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CARRIAGE OF *ENTEROCOCCI* SPECIES AMONG HUMAN AND PET ANIMALS

Hassan Falah Mohammed*, Nagham Mohammed Al-Gburi*
Zoonotic Diseases Unit, Coll. Vet. Med. Uni. Bagh. Iraq

Abstract

Enterococci are a part of the normal microbial flora in the gastrointestinal tracts of humans, animals, and the environment. They have been recognized as opportunistic pathogens that cause infections in humans and animals, especially when they are intrinsically more resistant to antimicrobial agents commonly used in hospitals. This study was conducted to investigate the carriage of *enterococci* species(spp) in oral, nasal, and fecal of human and their companion pets. Oral, nasal, and fecal swabs were collected from 25 dogs (25) and cats (25) when brought to the animal hospital, and oral, nasal swabs from their owners and from other owners (100) were collected , and fecal samples were collected from 50 other human group during a period from 2.Nov.2021to28.April.2022. Isolation and identification were done by traditional,Vitek2 system,and molecular methods using species-specific genes. The results of the current study showed that in human, *E.gallinarum* was the most predominant spp isolated from oral and nasal, followed by *E.faecalis* detected in oral and fecal samples , and *E. faecium* detected in oral and nasal samples. Males had a higher carriage and isolated *enterococci* spp reported than in females. According to age, the 10-19year old age group had a higher carriage and isolated enterococci spp than other groups.

In dogs, *E.gallinarum* followed by *E. faecium* were the most spp identified from nasal; *E. faecium* were the higher isolates followed by *E.gallinarum* in oral, and only *E.faecalis* were detected in fecal samples. All isolates from dogs males, puppies and adults dogs were given the highest positive carriage of *enterococci*.While in cats, *E.faecalis* isolated from oral higher than fecal samples and *E.gallinarum* isolated only from nasal samples. According to gender and age,Tom cats were higher carriage than Pussy cats ,and adults cats given the highest positive *enterococci*. The current study found that the most prominent *enterococci* spp in human, dogs and cats were *E.gallinarum* and *E.faecalis.* Furthermore, the isolation of the same *enterococci* spp in humans and their animals may be attributed to transmitted from these animals to their owners or vers versa and possible consider as zoonotic transmission.

Keywords: *Enterococci*, Human, Dogs Cats

Introduction

Enterococci spp are Gram-positive cocci, non spore-forming, facultative anaerobic lactic acid microorganism and catalase-negative, naturally found in the gastrointestinal tract of both human and animals (Escobedo-Hinojosa & Pardo-LópezL, 2017; Comerlato et al., 2020).In addition, enterococci spp are one of the sources of contamination of food especially antimicrobial strains(Dubin & Pamer, 2016; Zaheer et al., 2020; Arias & Murray, 2012). The important emerged species that healthcare-related , and one of the major causes of nosocomial infections are *E. faecium* and *E.faecalis*, they are after Staphylococcus aureus and coagulase-negative

Staphylococci and are associated with severe diseases in human and animals including birds, such as bacteremia, meningitis,endocarditid, intra-abdominal infections, wound infections,urinary tract infections(UTI), atherosclerosis and play a critical role in the riskiness or development of periodontitis particularly in a suitable mouth environment (Mendes et al., 2020; Xiong et al., 2021; Al-Hamdany & Al-Kennany, 2014; Bhardwaj et al., 2020). Companion animals such as dogs and cats can be infected with *enterococci* causing urinary tract infections, gastroenteritis, peritonitis, periodontitis, osteomyelitis, and endocarditis and or could be asymptomatic carriers *E. Faecalis* and *E. faecium* are the most spp reported in these animals, in addition, the *enterococcal* infections of these animals may be very difficult to treat (Výrostková et al., 2021; Alkhafaje et al., 2022). Enterococci spp can be transmitted to human via contaminated urin,feces,saliva, or via direct contact between pets and human, this transmission plays a main role in the distribution of resistant genes among microorganisms such as enterococci spp (Al-Shammary, 2019).Especially, pet owners are usually in constant contact with these animals, in addition to the habit of hugging and kissing them, from the mouth, or licking these animals their owners and helping many microorganisms such as enterococci spp to enter the human body through contamination of hands, skin abrasions, as well, the emergence of antimicrobial-resistant or virulent microorganisms in these pet animals act as a reservoir of antimicrobial-resistant microorganisms for humans, studies indicated that the mouth cavity could be an important source of antimicrobial resistant, virulent and biofilm forming enterococci strains and thus considered a major public health concern (Bhardwaj et al., 2020; Jackson et al., 2009; Bang et al., 2017). This study aimed to investigate the carriage of *enterococci* spp of oral,nasal and fecal samples from humans and their companion animals.

