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Human *Staphylococcus aureus* lineages among Zoological Park residents in Greece

E. Drougka^{1,2}, A. Foka^{1,2}, D. Posantzis³, N. Giormezis^{1,2}, E.D. Anastassiou^{1,2}, E. Petinaki⁴ and I. Spiliopoulou^{1,2,*}

¹Department of Microbiology, School of Medicine, University of Patras, Patras, Greece

²National Reference Laboratory for Staphylococci, University of Patras, Patras, Greece

³Attica Zoological Park, Athens, Greece

⁴Department of Microbiology, School of Medicine, University of Thessaly, Larissa, Greece

Abstract

Staphylococcus aureus is a part of the microbiota flora in many animal species. The clonal spread of *S. aureus* among animals and personnel in a Zoological Park was investigated. Samples were collected from colonized and infected sites among 32 mammals, 11 birds and eight humans. The genes *mecA*, *mecC*, *lukF/lukS-PV* (encoding Pantone-Valentine leukocidin, PVL) and *tst* (toxic shock syndrome toxin-1) were investigated by PCR. Clones were defined by Multilocus Sequence Typing (MLST), *spa* type and Pulsed-Field Gel Electrophoresis (PFGE). Seven *S. aureus* isolates were recovered from four animals and one from an employee. All were *mecA*, *mecC* and *tst*-negative, whereas, one carried the PVL genes and was isolated from an infected Squirrel monkey. Clonal analysis revealed the occurrence of seven STs, eight PFGE and five *spa* types including ones of human origin. Even though a variety of genotypes were identified among *S. aureus* strains colonizing zoo park residents, our results indicate that colonization with human lineages has indeed occurred.

Keywords: Carriers, Clones, Humans, *Staphylococcus aureus*, Zoological park.

Introduction

Mammals are colonized by a vast number of microorganisms which constitute the host's microbiota. *Staphylococcus aureus* is common within the bacterial community which inhabits the skin, mucous membranes and anterior nares in humans, as well as various animal species, birds, and reptiles (Walther *et al.*, 2008; Hasman *et al.*, 2010; Espinosa-Gongora *et al.*, 2012). Methicillin-resistant *S. aureus* strains (MRSA) pose a huge risk when they spread among animals of zoological parks, therefore treatment administration must be carefully determined for proper dosing, however, on several occasions the antimicrobials' administration is based upon estimated and not precise body weights (Espinosa-Gongora *et al.*, 2012; Vercammen *et al.*, 2012). Zoological parks provide an easily accessible and highly diversified habitat to study the distribution of *S. aureus* in different animal species, some of them being exotic. In a study from the Royal Zoological Society of Antwerp, in Belgium, no MRSA was identified, but the authors did not investigate *S. aureus* prevalence in the tested population (Vercammen *et al.*, 2012). In another study conducted in the Small Animal Clinic of the Veterinary Faculty at the Free University Berlin among small and exotic animals, 44.3% MRSA infections were identified (Walther *et al.*, 2008).

Greece is a country with relatively high prevalence of MRSA in human population (>25%), as already published (Maltezou *et al.*, 2009; Drougka *et al.*,

2014). Moreover, in the literature it has been shown that strains of human origin have been identified to be transmitted to animals causing infections, whereas, environmental factors were also found to be responsible (Walther *et al.*, 2008). *S. aureus* was characterized as a pathogen that has the ability to switch hosts, establishing its significance in the human and veterinary medicine (Espinosa-Gongora *et al.*, 2012).

The aim of this study was to investigate the occurrence and distribution of *S. aureus* and MRSA among humans and other animal species in contact with humans and birds at the Attica Zoological Park in Greece.

