



Article Staphylococcus aureus and Methicillin-Resistant Coagulase-Negative Staphylococci in Nostrils and Buccal Mucosa of Healthy Camels Used for Recreational Purposes

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Simple Summary: Animal-associated staphylococci have been isolated in human infections. Therefore, these strains may pose a zoonotic risk in addition to constituting a reservoir for antimicrobial resistance genes. In this study, we isolated *Staphylococcus aureus* and other species of staphylococci from camels used for recreational activities in the Canary Islands. Most *S. aureus* lacked the antimicrobial resistance genes, but some staphylococci species carried the *mecA* gene which confers resistance to methicillin. The carriage of this gene conferring resistance to methicillin in staphylococci isolated from camels may be a public health concern since there is a risk of bacterial transmission to humans during recreational activities. Furthermore, since the Canary Islands are the only camel exporter to the European Union, camels could constitute a source of zoonotic agents to the rest of the European countries.

Abstract: Several different species of animals host staphylococci as normal microbiota. These animals can be a source of staphylococci zoonotic infections. People with routine or occupational exposure to infected/colonized animals are at risk of a potential transmission. Therefore, we aimed to investigate the presence of *S. aureus* and other staphylococci in camels used for recreational purposes as well as their antimicrobial resistance, virulence factors and genetic lineages. A total of 172 samples were collected from 86 healthy camels (nose and mouth) from different farms located in the Canary Islands, Spain. Antimicrobial susceptibility testing was performed against 14 antimicrobial agents. The presence of virulence genes was studied by PCR. Multilocus sequence typing, *spa* typing and *agr* typing were performed in all *S. aureus* isolates. From the 86 camels tested, 42 staphylococci were



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Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). isolated, of which there were 11 *S. aureus*, 13 *S. lentus*, 12 *S. sciuri*, 3 *S. xylosus*, *S. epidermidis*, *S. hominis* and *S. chromogenes*. Staphylococci isolates were resistant to penicillin, ciprofloxacin, clindamycin and fusidic acid. All *S. aureus* isolates harbored the *hla*, *hlb* and *hld* virulence genes. *S. aureus* isolates were ascribed to three sequence types (STs) and three *spa* types. All *S. aureus* isolates belonged to *agr* type III. Camels from Gran Canaria used in recreational purposes have a moderate prevalence of *S. aureus* and other coagulase-negative staphylococci. Nevertheless, *S. aureus* isolates are susceptible to almost all antibiotics tested.

Keywords: *Staphylococcus aureus*; coagulase-negative staphylococci; methicillin-resistant; camels; antimicrobial resistance

1. Introduction

Camelids belong to the Camelidae family which comprises the genera Camelus, Lama and *Vicugna* [1]. The genera *Camelus* includes the species *Camelus dromedarius*, which is the one-humped camel, and the species *Camelus bactrianus*, the two-humped camel [1,2]. C. dromedarius is common in Africa, the Middle East, Asia and Australia, while C. bactrianus is dispersed in Central Asia, China, East Kazakhstan and Southern Russia [2,3]. In 2020, the camel population was 3,552,527 worldwide, with C. dromedarius accounting for approximately 90% of all camels [1,4]. Although Africa contains the largest population of the one-humped dromedary, since 1989, the Canary Islands have been the only region that provides dromedary camels in the European Union [5]. In 2013, the population of camels in the Canary islands was just under 1300 [6]. Camels were mainly used as a source of meat, milk, transportation, agricultural work and racing [7]. However, recently, camel-based tourism has become one of the main attractions in several countries which includes camel riding, trekking, excursions and picture taking [8]. These camel-human close interactive encounters may lead to the transmission of zoonotic agents [5]. Several studies have shown that camels are carriers of many important pathogens such as Salmonella, extended-spectrum beta-lactamase-producing Escherichia coli and Pseudomonas aeruginosa, Enterococcus spp. and *Staphylococcus aureus* [4,5,9–11].