Materials and methods

Two hundred fifty samples, including 100 human (group1) (100 nasal and 100 oral swabs) were collected from pet animal(dogs and cats) owners and other owners when visiting the Baghdad veterinary teaching hospital for their animals, most of them show flu-like symptoms, because of the difficulty in obtaining stool samples from this group, stool samples (50) were collected from another group as group2 from private laboratories. Samples were collected from both gender and of different ages. A total of 150 samples including (oral, nasal and fecal swabs were collected from dogs (25) and cats (25) from both gender and different ages and breeds. The animals were suffering from different clinical signs including emaciation, dyspnea, enteritis, difficulty, and lack of urine, diarrhea, abortion, nasal discharge, gingivitis, and gastritis. samples were collected from both gender and of different ages by sterile swab with transport medium, samples were labeled and placed in cooler boxes immediately before being sent to the laboratory during a period from 2.NOV.2021to28.April.2022.

Samples were inoculated in HiCrome *enterococci* broth (HiMedia) and incubated for 24 h at 37 °C. Then a loopfull of blue color cultures development that indicated positive *enterococci* were inoculated on HiCrome *enterococci* agar (HiMedia) and incubated at 37 °C for 24 h. The blue positive *enterococci* colonies were selected, and inoculated on 5% sheep blood agar and esculin

agar (HiMedia) and incubated at 37 °C for 24 h, bacterial smears were done for gram stained to confirm the presence of gram positive cocci, and then tested for catalase.Vitek2 compact system (Bio-merieux France) was used for complete biochemical identification. Molecular confirmation of *enterococci* spp was done using species-specific primers were selected for identification for *E.faecalis*,*E.faecium* and *E.gallinarum* (Table 1) according to (Dutka-Malen et al., 1995), from 24h old culturs,genomic DNA of the isolates were extracted according to the commercial Wizard Genomic DNA purification Kit (Korea), polymerase chain reaction (PCR) was performed at following steps: an initial denaturatin at 95°C for 5minute,followed by 35 cycles of denaturation for 45seconds,anneling at 60,55 and 58°C for 45seconds for *E.faecalis*,*E.faecium* and *E.gallinarum* respectively, extension at 72°C for 1minute ;and final extension at 72°C for 5minute.The reaction mixtures were composed of (5µl of Taq PCR PreMix, 1 µl of each of the two primers, 1.5µl of template (DNA), 16.5 µl of distill water to a final volume of 25 µl).PCR products were resolved by electrophoresis on a 1.5% of agarose gel stained with red stain,bands were
visualized using gel imaging system under UV transilliminated.

Table1: species-specific primers

Primer	Sequence	Gene sequence(5`-3`)	Length (bp)
Ddl E. faecalis	F	ATCAAGTACAGTTAGTCTT	941 bp
	R	ACGATTCAAAGCTAACTG	
Ddl E. faecium	F	GCAAGGCTTCTTAGAGA	550 bp
	R	CATCGTGTAAGCTAACTTC	
vanC E. gallinarum	F	GGTATCAAGGAAACCTC	822 bp
	R	CTTCCGCCATCATAGCT	

Statical Analysis

The data were analysis using Statical Analysis System(SAS version 9.1). The Chi-square test was used to assess the significant differences among proportions. Also the odds ratio (OR) along with 95% confedence interval was estimated (SAS, 2010).

