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Prevalence and antimicrobial resistance of *Staphylococcus* species isolated from cats and dogs

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Abstract

Background: Methicillin-resistant staphylococci (MRS) are an emerging global problem with serious public health concern.

Aims: This study investigated the prevalence and antimicrobial susceptibility of commensal *Staphylococcus* species isolated from healthy and clinical cats and dogs.

Methods: Nasal swab samples were collected from animals and processed using selective and semi-selective mediums. Presumptive isolates were subjected to biochemical testing and analyzed using the Phoenix automated identification and susceptibility testing system. PCRs protocols were used to screen for *mecA* and *pvl* genes.

Results: In total, 151 pets (103 cats and 48 dogs) were enrolled, of which 14 dogs (29%) and 24 cats (23%) were colonized with various *Staphylococcus* species mainly originated from healthy animals. A total of 38 staphylococci isolates were collected and distributed between 24 coagulase-negative and 14 coagulase-positive staphylococci. Only 13 staphylococci strains were identified as MRS, out of which only five isolates expressed that the *mecA* gene exclusively originated from healthy pets.

Conclusion: This is the first study reporting the prevalence and colonization status of staphylococci species and MRS strains isolated from cats and dogs in Libya. The study reports important information of medical and clinical importance on antimicrobial and multidrug resistance of different staphylococci strains, particularly the coagulase negative species.

Keywords: Coagulase-negative staphylococci, Libya, *mecA* gene, pet animals, *Staphylococcus*.

Introduction

Staphylococcus species are classic commensals bacteria of the skin and mucous membranes of mammals and birds (Grice and Segre, 2011). *Staphylococcus aureus* is the main pathogenic species; however, other staphylococci emerged, particularly the coagulase-negative staphylococci (CoNS), causing serious opportunistic infections in human and animals (Rich, 2005). In Africa, the incidence of CoNS varied across the continent and reported approximately from 68% and 62% of suspected human and animal infections, respectively, particularly from clinical cattle and hospitalized humans (Asante *et al.*, 2020). In Libya, methicillin-resistant *S. aureus* (MRSA) is the most reported and investigated nosocomial pathogens exclusively isolated from healthcare settings and human clinical sources (Ahmed *et al.*, 2017).

Cats and dogs can be colonized with various staphylococci species causing opportunistic infections and outbreaks (e.g., *S. aureus*, *Staphylococcus lentus*, *Staphylococcus schleiferi*, and *Staphylococcus pseudintermedius*) (van Duijkeren *et al.*, 2011; Gandolfi-Decristophoris *et al.*, 2013; Harrison *et*

al., 2014; Ma *et al.*, 2020). Methicillin-resistant staphylococci (MRS) were isolated from household pets associated significantly with antibiotic intake and veterinary hospitalization, expressing genetic determinants and clonal lineages similar to those typically identified in humans and other animals (i.e., staphylococcal cassette chromosome and Pantone–Valentine Leucocidin (*pvl*) gene) (van Duijkeren *et al.*, 2005; Han *et al.*, 2016; Rossi *et al.*, 2017; Loncaric *et al.*, 2019). Nevertheless, epidemiological and clinical information from Africa on healthcare-associated pathogens isolated from pet animals are very rare compared to the extensive knowledge on human and food-producing animals (Ahmed and Baptiste, 2018; Asante *et al.*, 2020). The aim of the current study was to investigate the prevalence and antimicrobial susceptibility of *Staphylococcus* species isolated from healthy and clinical cats and dogs in Tripoli, Libya.

Material and Methods

Criteria of sampling

The present study included household cats and dogs that were clinically healthy and showed no signs

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of systemic diseases, and were not being under any medication, including antimicrobials use for at least 3 months prior to enrolment in this study, and was referred to as the “healthy group”. The study also included clinical cats and dogs with gastrointestinal infections treated with antimicrobial therapy (i.e., penicillins, amoxicillin, enrofloxacin, gentamicin, and trimethoprim-sulfamethoxazole), and was referred to as the “clinical group”. Duplicate nasal swap samples were obtained from both nostrils of each animal using sterile, moist cotton swabs and transferred to the laboratory for isolation and characterization within 2–4 hours. Owners of animals were informed about the purpose of the study and were asked for their consent.

Isolation and molecular analysis of *Staphylococcus* spp.

Nasal samples of each animal were, respectively, cultured onto mannitol salt and Columbia blood agars and incubated at 35°C for 24 hours. A typical growing colony was selected from each plate and subjected to Gram-stain, catalase test, and coagulase reactivity. For the purpose of this study, only one presumptive isolate was selected to represent each sample and subjected for further testing. Presumptive isolates were further tested by the automated identification and susceptibility testing system BD Phoenix (PAMS, MSBD Biosciences, Sparks Md, USA) to identify and confirm the species of each isolate, and for the determination of susceptibility to antimicrobial agents. Confirmed staphylococci species were further screened for important genetic determinants frequently reported from healthcare-associated bacterial organisms using PCR-adopted protocols for methicillin encoding *mecA* *lukS/lukF* genes encoding Pantone–Valentine leucocidin (PVL) (Frebourg *et al.*, 2000; Jarraud *et al.*, 2002).