Materials and Methods

Sample collection and *S. aureus* identification

This research was conducted during 2012 among 32 mammals belonging to 16 species, 11 birds belonging to seven species and eight humans from the staff of the Attica Zoological Park (Table 1). The Park, located in Spata, is a self-funded creation, in an area of 20 hectares hosting 50 species of land mammals, 180 bird species, 30 reptile species and two species of marine mammals. As a member of the European Association of Zoo and Aquaria and the only zoological park in Greece, it receives 300 000-400 000 visitors of all ages annually. In an effort to preserve endangered species, about 60% of the animals are rare. It participates in 35 European Studbooks and 29 European Endangered species Programs, whereas, in collaboration with

*Corresponding Author: Iris Spiliopoulou. Department of Microbiology, School of Medicine, University of Patras, University Campus, Rion, Patras 26504, Greece. Tel: 0030-2610-996111. E-mail: spiliopl@upatras.gr

Table 1. Animal species included in this study for *S. aureus* carriage identification.

Studied population	Scientific names	Sampling site	Number of samples per animal	Number of samples	Number of <i>S. aureus</i> isolates
Mammals (n=40)					
Human (n=8)	<i>Homo sapiens</i>	Nostrils	1	8	1
Baringo giraffe (n=4)	<i>Giraffa camelopardalis</i>	Pile	1	4	0
Black and white ruffed Lemur (n=1)	<i>Varecia variegata</i>	Nostrils Pile	2	1 1	0 0
Black capped capuchin (n=1)	<i>Cebus apella</i>	Pile	1	1	0
Brown bear (n=4)	<i>Ursus arctos</i>	Nostrils Pile	2	4 4	0 0
Bottle-nosed dolphin (n=2)	<i>Tursiops truncatus</i>	Nostrils	1	2	0
Cretan wild goat (n=2)	<i>Capra aegagrus cretica</i>	Nostrils Pile	2	2 2	1 0
Grant's zebra (n=2)	<i>Equus quagga boehmi</i>	Pile	1	2	0
Pygmy hippopotamus (n=2)	<i>Choeropsis liberiensis</i>	Nostrils Pile	2/1	2 1	0 0
Ring tailed lemur (n=3)	<i>Lemur catta</i>	Nostrils Pile Skin lesion	3/1	1 2 2	0 0 0
Shetland pony (n=3)	<i>Equus ferus caballus</i>	Nostrils Pile	2	3 3	1 1
Skyrian horse (n=2)	<i>Equus caballus Skyros Poni</i>	Nostrils Pile	2	2 2	0 0
Squirrel monkey (n=1)	<i>Saimiri sciureus</i>	Nostrils Skin lesion	2	1 1	1 1
De Brazza's monkey (n=1)	<i>Cercopithecus neglectus</i>	Nostrils Pile	2	1 1	0 0
Javan langur (n=1)	<i>Trachypithecus auratus</i>	Nostrils Pile	2	1 1	0 0
Cow (n=1)	<i>Bos taurus</i>	Nostrils Pile	2	1 1	0 0
Rabbit (n=2)	<i>Oryctolagus cuniculus</i>	Nostrils Pile	2	2 2	1 1
Birds (n=11)					
Alexandrian parrot (n=2)	<i>Psittacula eupatria</i>	Skin	1	2	0
Blue and gold macaw (n=2)	<i>Ara ararauna</i>	Skin	1	2	0
Scarlet macaw (n=2)	<i>Ara macao</i>	Skin	1	2	0
Salmon-crested cockatoo (n=1)	<i>Cacatua moluccensis</i>	Skin	1	1	0
Military macaw (n=2)	<i>Ara militaris</i>	Skin	1	2	0
African grey parrot (n=1)	<i>Psittacus erithacus</i>	Skin	1	1	0
Eclectus parrot (n=1)	<i>Eclectus roratus</i>	Skin	1	1	0
Total (n=51)				72	8

Veterinary, Biology and Agriculture University Schools it participates in students' educational projects and practice, scientific studies and research programs. Moreover, educational and entertainment projects for visitors and pupils are consistently organized.

The study was approved by the Bioethics Committee of the University of Patras (No EB25). A written informed consent was obtained from the human individuals, who are working in the Park between two and 15 years. They all had companion animals such as dogs, cats and birds,

but none was hospitalized during the last year. All eight humans are working as animal caregivers without any rotation; three with big mammals, three with birds and another two with monkeys and felines.