Results

Enterococci spp were identified at the genus level by cultural characteristics, Gram stain, the catalase test, and the a esculin hydrolysis. The *enterococci* spp identification using Vitek 2 compact system and PCR results were in accordance, three species of enterococci were identified which are *E.faecalis*(27 isolates),*E.faecium*(6 isolates) and *E.gallinarum* (40 isolates)from human,dogs and cats figures 1and 2.

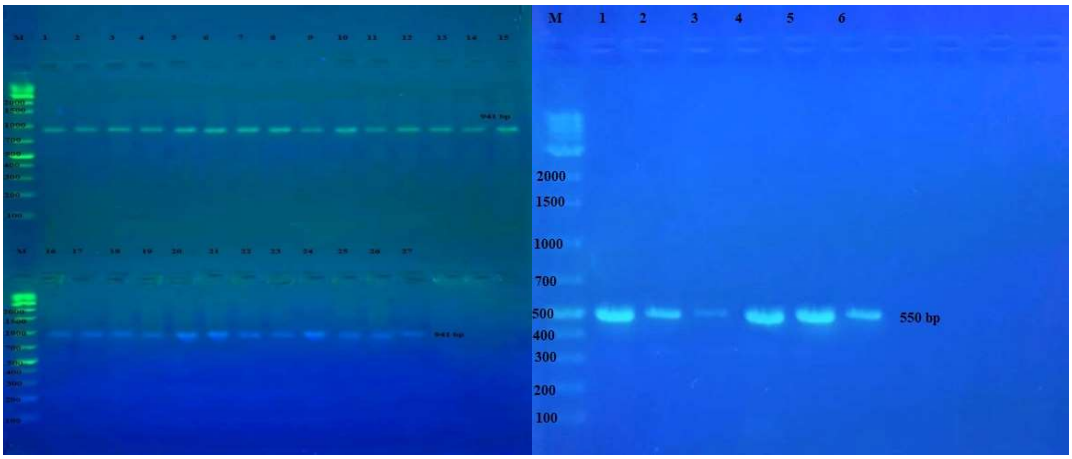

Figure (1) PCR product the band size 941bp presence of *E.faecalis*(left),550bp *E.faecium(*right*)*. The product was electrophoresis on 1.5% agarose at 5 volt/cm^2. 1x TBE buffer for 1:30 hours. M: DNA ladder (100).