The genus *Staphylococcus* currently comprises 81 species and subspecies [12]. Both S. aureus and coagulase-negative staphylococci, such as S. epidermidis, are commensals that colonize the skin and mucosal membranes of humans and several animal species [13]. Studies have shown that camels can also be colonized by *S. aureus*, reporting high carriage rates of around 55% [14,15]. The presence of other species of staphylococci, particularly methicillin-resistant staphylococci (MRS), has not yet been studied much in camels [14]. Furthermore, the prevalence of *S. aureus* and MRS has not yet been studied in camels from Europe. Staphylococci are also opportunistic pathogens that can acquire resistance to several or all classes of antimicrobials, threatening the ability to treat common infections [16]. Methicillin-resistant S. aureus (MRSA) is part of the World Health Organization global priority list [17]. Contrary to coagulase-negative staphylococci (CoNS), S. aureus produces a wide range of toxins that can act as virulence factors [18]. Nevertheless, lately, there was an emergence of nosocomial infections caused by CoNS which was more often observed in vulnerable patients with an increased risk for infections [19]. Staphylococci have been isolated from a wide range of hosts and environments including humans, livestock, pets, wild animals, air and surface waters [20–25]. Animal-associated staphylococci have been reported as infectious agents in humans. These strains pose a zoonotic risk in addition to constituting a reservoir for antimicrobial resistance genes [26]. Therefore, we aimed to investigate the presence of staphylococci in one-humped dromedary camels from the Canary Islands and to characterize the antimicrobial resistance, virulence and genetic lineages of the isolates.

2. Materials and Methods

2.1. Animals and Bacterial Isolates

Samples were collected from the nostrils and buccal mucosa of 86 one-humped camels from the Canary Islands, making a total of 172 samples as previously described [27]. Samples were collected from 37 camels from Gran Canaria in June 2019 and from 49 camels from Fuerteventura in November 2019 (Figure 1). All camels were domesticated and used in recreational activities. The swabs were placed into tubes containing BHI broth (LiofilChem, Via Scozia, Italy) with 6.5% of NaCl and incubated at 37 °C for 24 h [28]. Then, 150 μ L of inoculum was seeded onto Oxacillin Resistance Screening Agar Base Selective Supplement agar (ORSAB; Oxoid, Basingstoke, UK) supplemented with 2 mg/L of oxacillin and Baird-Parker agar (Oxoid, Basingstoke, UK) plates for methicillin-resistant staphylococci (MRS) and *S. aureus* isolation [28]. Up to 4 colonies showing different morphological characteristics were isolated from each plate. Confirmation and identification of staphylococci genera and species were conducted using MALDI-TOF MS [29].



Figure 1. Camel sampling sites: Gran Canaria and Fuerteventura.

2.2. Phenotypic Antimicrobial Resistance

Susceptibility to antimicrobial agents was carried out according to the Kirby–Bauer disk diffusion method against the following 14 antimicrobials (in μ g/disk): penicillin G (1 unit), cefoxitin (30), chloramphenicol (30), ciprofloxacin (5), clindamycin (2), ery-thromycin (15), fusidic acid (10), gentamicin (10), kanamycin (30), linezolid (10), mupirocin (200), tetracycline (30), tobramycin (10) and trimethoprim/sulfamethoxazole (1.25/23.75). The results were analyzed according to the criteria of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2018 except for kanamycin that followed the Clinical and Laboratory Standards Institute (CLSI) 2017 guidelines [30,31]. The reference strain *S. aureus* ATCC25923 was used as a quality control strain.

2.3. Antimicrobial Resistance and Virulence Genes

DNA extraction was performed as previously described [32]. According to the phenotypic resistance profiles, each isolate was screened for the presence of resistance genes, which included the penicillin resistance gene *blaZ*, the methicillin resistance gene *mecA*, the macrolide and licosamide resistance genes *ermA*, *ermB*, *ermC*, *ermT*, *mphC*, *msr*(A/B), *lnuA*, *lnuB*, *vgaA* and *vgaB* and the fusidic acid resistance genes *fusB*, *fusC* and *fusD* (Table S2).

All isolates were subjected to PCR for the detection of genes encoding Panton–Valentine leukocidin PVL (*lukF/lukS-PV*), hemolysins (*hla*, *hlb* and *hld*), exfoliative toxins (*eta* and *etb*)

and toxic shock syndrome toxin (*tst*). Additionally, the scn gene, which is the marker of the immune evasion cluster (IEC) system, was also investigated (Table S2).

