

Journal of the Hellenic Veterinary Medical Society

Vol 73, No 1 (2022)



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doi: [10.12681/jhvms.25239](https://doi.org/10.12681/jhvms.25239)

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To cite this article:

Razali, K., Kaidi, R., Giarratana, F., & Ait-Oudhia, K. (2022). Staphylococci and zoonotic potential : oral carriage and antibiotic susceptibility in stray dogs and cats in Algeria. *Journal of the Hellenic Veterinary Medical Society*, 73(1), 3621–3628. <https://doi.org/10.12681/jhvms.25239>

Staphylococci and zoonotic potential: oral carriage and antibiotic susceptibility in healthy dogs and cats in Algeria

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ABSTRACT: This study aimed at identifying species of staphylococci isolated from the oral cavity of dogs and cats, in Algeria, and to determine their antibiotic susceptibility.

Oral swabs were collected from 70 healthy animals (35dogs and 35cats) and were grown in Mannitol Salt Agar medium. Isolates were identified using API staph commercial kits and then confirmed with MALDI-TOF MS associated with SARAMIS software. Coagulase-positive staphylococci (CoPS) were tested for antibiotic susceptibility by disk diffusion method.

On the 70 sampled animals, 59 were carriers of staphylococci in the oral cavity and more than one species was detected in 11 of them. Seventy (70) staphylococcal isolates were obtained belonging to 10 different species. Coagulase-negative staphylococci (CoNS) were most prevalent (81.42%), of which the dominant species was *S.xylosum* (40%). Other coagulase-negative species, such as *S. simulans* (14.28%), *S. sciuri* (11.42%), *S.saprophyticus* (10%), *S.capitis* (2.85%), *S. cohnii* (1.42%), and *S. epidermidis* (1.42%) were also isolated. The remaining n.13 CoPS included : *S.intermedius* (2.85%), *S.pseudintermedius* (8.57%) and *S. intermedius* group (SIG) (4.28%). No strain of *S. aureus* was found.

Results of antimicrobial resistance showed that 61.53% of CoPS isolates were resistant to at least two drugs. The highest rate of resistance was observed against penicillin, ampicillin and tetracycline (53.84% for each drug), while amoxicillin-clavulanate was active on most isolates.

In Algeria, stray dogs and cats are carriers of many staphylococci species in the oral cavity, including multidrug resistant CoPS, which could be transmitted to humans through bites.

Keywords: Algeria, Antibiotic susceptibility; bites, Dog and cat; Oral cavity; Staphylococci.

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Date of initial submission: 05-11-2020
Date of acceptance: 15-10-2021

INTRODUCTION

Worldwide dogs and cats are responsible for the most animal bites (Damborg et al. 2016; Morzycki et al. 2019; Owczarczak-Garstecka et al. 2019) and for around 1% of all emergency department visits per year (Damborg et al. 2016; Goldstein et al. 2018; Morzycki et al. 2019). The most common complication of animal bites is secondary bacterial infection, requiring medical attention (Muniz et al. 2013; Oh et al. 2015; Tabaka et al. 2015; Rossi et al. 2017), which imply high costs to public health (Muniz et al. 2013; Holzer et al. 2019).

It is estimated that about 3-18% of dog bites and 20-80% of cat bites can lead to infections (Tabaka et al. 2015; Damborg et al. 2016), through the skin flora of the victim itself, but above all through the animal's oral flora that bites (Rothe et al. 2015; Damborg et al. 2016; Bula-Rudas and Olcott 2018); wounds are usually polymicrobial (Rothe et al. 2015; Goldstein et al. 2018; Bula-Rudas and Olcott 2018) and can contain up to five different bacterial species (Rothe et al. 2015; Bula-Rudas and Olcott 2018).

The natural oral flora of dogs and cats comprises abundant and complex bacterial species, including opportunistic pathogens (Muniz et al. 2013; Oh et al. 2015), such as *Staphylococcus* spp. (Muniz et al. 2013; Misic et al. 2015; Rossi et al. 2017) which may be transmitted to humans through bites (Muniz et al. 2013; Rothe et al. 2015; Rossi et al. 2017).

Members of the genus *Staphylococcus* are widely spread in the environment (Paharik and Horswill 2016). They are common inhabitants of the skin and nasal mucosa in humans (Misic et al. 2015; Paharik and Horswill 2016), while the principal carriage sites are the nares, mouth and perineum in animals, including dogs and cats (Misic et al. 2015; Iverson et al. 2015).