The study did not involve any experimentation, but was based on swabs collected by a veterinarian under routine diagnostic procedures from infected animals. Colonization samples were collected randomly among healthy animals at the Attica Zoological Park. *S. aureus* screening samples were obtained from the nostrils (n=23) and pile (n=27) or skin surfaces (n=11). Any sites of infection in animals were also sampled (n=3). Swabs were inoculated into 3 mL Trypticase Soy Broth (TSB) transport medium with 6% NaCl (Difco, Sparks, MD, USA), and transferred to the National Reference Laboratory for Staphylococci. One loop from each sample was inoculated into fresh TSB for enrichment, plated onto Trypticase Soy Agar with 5% Sheep Blood (Becton Dickinson, Le Pont de Claix, France) and incubated at 37°C for 48 h. Isolates were identified on the basis of colony morphology, Gram stain, catalase production and coagulase test (Slidex Staph Plus, bioMérieux, Marcy l'Étoile, France), whereas, verification was carried out by PCR for *nuc* gene (Boye et al., 2014).

Antimicrobial susceptibility testing

Susceptibility testing was performed according to EUCAST guidelines by the disc diffusion method for cefoxitin, tetracycline, gentamicin, rifampicin, kanamycin, erythromycin, clindamycin, fusidic acid and sulfamethoxazole-trimethoprim (SXT), (SirScan, i2a, Park Eureka, Montpellier, France) (The EUCAST, 2012). MICs of oxacillin, vancomycin, teicoplanin, daptomycin and linezolid were determined by a gradient method (Etest, bioMérieux) (The EUCAST, 2012).

Molecular analysis

All isolates were tested by PCR for the presence of *mecA*, *mecC* (Stegger et al., 2012), the genes encoding Panton-Valentine leukocidin (PVL) (*lukF/lukS-PV*) and toxic shock syndrome toxin-1 (TSST-1, *tst*) (Jarraud

et al., 2002), using the reference strains ATCC49775, LGA251, ATCC49775 and Fri913 as positive controls, respectively. DNA extraction was performed into agarose disks, whereas pulsed-field gel electrophoresis (PFGE) of *SmaI* DNA digests was carried out using a CHEF DR III apparatus (Bio-Rad Laboratories, Richmond, CA, USA). Visual interpretation of PFGE banding patterns and assignments of types were performed according to the published criteria (Tenover et al., 1995). PFGE types were named by capital letters and compared to previously identified pulsotypes among humans. A dendrogram was computed comparing molecular weights of strains' of DNA fragments by using FPQuest software (Bio-Rad, Cat.Num:1709300). Clustering was based on $\geq 80\%$ similarity (Fig. 1). Multi Locus Sequence Typing (MLST) was performed according to published protocols (<http://saureus.mlst.net/misc/info.asp>) (Enright et al., 2000). The polymorphic X-region of protein A gene (*spa*) was analyzed (Stegger et al., 2012) and the resulting *spa* types and clonal clusters were assigned using the software Ridom StaphType (Ridom GmbH, Wurzburg, Germany).

Results

A total of seven *S. aureus* were recovered from four animals among 43 tested (prevalence 9.3%) and one isolate among the personnel (12.5%), (Table 1). Twelve mammalian species and all birds were negative for *S. aureus* carriage or infection (Table 1). The MIC of oxacillin was ≤ 1.5 mg/L, whereas the MICs of vancomycin ranged from 0.5 mg/L to 1 mg/L, teicoplanin from 1.5 mg/L to 3 mg/L and linezolid from 1 mg/L to 2 mg/L. Only two out of eight isolates showed resistance to fusidic acid and one to tetracycline (Table 2). All isolates were negative for *mecA*, *mecC* and *tst* genes, whereas, one carried the PVL genes, recovered from a skin infection of a Squirrel monkey (*Saimiri sciureus*).