2.4. Molecular Typing

The polymorphic X of the *S. aureus* protein A gene (*spa*) was amplified as previously described [33]. The results were analyzed with the Ridom StaphType software (version 1.5, Ridom GmbH, Würzburg, Germany) to determine the *spa* type of each isolate. All *S. aureus* were subjected to multilocus sequence typing (MLST) by amplifying the 7 housekeeping genes (*arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi* and *yqi*L) by PCR followed by sequencing as described by Enright et al. [34]. The sequences were submitted to the MLST database (https://pubmlst.org/organisms/staphylococcus-aureus, accessed on 22 November 2021) to obtain the sequence types (STs) and clonal complexes (CCs). *S. aureus* isolates were characterized by *agr* typing (I–IV) by multiplex PCR [35].

3. Results and Discussion

The close contact between animals and humans offers favorable conditions for bacterial transmission [36]. The transmission of antimicrobial-resistant staphylococci has been shown between dogs and their owners and livestock and farm workers [37,38]. Therefore, a possible human-to-camel-to-human bacterial transmission may occur during recreational activities. In this study, we analyzed 172 samples recovered from 86 camels from the Canary Islands. A total of 42 staphylococci were isolated from the camels, with 21 staphylococci isolated from nasal samples and the other 21 from oral samples. It has been shown that the animal staphylococcal microbiota varies between anatomical sites due to the different microenvironmental conditions [39,40]. From the 86 camels tested, 11 (12.8%) S. aureus were isolated from 10 camels, since 1 camel carried 2 different strains of *S. aureus* (Table 1). S. aureus isolates were recovered from six oral samples and five nasal samples. A total of 4 (10.8%) S. aureus isolates were recovered from the 37 camels from Gran Canaria and 7 (14.3%) isolates were isolated from the 49 camels from Fuerteventura (Table S1). As far as we know, this is the first study reporting the presence of *S. aureus* and CoNS in camels in Europe. Nevertheless, a few studies have been conducted in healthy camels from the African and Asian continents. The frequency of *S. aureus* isolated from camels in our study is similar to other studies conducted in Egypt and Nigeria and higher than a recent study conducted in Tunisia [4,41,42]. However, two other studies conducted on healthy camels from Saudi Arabia and Algeria reported a much higher frequency of *S. aureus* of 56.2% and 53%, respectively [14,15]. Since most studies conducted on staphylococci from camels showed a prevalence of almost 100% of CoNS colonization, we decided to isolate only methicillin-resistant CoNS (MRCoNS) [14,42]. A total of 31 (18%) MRCoNS were recovered from the 172 samples and identified as S. lentus (n = 13), S. sciuri (n = 12), S. sylosus (n = 3), S. epidermidis, S. chromogenes and S. hominis. From the 100 camels tested in the study by Alzohairy, 8% were positive for MRCoNS, which is a lower frequency than that obtained in our study [14]. Co-carriage of two different species of staphylococci was identified in six animals and co-carriage of three species in two camels. The pattern of co-carriage was as follows: S. aureus/S. sciuri (n = 5), S. aureus/S. chromogenes (n = 1), S. aureus/S. lentus/S. sciuri (n = 1) and S. epidermidis/S. hominis/S.lentus (n = 1).

Antimicrobial susceptibility testing was performed in all isolates followed by the screening for antimicrobial resistance and virulence genes. Furthermore, all *S. aureus* isolates were typed by MLST, *spa* typing and *agr* typing. All *S. aureus* isolates were susceptible to all antibiotics tested except for isolate VS3144, which showed resistance to ciprofloxacin, in accordance with the study of Chehida et al. [4]. Other studies conducted in Asia and Africa revealed a higher number of antimicrobial resistances in *S. aureus* from camels [14,15,43]. These differences in results may be due to the different legislation for administering antibiotics to animals established in each continent and country. Furthermore, in our study, none of the *S. aureus* isolates showed methicillin resistance, which contrasts with the high frequency of MRSA found in other studies from Asia and Africa [14,41].