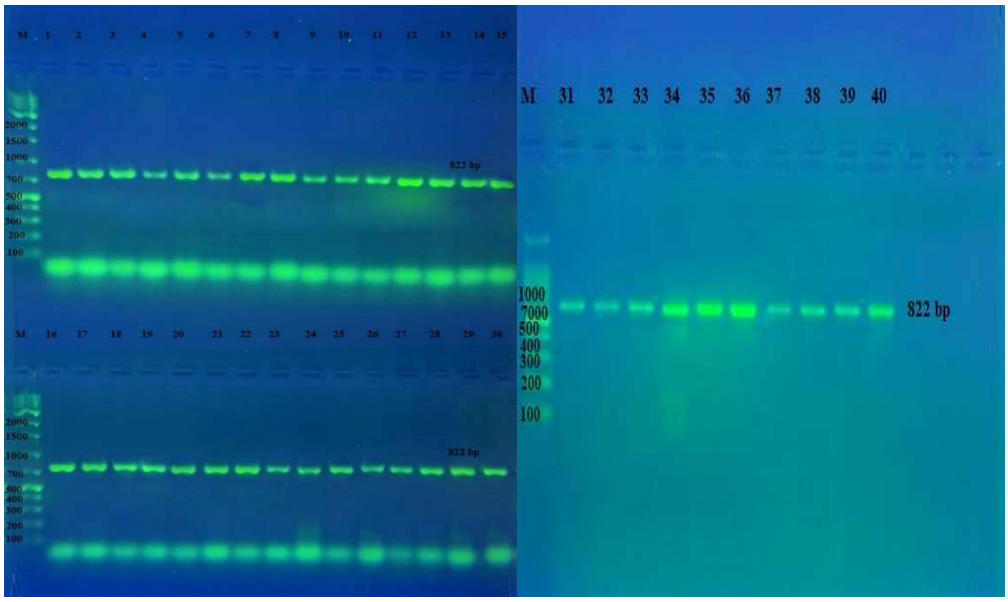

Figure (2) PCR product the band size822bp,presence of *E.gallinarum* . The product was electrophoresis on 1.5% agarose at 5 volt/cm^2. 1x TBE buffer for 1:30 hours. M: DNA ladder (100).

Human were carriag *enterococci* 20 out of 150 (13.4%), G1(oral and nasal samples) were carriage 17 (17%) and G2 (fecal samples) carriage 3/50 (6%). Thirty-one *enterococci* isolates were identified, 28 isolates from G1 which are 13(46.42%) from oral, and 15(53.57%) from the nasal, the *enterococcus* isolates from fecal samples (G2)were 3 (100%) and there was no significant differences in *enterococcus* distribution between oral, nasal, and fecal (Chi-square value 31, P-value<0.0001).The distribution of 28 enterococci isolates of G1,the most predominant spp were

E.gallinarum 20(71.42%) followed by *E.faecalis* and *E. faecium* .In oral, *E.gallinarum* and *E.faecalis* were reported the same percentage. In nasal, *E.gallinarum* was a higher 14(93.33%) followed by *E. faecium* . In contrast, only *E.faecalis* were reported in fecal samples, a significant correlation was seen in the distribution of *enterococcus* spp between oral, nasal, and fecal samples Table 2.

Table 2: Distribution of *enterococci* spp isolates according to samples sites in human

Site of Samples(No.of isolats)	***Enterococci* spp. (31 isolates)**			**Chi-square value**	**P-value**
	E.faecalis	*E.gallinarum*	*E. faecium*		
Oral (13)	6(46.15)	6(46.15)	1(7.69)	15.64	<0.001
Nasal(15)	0(0)	14(93.33)	1(6.66)		
Total(28)	21.42	71.42	7.14		
Fecal (3)	3(100)	0(0)	0(0)		

A significant differences were observed between males and females in *enterococci* carriage, the prevalence of positive carriage in males was 15(21.4%) more than in females. individuals who are 10-19 years old were carriage *entereococci* 6(20%), followed by 30-39 years old, and the results of the OR revealed that human at 10-19 years was more at risk (about four and seventy five fold) followed by 30-39 years and then 20-29years. Non significant difference found in *enterococcal* carriage between the age groups Table 3.

Table 3: Carriage of *enterococci* in human according to gender and age

Gender	**No. /positive (%)**	**Chi-square**	**P**	**OR**	**95%CI**
Male	70/15 (21.4)	7.44	<0.01**	Ref	
Female	80/5 (6.25)			0.24	0.08-0.71
Age group/years	No. /positive(%)				
1-9	20/1(5)	3.36	0.33NS	Ref	
10-19	30/6(20)			4.75	0.52-42.90
20-29	59/6(10.2)			2.15	0.24-19.04
30-39	41/7(17.1)			3.91	0.44-34.23

Positive carriage of *enterococci* were reported 9(36%) in dogs and 12(48%) in cats with non-significant differences between dogs and cats in carriage of *enterococci* (Chi-square value 0.74;Pvalue 0.38). Sixteen isolates were detected in dogs 8 (50%) of them were from nasal,7(43.75%) from oral, and 16(6.25%) from fecal samples, the most spp isolates were *E.gallinarum* 11(68.75%) followed by *E. faecium* 4(25%) and *E.fecalis* 1(6.25%). In cats, 26

isolates as follows: 8(30.76%) from oral and 9(34.62%) from nasal and fecal to each, the predominant spp were *E.faecalis* 17 (65.4%) followed by *E.gallinarum* 9(34.61%) . Non-significant differences in distribution of isolates among dogs and cats according to site samples(Chi-square value 4.39;Pvalue 0.11). The distribution of *enterococci* spp in dogs and cats according to sites samples listed in Table 4 showed a significant differences between the oral of dogs and cats, while non significant difference between nasal and fecal samples in both animals.

