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RESEARCH ARTICLE

Drug-Resistant Human *Staphylococcus Aureus* in Sanctuary Apes Pose a Threat to Endangered Wild Ape Populations

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Reintroduction of sanctuary apes to natural habitat is considered an important tool for conservation; however, reintroduction has the potential to endanger resident wild apes through the introduction of human pathogens. We found a high prevalence of drug-resistant, human-associated lineages of *Staphylococcus aureus* in sanctuary chimpanzees (*Pan troglodytes*) from Zambia and Uganda. This pathogen is associated with skin and soft tissue diseases and severe invasive infections (i.e. pneumonia and septicemia). Colonization by this bacterium is difficult to clear due to frequent recolonization. In addition to its pathogenic potential, human-related *S. aureus* can serve as an indicator organism for the transmission of other potential pathogens like pneumococci or mycobacteria. Plans to reintroduce sanctuary apes should be reevaluated in light of the high risk of introducing human-adapted *S. aureus* into wild ape populations where treatment is impossible. Am. J. Primatol. 00:1–5, 2012.

Key words: *Staphylococcus aureus*; wildlife; reintroduction; transmission; great apes; Africa

INTRODUCTION

Animal sanctuaries care for and protect endangered animals confiscated by national authorities from animal dealers or private holders. Reintroduction of wildlife housed in sanctuaries to their natural habitat is considered an important conservation tool for endangered species; however, for sanctuaries to have a net positive effect for species conservation, it is critical that reintroduction efforts do not endanger resident wildlife populations [Beck et al., 2007]. The close phylogenetic relationship between humans and apes, coupled with the high rates of human–ape contact characteristic of sanctuaries (i.e., especially infant and juvenile apes receiving comfort and play from humans which is necessary to ensure their survival and development, Figure 1) results in an exceptionally high potential for pathogen exchange [Calvignac-Spencer et al., 2012; Gillespie et al., 2008; Leendertz et al., 2006]. In contrast to wild apes, sanctuary apes may acquire protective antibody levels against human pathogens since they receive medical treatment when severe symptoms are observed. Although no studies have been conducted to determine the relative risk of ape reintroductions exposing naïve wild conspecifics to human-adapted pathogens, several sanctuaries have reintroduced great apes in areas with existing communities of wild conspecifics and the majority of ape sanctuaries are planning and/or implementing reintroductions [Faust et al., 2011].

Staphylococcus aureus is a common bacterium that asymptomatically colonizes humans and animals but can also cause a wide range of mild-to-severe diseases including skin and soft tissue infection, endocarditis, pneumonia, and sepsis [Wertheim et al., 2005]. Colonized individuals have a markedly higher risk to develop an *S. aureus* infection [von Eiff et al., 2001]. Decolonization of *S. aureus* requires

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Fig. 1. Sanctuary worker during routine work in contact with chimpanzees. Transmission of resistant bacteria from humans to animals can occur during close contact associated with care-giving. Infant and juvenile sanctuary apes require extensive care and contact from human care-givers to provide the body warmth, socialization, and play a mother would provide in the wild.

nasal application of mupirocin ointment and a strict hygiene regime or antibiotic treatments but recolonization is frequent [Lucet & Regnier, 2010].

To evaluate the risk of *S. aureus* transmission from humans to animals, we compared resistance pattern, genotypes, and the possession of Panton-Valentine leukocidin of *S. aureus* isolates from chimpanzees (*Pan troglodytes*) of two characteristic ape sanctuaries in Uganda and Zambia with isolates from sanctuary veterinarians and data published on classic human *S. aureus* isolates. We found a high proportion of typically human-related *S. aureus* in sanctuary apes and conclude that reintroduction of sanctuary apes poses a risk to naïve wild populations through the introduction of human-adapted pathogens.

METHODS

Samples

In a cross-sectional study, we collected nasal swabs from 23 chimpanzees housed in Chimfunshi Wildlife Orphanage, Chingola, Zambia in 2011. These swabs were stored in Amies medium until culture. In addition, oral swabs were obtained from 39 chimpanzees from Ngamba Island Chimpanzee Sanctuary, Entebbe, Uganda in 2007. These swabs were stored in RNAlater (Qiagen) in liquid nitrogen until culture. Swabs from animals were taken as part of routine health checks at participating sanctuaries and analyzed on request. The research complied with protocols approved by the Institutional Animal Care Committees of each sanctuary and country.