Among staphylococci species, *Staphylococcus aureus* and other coagulase-positive staphylococci (CoPS) may be opportunistic pathogens (Paul et al. 2014; Lozano et al. 2017; Agabou et al. 2017). Several reports suggest that CoPS are the most commonly detected aerobic bacteria in infected bite wounds (Abrahamian and Goldstein 2011; Muniz et al. 2013; Katica et al. 2019), accounting for 10-50% of bacterial isolates (Abrahamian and Goldstein, 2011; Katica et al. 2019).

The choice of effective treatment for this microbial

infection focuses on the knowledge of staphylococcal species present in the mouth of dogs and cats (Muniz et al. 2013; Rossi et al. 2017), as well as their antibiotic susceptibility (Rossi et al. 2017).

In Algeria, the number of stray dogs and cats roaming freely through the streets and urban areas, has been steadily increasing in recent years (Kardjadj and Ben-Mahdi 2019) exposing people to the risk of bites. Each year about 120'000 people are exposed to animal bites, 80% of which are bitten by stray dogs (Yahiaoui et al. 2018). Despite this, no study has yet investigated the oral carriage of staphylococci in dogs and cats.

For all these reasons, the aim of this study was to identify staphylococci species found in the oral cavity of stray dogs and cats collected in Algeria, and to determine their susceptibility to antibiotics.

MATERIAL AND METHODS

Study area

The study was conducted in the department of Algiers situated on the central coast of Algeria between 3°2'31.09" east longitude and 36°45'9" north latitude. There are 57 districts in this department, covering a total area of 1190km² and a population of over 2.9 million inhabitants. Algiers is bordered from the north by the Mediterranean Sea, from the south by the department of Blida, from the east by the department of Boumerdes, and from the west by the department of Tipaza.

Ethics statement

The protocol was approved by the ethics committee and decision board of Public Industrial and Commercial Company (P.I.C.C.) and Urban Hygiene and Environmental Protection (U.H.E.P.) of Algiers (application no.01/2018). P.I.C.C-U.H.E.P. is an affiliate of the Algerian Ministry of Water Resources and Environment. It controls zoonoses and vector-borne diseases such as rabies and leishmaniasis.

In 1996, an Algerian Rabies Prevention Program was launched (Yahiaoui et al. 2018). It is in this context that the P.I.C.C-U.H.E.P. catches stray dogs and cats in the 57 districts of the department of Algiers (Yahiaoui et al. 2018; Kardjadj and Ben-Mahdi 2019). During the legal period (7 days) before euthanasia, captured animals are then sheltered in the dog-pound of El-Har-rach (Zaidi et al. 2018). The geographical position in Algiers of the dog-pound and the animal capturing

radius is shown in Fig.1.

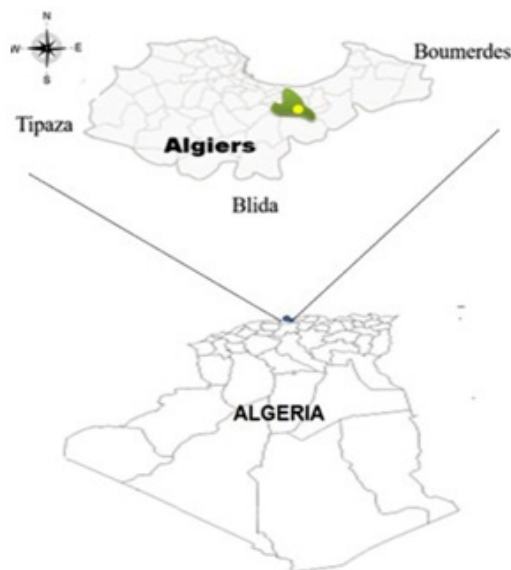


Fig.1 Map of Algeria showing the location of the study area in Algiers

Samples collection

In the period from January 2018 to July 2019, oral swabs were collected from 70 stray animals (35 dogs and 35 cats) which were randomly selected without distinction of age, sex, or breed. All sampled animals were caught by the P.I.C.C-U.H.E.P. from the 57 municipalities of Algiers during the study period, they were apparently healthy and didn't receive any previous antibiotic.

Before swabbing, retaining clip was placed at the animal's neck to immobilize. Subsequently, a dry and sterile cotton swab (without transport medium) was inserted in the oral cavity and it was rubbed over the sublingual area for few seconds to obtain oral fluid.

