the contact people. In this study table (2) showed that, in apparently healthy male dogs the percent of isolation of coagulase negative *staph aureus* was (40%) while *Strept. zooepidemicus*, *E. coli* and *P. aeruginosa* were (20%) of each. In apparently healthy female dogs, coagulase positive *staph. aureus* , coagulase negative *staph aureus*, *Strept. pyogens*, *Strept. zooepidemicus* were (12.5%) of each while *E. coli* and *P. aeruginosa* were (25%) of each.

On the other hand, the urinary tract affected male dogs showed coagulase positive *staph. Aureus* (14.7%), *Strept. Pyogens* (11.7%), *Strept. Zooepidemicus* (8.8%), *Strept. bovis* (8.8%), other not identified strepcoccus spp. (5.8%), *E. coli* (23.5%), *Klebsiella* (8.8%) and *P. aeruginosa* was (17.6%). the urinary tract affected female dogs showed coagulase positive *staph. Aureus* (13.7%), *Strept. pyogens* (17.2%), *Strept. zooepidemicus* (13.7%), *Strept. bovis* (6.9%), other not identified strepcoccus spp. (10.3%), *E. coli* (20.7%), *Klebsiella* (6.9%) and *P. aeruginosa* was (10.3%) The percentage calculated according to the total number of isolates showed that male dogs were more predisposed than females. These results are slightly higher than reported by (**Papini *et al.,* 2006).** Who isolated *staph. aureus* (6.27%), *E. coli* (17.48%), *Klebsiella* (0.89%) and *P. aeruginosa* (17.7%) from dogs with urinary tract infection. On the other hand our results are nearly agree with **(Gerald *et al., 2001*)** who isolated *E. coli* (44.1%), *Staphylococcus* spp. (11.6%), *Klebsiella* spp. (9.1%) and *Streptococcus* spp. (5.4%)from dogs with urinary tract infection. The same authors failed to identify some isolates of streptococcus spp. by traditional methods, in spite of females were more predisposed to infections. The difference of results could be attributed to difference in climatic conditions, veterinary care, educational and economical culture.

Table (3) illustrated the prevalence of microorganisms isolated from contact patient children, coagulase positive *staph. Aureus* was (60%), *Strept. pyogens*(20%) and other not identified strepcoccus spp. (20%).

The non-identified isolates in table (2) and (3) were identified by using Vitek 2 compact system as shown in sheet one after confirmation that they were *streptococcus* by PCR for detection of *16S rRNA* gene. The results revealed that these isolates *S. gallolyticus ssp. gallolyticus* with a prevalence of (5.8%), (10.3%) and (20%) for infected male, female dogs and contact patient respectively. The results agree with that reported with **(Sillanpää *et al.,* 2008)** who

reported that *S. gallolyticus ssp. gallolyticus* was responsible for24% of human acute endocarditis. Vitek 2 compact system also, used to identify *S. gallolyticus ssp. gallolyticus* from contact hands, dogs buccal cavities and urine sand boxes table (4) with a percentage of (15%), (25%) and (15%) respectively. These results agreed with **(Dumke *et al.,* 2015)** who isolated *S. gallolyticus ssp. gallolyticus* from surrounding environmental dusts and stored manure.

Photo (1) and (2) illustrated the confident conformation of streptococcus isolates from different samples sources of the study under investigation by using PCR for *16S rRNA* gene.
In table (5) *S. gallolyticus ssp. gallolyticus* isolates were highly sensitive to Ampicillin, amoxicillin/clavulanic acid, Cefotaxime, Erythromycin, Penicillin G and Vancomycin . while it was resistant to Ciprofloxacin, Clindamycin and Tetracyclin the same results were mentioned by **Dumke *et al.,* (2015)**. Furthermore the results agreed with that reported by **Ryohei *et al.,* (2013)** who examined the antimicrobial susceptibility of *S. gallolyticus* isolates from human and animals and the results interpreted that, all the tested isolates were susceptible to vancomycin, penicillin G, and ampicillin. Tetracycline resistance was prevalent in the isolates from human patients, diseased animals, and healthy broiler chickens.

Conclusion: house hold dogs must be undergoing regular veterinary inspection for early and accurate diagnosis of diseases with application of vaccination programs. Contact persons must be aware with personal hygiene, and biosafety measures to protect them from zoonotic diseases transmission. Finally *S. gallolyticus* subsp. *gallol-yticus* is one of zoonotic diseases that may be transmitted to dogs contact persons and further investigations should be carried for more confirmation.

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