Regarding the presence of virulence genes, all *S. aureus* isolates carried the *hla*, *hlb* and hld genes that encode for the alfa-, beta- and delta-hemolysins, which is not surprising since these toxins are present in most S. aureus strains, mainly because they are located in very stable regions of chromosomal DNA [44]. Similar results were found in the study of Chehida et al. [4]. However, most studies conducted on camels did not investigate the presence of resistance or virulence genes in staphylococci isolates. S. aureus isolates were ascribed to three STs (ST7345, ST88 and ST8) and three spa types (t1773, t3221 and t008), showing a low diversity of clonal lineages. Furthermore, S. aureus ST7345 and t1773 were isolated from both Gran Canaria and Fuerteventura camels, suggesting either a dominance of these lineages in camels or in the study region. S. aureus ST7345 was first described in this study and is a double loci variant of ST130 with mutations in the *aro*E and *pta* loci. S. aureus ST130 is frequently associated with ruminants but it has also been isolated from humans and wildlife, usually associated with *mec*C-carrying MRSA isolates [45–47]. The spa type t1773 was previously reported to be associated with CC130 and common among farm animals and as a frequent cause of ovine mastitis [48–51]. Three S. aureus isolates were ascribed to ST88 which is a relatively rare lineage distributed globally among MRSA and MSSA [52]. This clonal lineage is highly related to community-acquired MRSA strains and is predominant in sub-Saharan Africa [53]. Nevertheless, and in accordance with our results, both S. aureus ST130 and ST88 were the predominant clones among samples of healthy camels in Algeria [15]. Furthermore, isolates belonging to ST130 have also been detected in camel's milk and fermented milk [54,55]. One *S. aureus* isolate was ST8-t008 which is highly related to the CA-MRSA epidemic clone USA300 [21]. Since S. aureus ST8-t008 is a classical human pathogen, a possible human-to-animal transmission may have occurred.

Isolate	Antimicrobial Resistance			Molecular Typing		
	Phenotype	Genotype	Virulence	ST (CC)	spa	agr
VS3140	Susceptible	-	hla, hlb, hld	7345	t1773	III
VS3141	Susceptible	-	hla, hlb, hld	7345	t1773	III
VS3142	Susceptible	-	hla, hlb, hld	7345	t1773	III
VS3143	Susceptible	-	hla, hlb, hld	7345	t1773	III
VS3144	CÎP	-	hla, hlb, hld	7345	t1773	III
VS3145	Susceptible	-	hla, hlb, hld	7345	t1773	III
VS3146	Susceptible	-	hla, hlb, hld	7345	t1773	III
VS3147	Susceptible	-	hla, hlb, hld	88	t3221	III
VS3148	Susceptible	-	hla, hlb, hld	88	t3221	III
VS3149	Susceptible	-	hla, hlb, hld	88	t3221	III
VS3150	Susceptible	-	hla, hlb, hld	8 (8)	t008	Ι

Table 1. Genetic characterization and molecular typing of *S. aureus* isolates from healthy camels.

Abbreviations: CIP: ciprofloxacin; ST: sequence type; CC: clonal complex.

Studies reporting the frequency and antimicrobial resistance of *S. aureus* in healthy camels are scarce, but studies showing the frequency and antimicrobial resistance in CoNS are even scarcer. In our study, among the 31 MRCoNS isolates, 13 *S. lentus* and 12 *S. sciuri* were isolated from 12 camels each (Table 2).

In other studies, *S. lentus* has been frequently identified in samples from livestock and from people with occupational exposure to livestock [56–58]. *S. sciuri* has a wider host range and is adapted to very different habitats [59,60]. One nasal sample from one camel was positive for *S. lentus* (VS3158) and *S. sciuri* (VS3168), and both isolates showed the same resistance pattern. Another camel simultaneously carried *S. lentus* (VS3155), *S. epidermidis* (VS3152) and *S. chromogenes* (VS3153) in the nasal mucosa. Additionally, the same animal was the only one to carry the same staphylococci species (*S. lentus*) in both the mouth and nose. Nevertheless, the isolates differed in the resistance profile, with the *S. lentus* (VS3166) isolated from the oral sample having resistance to penicillin and clindamycin conferred by the genes *mecA* and *mphC*, and the *S. lentus* isolated from the nasal sample