All swabs were put in the icebox (+4°) and brought back to the microbiology laboratory, within 60 minutes after collection.

Isolation and biochemical identification of staphylococci

Once in the laboratory, each oral swab was immediately put in Brain-Heart Infusion Broth (BHIB) (Oxoïd, Basingstoke, UK) and incubated at 37°C for 24 h before isolation on Mannitol Salt Agar (MSA) (Oxoïd, Basingstoke, UK). From BHIB 10 µl with a loopful were streaked on MSA and then incubated aerobically for 24 h at 37°C.

After purification, presumptive staphylococci col-

onies were identified using Gram stain (Gram-positive cocci), catalase test (positive) and API-staph commercial kits (Biomerieux, Marcy Etoile, France).

Each recovered staphylococcal isolate was tested for coagulase activity using rabbit plasma (BioRad) and finally placed in eppendorf tubes containing BHIB and 15% of glycerol and stored at -80°C until use.

Proteomic identification (MALDI-TOF MS)

The API identification was confirmed by Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) as described in other studies (Lee et al. 2015; Kaspar et al. 2018).

Frozen isolates were subcultured on Nutrient Agar (Biolife, Milan, Italy) supplemented with 5% sheep blood (Biolife, Milan, Italy) and incubated aerobically for 24 h at 37°C.

From the obtained culture, a loopful (1 µl loop) from a single colony of each isolate was smeared onto the FlexiMass MALDI target plates containing 48-well sample spots (bioMerieux, Firenze, Italy).

The well containing the colony was covered with 1 µl of CHCA solution (alpha-cyano-4-hydroxycinnamic acid dissolved in 500 mL/L acetonitrile, and 25 mL/L tri-fluoroacetic acid) (bioMerieux, Firenze, Italy) and air-dried for about 5 minutes at room temperature for the co-crystallization (CHCA solution and microbial material). Each strain was analysed 3 times in distinct and separate runs.

As required by the instrument the *Escherichiacoli* ATCC 8739 (bioMerieux, Firenze, Italy) was used as calibrator and internal ID control and inoculated on distinct and separate calibration spots (G3 and G4 position). This strain was grown on Nutrient Agar (Biolife, Milan, Italy) supplemented with 5% sheep blood (Biolife, Milan, Italy) and incubated aerobically for 24 h at 37°C.

The mass spectra, ranged from 2.000 to 20.000 Da, were evaluated using a VITEK MS Axima Assurance mass spectrometer (Biomerieux, Firenze, Italy) in positive linear mode at a laser frequency of 50 Hz with an acceleration voltage of 20 kV with an extraction delay time of 200 ns.

The obtained mass spectra were automatically transferred into the SARAMIS software (Spectral Ar-

chive and Microbial Identification System - Database version V4.12- Software year 2013, bioMerieux, Firenze, Italy) where they were compared to the database hosting the reference spectra and Super Spectra of bacteria, giving a results reported as confidence level, represents specific peaks between the generated spectrum and the database spectra of their similarity in terms of presence or absence. For confidence levels ranging from >60% to 99.8%, the identification was considered a “good ID” because the spectrum was adequately close to that of a reference spectrum, while for a value of <60%, “no identification” (no ID) was given.

Antimicrobial susceptibility testing

CoPS were tested for susceptibility to five antibiotics frequently recommended for bite- wounds treatment. Resistance to Amoxicillin/clavulanate 30µg (Oxoïd, Basingstoke, UK), Penicillin10IU. (Oxoïd, Basingstoke, UK), Ampicillin 10µg (Oxoïd, Basingstoke, UK), Tetracycline 30µg (Oxoïd, Basingstoke, UK) and Erythromycin 5µg (Oxoïd, Basingstoke, UK) was determined on Muller-Hinton agar (Oxoïd, Basingstoke, UK) using the standard Kirby-Bauer disk diffusion method.

Statistical analysis and Map conception

Pearson’s Chi-square test and Fisher exact test ($\alpha=5\%$) were applied to compare staphylococci prevalence, using Microsoft excel 2007. Differences were statistically significant when P value was less than 0.05 ($p\leq 0.05$).

Map of the study area was designed using Microsoft PowerPoint 2007, available in Microsoft Office software.

RESULTS

Of the 70 sampled animals, 59 (84.28%) carried

Staphylococcus spp. in oral cavity, and among these, 48 (68.57%) carried only one *Staphylococcus* species, while the remaining n. 11 (15.71%) carried two different species (Table 1).