Table 4: Distribution of *enterococci* spp in dogs and cats according to sites

Animal	**Sit (No;% of isolates)**	**No. (%) of *enterococcus* spp isolates**			**Chi-square value**	**P-value**
		E.fecalis	*E.gallinarum*	*E. faecium*		
Dogs	Oral (7; 43.75)	0(0)	3(42.85)	4(57.14)	15.00	<0.001
Cats	Oral (8; 30.76)	8(100)	0(0)	0(0)		
Dogs	Nasal(8;50)	0(0)	8(100)	0(0)	0.01	0.99NS
Cats	Nasal(9;34.62)	0(0)	9(100)	0(0)		
Dogs	Fecal (1; 6.25)	1(100))	0(0)	0(0)	0.02	0.27NS
Cats	Fecal (9;34.62)	9(100)	0(0)	0(0)		

All positive carriage *enterococci* isolates were from male in dogs samples 9(36%) with significant differences. less 1year and 6-12years old of age were given the highest positive *enterococci* 2 (40%), and 6(40%) respectively than 1-5 years, police dogs (K9) reported the highest positive rate of *enterococci* 6(54.54%) than other breeds Table 5.

Table 5: Positive carriage of *enterococci spp* in dogs according to gender, age, and breed

Gender	**No. /positive (%)**	**Chi-square value**	**P-value**	**OR**	**95%CI**
Male	25/9(36)	-	-		
Female	0(0)				
Age group					
(less 1 year)	5/2(40)	0.69	0.70NS	Reference=1	
(1-5years)	5/1(20)			0.37	0.02-6.34
(6-12years)	15/6(40)			1.00	0.12-7.89
Breed					
Police dogs(K9)	11/6(54.54)	2.96	0.39NS	Reference=1	
Husky	4/1(25)			0.27	0.02-3.57

Local	5/1(20)	0.20	0.01-2.51
Terrier	5/1(20)	0.20	0.01-2.51

In cats, the positive carriage of *enterococci* in males was 6 (60%) higher than in females 6(40%), 6-12years old age cats were highest positive for *enterococci* 5(62.5%) followed by 1-5 years old of age 3(42.9%), Persian, Shirazi give higher positive 1(50%) and 5(50%) respectively than other breeds Table 6.

Table 6: Carrige of *enterococci spp* in cats according to gender, age, and breed

Gender	**No./ positive (%)**	**Chi-square value**	**P-value**	**OR**	**95%CI**
Male	10/6(60)	0.96	0.32NS	Reference=1	
Female	15/6(40)			0.44	0.08-2.27
Age group					
less1year	10/4(40)	1.00	0.60NS	Reference=1	
1-5years	7/3(42.9)			1.12	0.15-7.98
6-12years	8/5(62.5)			2.50	0.37-16.88
Breed					
Persian	2/1(50)	0.37	0.94NS	Reference=1	
Shirazi	10/5(50)			1.00	0.04-20.83
Local	4(40)			0.67	0.03-14.03
Scottish	1(33.33)			0.50	0.01-19.56