showing only resistance to penicillin. Although S. epidermidis and S. hominis strains have been isolated from animal samples, these species are the most prevalent CoNS at the clinical level and as part of the normal nasal microbiota of healthy individuals, which may suggest a possible human origin [22,61,62]. All MRCoNS were resistant to penicillin and harbored the mecA gene. The presence of the mecA gene among staphylococci of the S. sciuri group (S. sciuri, S. lentus, S. vitulinus and S. fleurettii) is common since it is believed that they played an important role in the origin, evolution and dissemination of mecA [63]. None of the MRCoNS showed phenotypic resistance to cefoxitin. In fact, it has been shown that some CoNS carry a homologue of the mecA gene which does not confer resistance to β -lactams [64]. Despite all isolates being resistant to penicillin, all isolates lacked the *blaZ* gene, which suggests the presence of other unknown resistance mechanisms or that the breakpoints used for susceptibility testing are not accurate for CoNS [22]. Contrary to what was obtained in the study by Alzohairy, none of our MRCoNS isolates displayed a multidrug resistance profile [14]. Finally, only 7 out of 31 MRCoNS isolates carried virulence genes. The *hld* gene was detected in six isolates, while *hla* was detected in two. S. epidermidis was the only isolate that carried both genes. Although CoNS carry fewer virulence genes than S. aureus strains, studies have shown that CoNS are a heterogeneous group with distinct virulence potential levels [19,65].

 Table 2. CoNS species identification, antimicrobial resistance and virulence.

	Inclute Country	Antimicrobia			
	Isolate Species	Phenotype	Genotype	 Virulence Factors 	
VS3151	chromogenes	PEN	mecA		
VS3152	epidermidis	PEN	mecA	hla, hld	
VS3153	hominis	PEN	mecA		
VS3154	lentus	PEN, ERY, CD	mecA, mphC	hla	
VS3155	lentus	PEN	mecĂ		
VS3156	lentus	PEN, FD	mecA		
VS3157	lentus	PEN	mecA		
VS3158	lentus	PEN	mecA		
VS3159	lentus	PEN	mecA		
VS3160	lentus	PEN	mecA		
VS3161	lentus	PEN	mecA		
VS3162	lentus	PEN	mecA		
VS3163	lentus	PEN, FD	mecA	hld	
VS3164	lentus	PEN, FD	mecA		
VS3165	lentus	PEN	mecA		
VS3166	lentus	PEN, CD	mecA, mphC		
VS3167	sciuri	PEN	mecA		
VS3168	sciuri	PEN	mecA		
VS3169	sciuri	PEN	mecA		
VS3170	sciuri	PEN	mecA		
VS3171	sciuri	PEN	mecA		
VS3172	sciuri	PEN	mecA		
VS3173	sciuri	PEN	mecA	hld	
VS3174	sciuri	PEN	mecA		
VS3175	sciuri	PEN	mecA		
VS3176	sciuri	PEN	mecA	hld	
VS3177	sciuri	PEN	mecA	hld	
VS3178	sciuri	PEN, CD, FD	mecA, mphC		
VS3179	xylosus	PEN	mecĂ		
VS3180	xylosus	PEN	mecA		
VS3181	xylosus	PEN	mecA	hld	

Abbreviations: PEN, penicillin; ERY: erythromycin; CD: clindamycin; FD: fusidic acid.

4. Conclusions

In this study, a moderate frequency of *S. aureus* and MRCoNS was detected among healthy camels. However, our findings show that, in general, European camels have fewer resistance and virulence genes than healthy camels from Africa and Asia. This study demonstrates a low diversity of *S. aureus*. The predominant lineage was ST7331, followed by ST88, which has already been reported among healthy camels, suggesting that these lineages may be dominant in camels. The carriage of *mec*A-positive staphylococci by camels may be a public health concern since there is a risk of bacterial transmission to humans during recreational activities. Furthermore, since the Canary Islands are the only camel exporter to the EU, camels could constitute a source of zoonotic agents to the rest of the EU.

Supplementary Materials: The following supporting information can be downloaded at https: //www.mdpi.com/article/10.3390/ani12101255/s1. Table S1: Distribution of staphylococci isolates according to the geographical location and anatomical isolation site; Table S2: Primer pairs used for molecular typing and detection of antimicrobial resistance genes in staphylococci strains. References [35,66–79] are cited in the Supplementary Materials.

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