In the 59 positive animals (28 dogs and 31 cats) a total of 70 strains of *Staphylococcus* were isolated (35 in dog and 35 in cat), with a prevalence of CoNS (n. 57 - 81.42%) compared to CoPS, represented by only 13 isolates (18.57%) (Table2).

Among CoNS, *S.xylosus* was the most frequently isolated (45.71% in dog and 34.28% in cat), followed by *S.simulans* in dog (14.28%) and *S.saprophyticus* and *S.sciuri* in cat (17.4% for each specie).

CoPS were represented by *S.pseudintermedius* (8.57%), *S. intermedius* group (SIG) (4.28%) and *S.intermedius* (2.85%).No *S.aureus* strain was isolated.

The isolation rate of *S.intermedius* was similar in dog and cat (2.85%), while *S.pseudintermedius* and SIG were isolated with higher frequency in dog (14.28%) compared to cat (5.71%).

Regarding the results of the antibiotic sensitivity, the majority (61.53%) of isolated CoPS were resistant to at least two drugs, while only five CoPS isolates (38.46%) were susceptible for all tested drugs. Multi-drug resistance profiles (to three or more drugs) was observed in 46.15% (n=6/13) of the isolates (Table3).

Almost all strains were susceptible to amoxicillin-clavulanate and erythromycin (92.3% and 84.61% respectively), while a high resistance to penicillin, ampicillin, and tetracycline was found (53.84% for each) (Table 4).

Table 1. Prevalence of staphylococci in the oral cavity of dogs and cats

Characteristics	Total population		Dog		Cat	
	No	%	No	%	No	%
Sampled animals	70	100	35	100	35	100
Animals negative for staphylococci	11	15.71	7	20	4	11.42
Sataphylococci carriers	59	84.28	28	80	31	88.57
1 specie	48	68.57	21	60	27	87.09
2 species	11	15.71	7	25	4	12.9

Table 2. Distribution of staphylococcal species isolated from oral cavities of dogs and cats

Staphylococci	Total population		Dog		Cat	
	No	%	No	%	No	%
No of isolates	70	100	35	100	35	100
CoNS	57	81.42	27	77.14	30	85.71
<i>S. capitis</i>	2	2.85	2	5.71	-	-
<i>S. cohnii</i> subsp <i>cohnii</i>	1	1.42	1	2.85	-	-
<i>S. saprophyticus</i>	7	10	1	2.85	6	17.14
<i>S. epidermidis</i>	1	1.42	-	-	1	2.85
<i>S. simulans</i>	10	14.28	5	14.28	5	14.28
<i>S. sciuri</i>	8	11.42	2	5.71	6	17.14
<i>S. xylosus</i>	28	40	16	45.71	12	34.28
CoPS	13	18.57	8	22.85	5	14.28
<i>S. intermedius</i>	2	2.85	1	2.85	1	2.85
<i>S. pseudointermedius</i>	6	8.57	5	14.28	1	2.85
<i>S. pseudointermedius/delphini/intermedius</i> (SIG)	3	4.28	2	5.71	1	2.85
Not identified	2	2.85	-	-	2	5.71

Table 3. Antibiotic resistance patterns of CoPS isolated oral cavities in dogs and cats

Antibiotic resistance patterns	Total (n=13)	Dog (n=8)	Cat (n=5)
S ^{ble} (%)	5 (38.46)	4 (50)	1 (20)
Pen-Amp	1 (7.69)	-	1 (20)
Ery-Tet	1 (7.69)	-	1 (20)
Pen-Amp-Tet	4 (30.76)	2 (25)	2 (40)
Pen-Amp-Tet-AMC	1 (7.69)	1 (12.5)	-
Pen-Amp-Tet-Ery	1 (7.69)	1 (12.5)	-

Pen penicillin, *Amp* ampicillin, *Ery*, erythromycin, *Tet* tetracycline, *AMC* amoxicillin-clavulanate

Table 4. Antibiotic resistance of CoPS isolates among dog and cats

Staphylococci isolates	Total (n=13)		Isolates from dogs (n=8)		Isolates from cats (n=5)	
	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)
Antimicrobial agents						
Penicillin	7 (53.84)	6 (46.15)	4 (50)	4 (50)	3 (60)	2 (40)
Ampicillin	7 (53.84)	6 (46.15)	4 (50)	4 (50)	3 (60)	2 (40)
Amoxicillin-Clavulanate	1 (7.69)	12 (92.3)	1 (12.5)	7 (87.5)	-	5 (100)
Erythromycin	2 (15.38)	11 (84.61)	1 (12.5)	7 (87.5)	1 (20)	4 (80)
Tetracycline	7 (53.84)	6 (46.15)	4 (50)	4 (50)	3 (60)	2 (40)