Discussion

Enterococci are one of the commensal flora bacteria on the human intestine and airways and are responsible for numerous infections (Mendes et al., 2020; Mustafa et al., 2021). In human, the most prevalent spp were *E.gallinarum*, followed by *E.faecalis; E.gallinarum* were more than *E. faecium* detected from nasal , whilst in oral, *E.gallinarum* and *E.faecalis* were detected higher than *E. faecium*. These results may agree or partially agree with other studies, in children patients *enterococci* were isolated from the nasal at 26.4%, *E. faecium* was more than *E.faecalis*; in perirectal was 50.9% and *E.faecalis* were more than *E. faecium* (Yameen et al., 2013). *Enterococci* were reported in the oral of healthy persons at 47.91%, the most predominant were *E. faecalis* 88.7% and a lower percentage of *E. faecium* 1.7% (Komiyama et al., 2017) which agrees with this study. Farmers were reported to harbor *E.faecalis* and *E.faecium* in nasal and fecal samples 35%,6%;62%,and 54% respectively reported by (Tan et al., 2018).In addition, *enterococci* were isolated at 76% of stool from gastrointestinal tract infections, 19.2% of them were *E.faecalis* and 53.5% were *E.faecium,* while *E. faecium* isolated from human fecal samples at 80% more than *E. faecalis* at 1.5%, and *enterococci* spp were isolated from oral cancer patients at10.90% (Ekin et al., 2018; Iseppi et al., 2020; Nawar et al., 2021). *E.faecalis* is one of

the nonoral bacteria, usually present in the nares and intestine of human, but it can accidentally entrance into the mouth through contact with animals, contaminated food and water, mouthing or chewing contaminated materials leading to infection, systemic colonization and infections associated with non-oral microorganisms including *E.faecalis* from oral cavity have been recorded, thus the oral cavity considers an additional hospital reservoir (Arirachakaran et al., 2019; Ghapanchi et al., 2019; Kearney et al., 2020). *E.gallinarum* were isolated in high percentages, which contradicts the other studies that both *E.faecalis* and *E.faecium* are the predominant spp, there are no previous studies isolates of *E.gallinarum*, and this spp is a natural flora in human and animals gastrointestinal tract (Oladipo et al., 2014), and because of the rising use of broad-spectrum antibiotics and invasive medical equipment and devices, infections by *E. gallinarum* have gradually increased, and multi drugs resistance has acquired more attention, *E. gallinarum* isolated from different clinical cases and from foods (Zhao et al., 2018; Atiyah AS, Alkhafaji, 2020; Çalgin & Çetinkol, 2018; Dias et al., 2019). The differences in isolation rate, and isolated spp were found to be influenced by the geographical region and the clinical samples used in the study (Shridhar & Dhanashree, 2019), in addition to variation in the methods used for the detection of *enterococci*, number and type of samples, and healthy or diseased cases and hygiene habits of the individuals.

Although there was a non-significant difference in enterococci carriage among the age groups, this result was not in accordance with (Yameen et al., 2013) who found patients with an age group less one years were more colonized with enterococci at 62.7%, and partially in line with the study found that carried oral enterococci reported in healthy individuals were higher 30% among 30–59 years (Komiyama et al., 2016).Males carriage *enterococci* more than females, and the carriage increased with age in the current study this may be because the males have more contact with these pets, the environment or poor or hygiene status of owners or they are smoking, a study found the presence of enterococci was to be directly correlated with periodontitis and smoking, and enterococci were found common in persons whose oral hygiene is poverty, low socioeconomic status and who are smokers, another study found the oral cavity consider an important reservoir of rather virulent and antimicrobial enterococci, with a growing degree of carriage in the adults and elder (Bhardwaj et al., 202; Komiyama et al., 2016).

Even though pet animals such as dogs and cats have psychosocial benefits to their owners, they can carry serious pathogenic bacteria to human (Abdel-Moein & Samir, 2011).Although there was no significant difference between cats and dogs in the carriage of *enterococci*, cats were higher in carriage compared with dogs. The carriage of *enterococci* in dogs' nasal cavities was higher than oral, and a lower prevalence in fecal samples. While, in cats, the prevalence was similar percentages in nasal and fecal samples and close to oral. Current results were lower compared to the findings, (Jackson et al., 2009) found 80% of the dogs and 60% of the cats were carriages of *enterococci*, and have been isolated from dogs 83.3% and from cats 43.8% reported by (Kataoka et al., 2014), which is in line with this study in cats found carriage *enterococci* at 48%. In contrast, in dogs *enterococci* were 31% in the rectal, and l.45% in the nasal; in cats, 36.55% in the rectal,

and 2.07% in the nasal were reported by (Jackson et al., 2009), while (Bertelloni et al., 2015) found that 77.3% of fecal swabs of clinically healthy dogs were shedding *enterococci*.