Statistical analysis

The analysis of positive samples was carried out using Epi Info™ of the Center for Disease Control and Prevention using the chi-square test at $p \leq 0.05$.

Ethical approval

This study obtained approval from the Department of Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Tripoli, Libya.

Results

A total of 151 pets were involved in the study, including 103 cats (62 healthy and 41 clinical) and 48 dogs (37 healthy and 11 clinical). Of these, 38/151 (25%) carried various *Staphylococcus* species distributed between 23% (24/103) of cat samples (23 from healthy and 1 from clinical) and 29% (14/48) of dog samples (10 from healthy and 4 from clinical). Healthy cats were significantly higher carrier of staphylococci than healthy dogs ($p < 0.05$); 37% of healthy cats ($n = 23/62$) compared with 26% of healthy dogs ($n = 10/37$). Furthermore, there were no significant differences of CoNS, CoPS, and *mecA*-MRSA carriage between cats and dogs ($p > 0.05$).

A total of 38 confirmed staphylococci isolates were collected, characterized, and distributed between 24 (63%) coagulase-negative (CoN) and 14 (37%) coagulase-positive (CoP) staphylococci. *Staphylococcus felis* ($n = 12$; 31%) was the most identified CoNS, followed by *S. sciuri* group (including *S. lentus*) ($n = 9$; 21%), *S. capitis* ($n = 2$; 5%), and *S. cohnii* ($n = 1$; 3%). *S. aureus* ($n = 8$; 21%) was the most identified CoPS, followed by *S. intermedius* ($n = 5$; 13%) and *S. schleiferi* ($n = 1$; 3%) (Table 1). *S. felis*, *S. intermedius*, *S. capitis*, *S. schleiferi*, and *S. cohnii* isolates were exclusively of cat origins, whereas *S. sciuri* isolates were exclusively from dogs, but *S. aureus* isolates were isolated from both animals. The collection expressed high resistances to penicillins, followed by amoxicillin-clavulanate, cephalosporins (cefotaxime and cefotaxime), and very low resistance to gentamicin, trimethoprim-sulfamethoxazole, and ciprofloxacin. Three isolates expressed resistance to clindamycin, of which a single *S. lentus* isolate was also resistant to erythromycin and characterized as macrolide-lincosamide-streptogramin B resistance (MLS_B) resistance phenotype, originated from a clinical dog. Resistance to mupirocin was not detected (Table 2).

The Phoenix automated system characterized 34% (13/38) of isolates expressing methicillin resistance phenotype distributed over 11 CoNS [*S. sciuri* ($n = 8$), *S. capitis* ($n = 1$), *S. cohnii* ($n = 1$), *S. lentus* ($n = 1$)] and two *S. aureus* isolates. PCRs confirmed 13% (5/38) of isolates positive for the *mecA* gene (*S. aureus*, *S. intermedius*, *S. cohnii*, *S. capitis*, and *S. sciuri*) originated from healthy pets mostly of cat origins. All these *mecA*-MRS strains were also characterized by the Phoenix system as MRS, except a *S. intermedius* isolate. The *pvl* gene was not identified.

Discussion

Cats and dogs are frequently colonized by various staphylococci showing variable dynamics (Davis *et al.*, 2014; Ruzauskas *et al.*, 2015; Kaspar *et al.*, 2018). A recent study reported a high colonization rate of diverse staphylococci species among cats and dogs, particularly with CoNS (i.e., 20 different species), and significantly higher in dogs than cats (Ma *et al.*, 2020). On the contrary, the current study revealed that cats were highly colonized with six diverse staphylococci species, particularly of the CoNS group (i.e., three different species) representing also 63% of the collected isolates mostly from healthy pets. The very low colonization rate of staphylococci and absence of MRS strains from clinical animals in the current study might be related to the sample size and/or attributed to the ability of staphylococci to persist throughout atypical environmental conditions, including antimicrobial therapies.

MRS are a major global and health concerns increasingly reported from humans and animals (Weese *et al.*, 2007; Loeffler *et al.*, 2011; WHO, 2017). MRS

Table 1. Proportion and features of staphylococci species isolated from cats and dogs.

No. of staphylococci	No. of staphylococci by automated system	No. of MRS by automated system	No. of confirmed <i>mecA</i> -MRS
Cats (NO = 24) ^a	CoN=15;	CoN=2	CoN=2
	12; <i>S. felis</i>	1; <i>S. capitis</i>	1; <i>S. capitis</i>
	2; <i>S. capitis</i>	1; <i>S. cohnii</i>	1; <i>S. cohnii</i>
	1; <i>S. cohni</i>		
	CoP=9;	CoP=2	CoP=2
	5; <i>S. intermedius</i>	2; <i>S. aureus</i>	1; <i>S. aureus</i>
	3; <i>S. aureus</i>	1; <i>S. intermedius</i>	
	1; <i>S. schleiferi</i>		
Dogs (NO = 14) ^b	CoN=9;	CoN=9	CoN=1
	8; <i>S. sciuri</i>	8; <i>S. sciuri</i>	1; <i>S. sciuri</i>
	1; <i>S. lentus</i>	1; <i>S. lentus</i>	
	CoP=5;	CoP=0	CoP=0
	5; <i>S. aureus</i>		

(^a) = 1 clinical and 23 healthy; (^b) = 4 clinical and 10 healthy; (No.) = Number, (MRS) = methicillin-resistant staphylococci; (CoN) = Coagulase-negative; (CoP) = Coagulase-positive.