DISCUSSION

Staphylococci are common inhabitants of the skin, nasal and oral mucous membranes in humans and many mammals, including dogs and cats, (Iverson et al. 2015; Paharik and Horswill 2016)

Oral carriage of *Staphylococcus* spp. among healthy dogs and cats was confirmed in this study, due to the high incidence of strains isolated from the mouth of the studied animals (84, 28%). This is consistent with other studies that isolated *Staphylococcus* spp. from the oral cavity of more than 60% of dogs and cats (Paul et al. 2014; Muniz et al. 2013; Bean and

Wigmore 2016; Rossi et al. 2017)

Unlike the CoPS commonly associated with clinical infections (Natsis and Cohen 2018), CoNS are very common in healthy animals, including dogs and cats. (Muniz et al. 2013; Rossi et al. 2017; Kaspar et al. 2018). It was therefore not surprising, that the rate of CoNS (51.42%) detected in this study was 4 times higher than that of CoPS (18.57%). This result was compatible with that reported by Muniz et al. (2013) showing a high rate of CoNS (89.6%) in healthy cats, compared to CoPS (10.4%).

Likewise, *S.xyloso* is known to be widely spread in the environment and animals (Malik et al.2005), including healthy dogs and cats (Muniz et al.2013; Rossi et al.2017 ; Kaspar et al.2018). As shown in this study, *S.xyloso* was the most commonly found in dogs and cats among CoNS (45.71% and 34.28% respectively). This result is in agreement with Hariharan et al. (2011), who reported a frequency of 33.33% in feral cats, but much higher than that reported by Rossi et al. (2017) in pet cats (5.3%).

Muniz et al. (2013) recorded a higher frequency (50.9 %) of *S.xyloso* in pet cats in Brazil, while Kaspar et al. (2018) found a lower prevalence (23.1 %) in pet dogs.

In addition to *S.xyloso*, dog and cat were found to be carriers of several other CoNS species: *S.simulans* (14.28%), *S.siuri* (11.42%), *S.saprophyticus* (10%), *S.cohniisubspcohnii* (1.42%) and *S. epidermidis* (1.42%). Statistical analysis showed no significant difference between dog and cat in the distribution of CoNS species. Those staphylococci species were already detected in several previous studies (Hariharan et al. 2011; Muniz et al.2013; Mistic et al.2015; Rossi et al.2017), whose frequencies and distributions vary from one report to another, depending on several factors such as: age, diet, environment, geographic location and study methodology (Paul et al.2012).

It is well known that CoNS are not as pathogenic as CoPS (Natsis and Cohen, 2018), but recent studies (Nemeghairet al.2014; Tous Romero et al.2016; Shields et al.2016; Natsis and Cohen2018) showed that certain species of CoNS such as *S.sciuri* and *S. simulans*, with multiple virulence factors and genes of resistance, can cause serious infections in humans, such as endocarditis, septicemia, urinary tract, soft tissue and osteoarticular infections. Thus, a high proportion of CoNS in dog and cat saliva may be a potential risk to public health.

Among staphylococci species, *S.aureus* is known to be the most significant pathogen (Gharsa et al.2015b; Velázquez-Guadarrama et al.2017), causing a broad variety of infections in humans and animals (Gharsa et al.2015b; Agabou et al.2017).Neither the dog nor the cat harboured *S.aureus* in the mouth in this research. In contrast to our finding, Muniz et al. (2013) and Rossi et al. (2017), recorded very low *S.aureus* prevalence in brazilian pet cats (4.7% and 2.66%, respectively). In a study by Kaspar et al. (2018), *S.aureus* was found in the oral cavity of 4.68% and 5.33%

of pet dogs and cats, respectively. According to Mistic et al. (2015), *S.aureus* is optional in pets, especially dogs that are colonized by other coagulase-positive staphylococci such as *S.pseudintermedius*. In addition, *S.aureus* is a common inhabitant of the skin and nares in humans, that can be transmitted to pets through close contact (caressing, kissing, licking the skin) with the owners. Thus, *S.aureus* is significantly more abundant in pets compared to stray animals (Bierowiec et al.2016). All of the animals in our sample are stray, have no regular contact with humans, and that may explain our finding without *S.aureus*.