The human *S. aureus* strain belonged to ST80 clone *spa* type t053, PFGE type C and negative for PVL genes (Table 2, Fig. 1). Among the strains of animal origin,

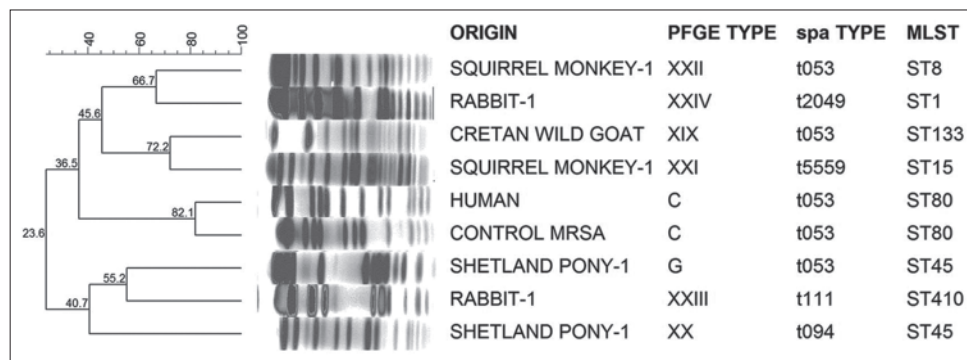


Fig. 1. Dendrogram of PFGE banding patterns of the isolated *S. aureus* strains and the respective MLST and *spa* types. One methicillin-resistant *S. aureus* strain (MRSA) of human origin, ST80-t053 and PFGE type. C is included as control.

Table 2. Phenotypic and molecular characteristics among *S. aureus* isolates from four animals and an employee at the Attica Zoological Park.

Species	MLST ST	MLST group	spa type	spa CC	PFGE type	PVL toxin	Antimicrobial resistance patterns	Colonized/infected (C/I)
Cretan wild goat	133	10	t053	S	XIX	N	Fusidic acid	C
Shetland pony-1	45	3	t053	S	G	N	Susceptible	C
Shetland pony-1	45	3	t094	S	XX	N	Susceptible	C
Squirrel monkey-1	15	1	t5559	S	XXI	N	Tetracycline, fusidic acid	C
Squirrel monkey-1	8	1	t053	S	XXII	P	Susceptible	I
Rabbit -1	1	1	t2049	Common	XXIV	N	Susceptible	C
Rabbit -1	410	New	t111	CC	XXIII	N	Susceptible	C
Human	80	14	t053	S	C	N	Susceptible	C

ST: Sequence types assigned by multi-locus sequence typing (MLST), MLST Groups: Classified according to sequence types by eBURST (<http://saureus.mlst.net/misc/info.asp>), CC: Clusters assigned according to *spa* types by BURP clustering with Ridom StaphType (Ridom GmbH, Wurzburg, Germany): t111 and t2049 are assigned to a common CC, PFGE: Pulsed-field gel electrophoresis types, PVL: Panton-valentine leukocidin genes detected by PCR, S: Singleton, N: negative, P: positive. Antimicrobials tested: ceftiofur, tetracycline, gentamicin, rifampicin, kanamycin, erythromycin, clindamycin, fusidic acid, sulfamethoxazole-trimethoprim, oxacillin, vancomycin, teicoplanin, daptomycin and linezolid.

MLST revealed the occurrence of six STs (133, 45, 15, 8, 1 and 410), which were classified into three major MLST Groups (10, 3 and 1), whereas ST410 was new in our setting (eBURST, <http://saureus.mlst.net/misc/info.asp>), (Table 2). Five *spa* types were detected; t053 predominated (three strains), followed by t094, t111, t5559, and t2049 (one strain each). Seven PFGE types were identified, including type G that was previously characterized in humans (Drougka *et al.*, 2014). The remaining five pulsotypes were detected in our setting for the first time in our present study (Fig. 1, Table 2).