Table 2. Antimicrobial susceptibility profiling and phenotypes of staphylococci as identified by the Phoenix Automated Identification System ($n = 38$).

Antibiotic agent	No. and Proportion (%)
Gentamicin	2 ^{MRS} (5.2%)
Imipenem	13 ^{MRS} (34.2%)
Cefoxitin	13 ^{MRS} (34.2%)
Cefotaxime	12 ^{MRS} (6%)
Ampicillin	33 (86.8%)
PenicillinG	33 (86.8%)
Oxacillin	13 ^{MRS} (34.2%)
Amoxicillin-Clavulanate	13 ^{MRS} (34.2%)
Daptomycin	0 (0%)
Trimethoprim- Sulfamethoxazole	1 ^{MRS} (2.6%)
Teicoplanin	0 (0%)
Vancomycin	2 (5.2%)
Clindamycin	3 (7.8%)
Erythromycin	1 ^{MRS-MLS_B} (2.6%)
Fusidic acid	0 (0%)
Linezolid	0 (0%)
Mupirocin	0 (0%)
Nitrofurantoin	0 (0%)
Ciprofloxacin	1 ^{MRS} (2.6%)
Moxaifloxacin	0 (0%)
Rifampin	0 (0%)
Tetracycline	0 (90%)

(MRS) = methicillin-resistant staphylococci; (MLS_B) = macrolide-lincosamide-streptogramin B resistance.

of pet's origin was first reported from healthy cats and emerged since after of clinical and nonclinical sources (Walther *et al.*, 2012; Cain, 2013; Somayaji *et al.*, 2016). The methicillin-resistant CoNS are emerging worldwide, including from Africa, and are isolated from various sources, including healthy and clinical pets expressing multidrug resistance patterns and frequent antimicrobial resistance genes, i.e., *mecA* and *pvl* genes (Chah *et al.*, 2014; Ibadin *et al.*, 2017; Qekwana *et al.*, 2017; Teixeira, 2019). In the current study, 13% of pets were *mecA*-MRS carriers exclusively originated from healthy animals; four *mecA*-MRS isolated from cats (two of CoN and CoP staphylococci each) and one *mecA*-MR *S. sciuri* isolated from a dog. The CoN group of staphylococci (particularly the *S. sciuri* group) was proposed as an evolutionary precursor of species playing a potential role as a reservoir of important genetic and virulence elements that are frequently reported in *S. aureus* (i.e., *mecA* gene) (Couto *et al.*, 2003; Zhou *et al.*, 2008; Nemegehaire *et al.*, 2014). Also, the identification of MLS_B resistance phenotype among CoNS demonstrates the potential spread of critical and cross-multidrug resistance patterns which may become potential gene reservoir for other bacterial organisms. Such strains can be transferred and shared between householders, veterinary personnel, and other companion animals, posing a serious public health and medical concerns (Sing *et al.*, 2008; Couto *et al.*, 2016; Drougka *et al.*, 2016).

Staphylococcus species of veterinary relevance are difficult to identify and characterize due to the underdeveloped diagnostic protocols. The Phoenix automated system has been widely reported as an effective tool for the identifications and antimicrobial susceptibility of staphylococci; however, a few

species are not distinguishable using this advanced system (Bannoehr *et al.*, 2007). For instance, *S. pseudintermedius* is easily misdiagnosed with *S. aureus*, due to very close phenotypic characteristics requiring molecular methods for definite identification, such matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) or PCR methods (Guardabassi *et al.*, 2017). In the present study and due to limited available resources only the most significant genes associated with clinical complications were screened (i.e., *mecA* and *pvl* genes) and further molecular and epidemiological analysis are required.

In conclusion, cats and dogs may harbor various staphylococci species and MRS strains of medical and public health concerns. The reported antimicrobial resistance among the CoNS warrants further attention and continuous monitoring by veterinarians and public health professionals. Preventative measures and antimicrobial stewardship are required to control and minimize the spread of MRS particularly in veterinary and animal medicine in Libya.

Conflict of interest

The authors declare that there is no conflict of interest.

Authors' contribution

Mohamed O. Ahmed designed the study, analyzed data, and wrote the article. Hiam Elnageh and Murad Hiblu collected the samples and data, and performed most of laboratory protocols. Mohamed Abbassi and Yousef Abouzeed contributed to laboratory works and analysis of information. All authors approved the final manuscript.

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