Other than *S.aureus*, some CoPS are also recognized as opportunistic pathogens, such *S.intermedius* group members, which include: *S.intermedius*, *S.pseudintermedius* and *S.delphini* (Gharsa et al.2015a; Kaspar et al.2018). These bacterial species are zoonotic agents, especially *S.pseudintermedius*, whose transmission between dogs and owners has been already documented (Gharsa et al.2015a; Magleby et al.2019). In this study, *S. Pseudintermedius* was detected in the oral cavity of 5/35 (14.28%) dogs, accounting for 62.5% of all CoPS isolates in this animal. This result is in agreement with the literature (Paul et al.2012; Paul et al. 2014; Magleby et al. 2019) stating that *S.pseudintermedius* is the most common species of all coagulase-positive staphylococci in dogs colonizing puppies immediately after birth (Paul et al.2014). The most relevant sites of colonization are the oral cavity and perineum, with a carriage rate between 23% and 92% (Paul et al.2012; Paul et al.2014; Kaspar et al.2018). Several authors (Muniz et al.2013; Rossi et al. 2017; Kaspar et al.2018) did not find any *S. pseudintermedius* in pet cats. In our research, only one cat was found to bear this bacterium in the oral cavity. According to Gandolfi-Decristophoris et al. (2013), *S.pseudintermedius* is more common in healthy dogs than in healthy cats. Thus, dogs are considered to be the primary source of *S.intermedius* for humans.

In addition, *S.pseudintermedius* is a zoonotic agent, that is frequently transmitted to humans by canine bites or by daily dog contact (Paulet al. 2014; Gharsa et al. 2015a; Lozano et al. 2017; Magleby et al. 2019), causing local or systemic infections such as bacteremia, brain abscess, endocarditis or pneumonia (Lozano et al. 2017).

Antibiogram results showed that the majority (61.53%) of isolated CoPS were resistant to at least two drugs. Resistance to penicillin, ampicillin and tet-

racycline was the most common (53.84% for each). This finding is in agreement with several studies (Muniz et al.2013; Bean et al.2016; Rossi et al.2017; Kaspar et al.2018) reported that dog and cat staphylococci isolates have become resistant to several antibiotics in recent years, including, ampicillin, penicillin and tetracycline. Although these molecules are the most recommended drugs for the treatment of infected bite wounds (Muniz et al.2013; Bula-Rudas and Olcott2018), the results of this study show that in the case of infections involving staphylococci, the risk of resistant strains is very high, leading to ineffective antibiotic therapy. In contrast, almost all CoPS were sensitive to amoxicillin-clavulanate (92.30%). This finding is in accordance with that reported by Muniz et al. (2013) in Brazil (81.81%). β -lactamases are the most common resistance mechanism to β -lactam antibiotics, such as penicillins (Klachbrenner 2017), therefore the use of amoxicillin combined with clavulanic acid which is a beta-lactamase inhibitor is recommended for multidrug-resistant staphylococci (Klachbrenner 2017; Bula-Rudas and Olcott 2018).

Staphylococci carry several plasmid-encoded antibiotic resistance genes (Muniz et al.2013; Rossi et al.2017), which may be transferred horizontally (conjugation) to other bacteria, including zoonotic pathogens (Muniz et al.2013; Velázquez-Guadarrama et al.

2017; Rossi et al. 2017; Kaspar et al. 2018;).

Dogs and cats carrying antibiotic-resistant staphylococci pose therefore a possible danger to humans being in close contact with these animals and their saliva (Gandolfi-Decristophoris et al.2013; Muniz et al.2013; Bierowiec et al.2016; Kaspar et al.2018).

CONCLUSION

This study indicates that there are many species of staphylococci in the oral cavity of stray dogs and cats in Algeria, including multidrug resistant CoPS, which could be a potential risk to public health.

S.aureus which is considered to be the most pathogenic among staphylococci species, was not revealed in this report. However, *S.pseudintermedius* was common in dogs.

Amoxicillin-clavulanate is recommended for the treatment of infected bites due to staphylococci due to the high sensitivity of staphylococci species to this drug. In people with penicillin allergies, high tetracycline resistance discourages the treatment of infected bites.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare that are relevant to the content of this article.

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