Discussion

The prevalence of *S. aureus* carriage in animals and personnel of the zoo were 9.3% and 12.5%, respectively. This is much lower compared to published colonization rates of people in Greece (16.7% among hospital residents and 33.3% in health care workers) (Maltezos *et al.*, 2009). Espinosa-Gongora *et al.* (2012) identified 35% *S. aureus* carriage among zoo animals that was comparable to their human population results. However, the aforementioned result can be explained by the low number of human participants that constitutes a limitation of our study. All *S. aureus* were methicillin-susceptible in this setting. The absence of MRSA has previously been detected among mammals of the zoological park in Belgium (Vercammen *et al.*, 2012). Resistance to fusidic acid was detected in two isolates and to tetracycline in one; both are antimicrobials that are rarely used in the Zoological Park for local therapy only, whereas, this resistance phenotype is correlated with the human MRSA ST80 clone spread in the country (Drougka *et al.*, 2014).

Molecular analysis classified the human *S. aureus* isolate as ST80, t053, PFGE type C and this isolate

was PVL-negative. The ST80 *spa* t053 clone is the predominant clone among MRSA in Greece, it was first identified as a hospital-associated pathogen, and it is mainly associated with skin and soft tissue infections in the community, but has also infiltrated the healthcare system causing nosocomial infections (Aires de Sousa *et al.*, 2003; Drougka *et al.*, 2014). Among the isolates of animal origin, ST45 has been identified in two strains, both originating from the Shetland pony (*Equus ferus caballus*) and was classified into different PFGE and *spa* types (t053 and t094). A search of the MLST database showed that ST45 has been found among human nasal carriers and is associated with hospital infections. Both MRSA and MSSA ST45 strains have been reported from several European countries (www.saureus.mlst.net/sql/all_allelicprofileresults.asp). Espinosa-Gongora *et al.* (2012) identified ST45 strains in horses at the Copenhagen zoo. The identification of the human pulsotype G in the ST45 clone (Drougka *et al.*, 2014), suggests that these strains may be of human origin and transmission of this clone might be associated with the close contact these animals have with personnel and visitors of the park.

Strains characterized as ST8 and ST15 clones were assigned into MLST Group1 and were isolated from the Squirrel monkey. These STs are mainly associated with human infections (Tavares *et al.*, 2014), reinforcing the aspect that these strains might have been recently introduced to animals by humans and have not yet evolved for eliciting host specific traits. In Portugal, 4.7% of MSSA strains were classified to ST8-t008 clone (Tavares *et al.*, 2014). In the present study, the strain characterized as ST8 belonged to *spa* type t053, a human lineage in Greece, previously identified in

MRSA (ST8-SCC*mec*-IV), carrying also PVL genes (Drougka *et al.*, 2014). Moreover, the ST8-t053 strain originating from the skin infection of a Squirrel monkey was PVL-positive, reinforcing the aspect of human to animal transfer and the importance of surveillance for *S. aureus* and not only for MRSA transmission.

ST133 isolated from a goat has been previously identified in cows, goats and sheep (Smyth *et al.*, 2009). Two STs were identified from the rabbit: ST1, which was originally a human clone but has also colonized some other animal species (Gómez-Sanz *et al.*, 2013), and ST410, a clone rarely reported in the literature (Smyth *et al.*, 2009).

The predominant *spa* type t053 has been identified among strains of human origin and more specifically associated with the ST80 clone that is common in our country (Table 2) (Drougka *et al.*, 2014), whereas worldwide, its frequency is very low (0.02%). It has previously been detected among ST5 strains in France, Germany, Romania and Spain (<http://spa.ridom.de/frequencies.shtml>). Moreover, in the present study, t053 was characterized among strains of different STs (Table 2), suggesting that zoo park animals are colonized by *S. aureus* clonal lineages of human origin.

Conclusions

Worldwide, studies investigating the distribution of *S. aureus* in zoo park population have been very scarce and this is the first study conducted in Greece. A variety of STs were identified among *S. aureus* colonizing the zoological park's inhabitants, interpreted by the fact that in most cases they do not interact with each other, thus having no chance to transmit bacteria between different species. However, our results indicate that some animals are colonized with lineages of human origin. No transmission of infection among animals and personnel has been detected. Further studies should be carried out in order to provide additional information of *S. aureus* ecology in the animal kingdom.

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Conflict of interest

Authors E.D. and A.F. have received funding from the National Staphylococcal Reference Laboratory.

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