The variation in percentages in the present and other results may be because of differences in the number of animals and samples, the difference in the site of sampling, the condition of the animals, and the method of isolation, some studies focus on certain spp especially, *E.faecalis* and *E. faecium*, cats isolates were high compared to dogs this may be attributed to environmental contact outside of the house than dogs. In addition, cats usually clean themselves by licking their body and the anus, especially after defecation, these results show that different areas of the animals especially the mouth, nose, and rectal can be contaminated with *enterococci* at any time. *E. faecalis* and *E. faecium* are the most detected in both humans and animals (Ramos et al., 2020). In the current study, the most strain isolates from dogs as total were *E.gallinarum* isolates from oral and nasal followed by *E. faecium* from oral and *E.fecalis* from fecal samples only. In contrast, in cats the predominant strain was *E.faecalis* which was isolated from oral and fecal followed by *E.gallinarum* isolated from nasal only which partially agrees with other studies, oral occurrence rates of *E.faecalis* in dogs were 3.2% and in cats were 5.3%, while *E.faecium* was 22.2% , and 15.8% in dogs and cats respectively reported by (Abdel-Moein et al., 2018). *E. faecalis* was reported as the predominant spp among the dogs 68%; from nasal 17% *E.faeclis* and 1% *E. gallinarum*, from rectal were 60% *E.faecalis* and 8% *E. faecium*. In cats, 45% *E.faecalis*,2% *E. gallinarum* in the rectal, and *E.fecalis* were not isolated from cat nasal (Jackson et al., 2009). *E. faecalis* were isolated from fecal samples and oral of most cats in this study, this may explain the presence of *E. faecalis* in oral which may be transmitted by contaminating the rectal area during cats clean themselves by licking their body and the anus. The differences in isolation rate and isolated spp were found to be influenced by the geographical region and the clinical samples used in the study (Shridhar & Dhanashree, 2019). In addition to, variations in the study participants and the methods employed for the detection of *enterococci*, number and type of samples, and health status.

Since the dog samples were all males, it is not possible to compare. puppies and adults dogs giving the same positive carriage of *enterococci*,and K9 reported a higher rate of carriage than others. Also in cats, males were higher than in females, and the highest positive in aduls cats . Persian and Shirazi give positive more than local cats, although local cats carry the high isolates followed by Shirazi. There are no studies to compare with these results; studies were partial in the line with the present study, it is found a higher isolates in puppies(less than 3.5 months of age) than dogs 6 months old of age, while kittens (less than 2 months age) were less than cats 6 months old of age (Kataoka et al., 2013). In military working dogs, in male *enterococci* was 87.50%, in females 87.88% with not significant, and more prevalence in 2-7 months of age followed by 1–6 years, and notice that the *E.faecalis* prevalent decreased with age, whilst *E.faecium* increased with age . This may be attributed to the rise in antibiotics utilization with age (Bang et al., 2017). K9 has been trained to assist the police in their work and used to check the bags of passengers and passengers, in the fight against crimes. Thus, it is more exposed to the environment and more in contact with people or contaminated materials, this is what can be attributed to isolating the highest

percentage from K9, in addition to the use of medications with age or poor care, and in the local cats were a higher number of isolates this may attribute to these local cats in study more contact with the environment.

Conclusion

The current study found that the most prominent *enterococci* spp in were *E.gallinarum* and *E.faecalis,* isolated enterococcus from infected dogs and cats may be act as source of this bacteria for their owners especially immunocompromised persons leading to severe infections.And the isolation of the same *enterococci* spp in humans and their animals may be attributed to transmitted from these animals to their owners or vers versa and possible consider as zoonotic transmission.

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Conflict of interest

Non

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