We also collected nasal samples from veterinarians working in various African great ape sanctuaries ($N = 30$) during a conference in Johannesburg, South Africa in 2010. Nasal swabs were stored in Amies medium until culture within 5 days.

Ethical clearance for human participation was obtained from the independent ethics committee of the medical faculty, University of Münster (2009–227-b-S). Informed consent was obtained from all participants.

The noninvasive samples from animals were collected in accordance with the international guidelines and under the permission of the national authorities. This research adhered to the American Society of Primatologists principles for the ethical treatment of primates. The results of this study have been shared with the authorities of the sanctuaries, which exchange information with the respective governmental bodies.

Bacterial Analyses

Samples were cultured on Columbia blood agar and SAID agar (bioMérieux, Marcy l'Etoile, France). We used Vitek2 automated systems (bioMérieux) for species identification and susceptibility tests. Species of *S. aureus* was confirmed by 16s rRNA gene sequencing [Becker et al., 2004]. Resistance against penicillin and methicillin was confirmed by detection of *BlaZ* and *mecA*, respectively [Becker et al., 2006; Kaase et al., 2008]. Genes encoding Panton-Valentine leukocidin (*lukF*-PV and *lukS*-PV) were detected as published [Lina et al., 1999]. Sequence-based typing of the hypervariable region of *S. aureus* protein A (*spa* typing) and multilocus sequence typing (MLST) was performed for each isolate [Enright et al., 2000; Mellmann et al., 2006]. In general, *spa* typing is considered to be more discriminatory than MLST [Strommenger et al., 2006]. To assign the MLST ST to a known clonal complex, we compared the ST of our study with the STs of the whole MLST database of *S. aureus* using the stringent group definition of six shared identical alleles (http://saureus.mlst.net). Different *spa* types were clustered in spa clonal complexes (spa CC) using the BURP algorithm implemented in Ridom StaphType software (Version 2.2.1) with preset parameters as published [Mellmann et al., 2007].

TABLE I. *Staphylococcus Aureus* from Humans and Chimpanzees Living in African Sanctuaries

				S. aureus from humans ($n = 10$)		*S. aureus* from chimpanzees ($n = 36$)	
MLST-CC	ST	Spa-CC	*Spa* type	Isolates (n)	Antibiotic resistance pattern	Isolates (n)	Antibiotic resistance pattern
6	6	304/701	t701	1	Pen	-	-
	2020	304/701	304	-	-	1	Pen
15	15	084	t279	2	Pen-SXT[a]-Tet	1	Pen-Tet
			t7723	-	-	4	Pen
		Singleton	t1877	1	Pen-SXT	-	-
	2126	084	t084	-	-	1	Pen
	2168	Singleton	t1247	-	-	16	Pen
30	30	012	t012	1	Pen	-	-
			t021	1	Pen	-	-
			t122	1	Pen	-	-
	2178	012	t2864	-	-	1	Pen-Ery-Clinda-SXT
80	80	Singleton	t934	-	-	9	Tet
88	88	186/1855	t186	1	Pen	-	-
			t1855	1	Pen-Oxa	-	-
	2167	186/1855	t186	1	Pen-Oxa-Ery-Clin-Tet-SXT	-	-
101	101	Singleton	t7722	-	-	2	Pen-Tet
Singleton	1948	Singleton	t224	-	-	1	Pen-Tet

Penicillin (Pen), oxacillin (Oxa), erythromycin (Ery), clindamycin (Clin), tetracyclin (Tet), SXT (trimethoprim/sulfamethoxazol).
[a]Only one isolate was SXT resistant.

RESULTS

Antimicrobial Susceptibility

S. aureus was found in 36 chimpanzees (58%) and 10 humans (33%). Resistance rates were slightly higher in isolates from humans compared to chimpanzees for penicillin (100% vs. 75%), oxacillin (20% vs. 0%), trimethoprim/sulfamethoxazol (SXT; 40% vs. 3%), erythromycin (10% vs. 3%), and clindamycin (10 vs. 3%). Higher resistance rates in animals compared to humans were found for tetracyclin (36% vs. 20%). No resistance was detected to glycopeptides, fluorquinolones nitrofurantoin, fosfomycin, rifampicin, and aminoglycosides (Table I). The resistance to antibiotic agents was not restricted to specific clonal lineages but was present in all clonal complexes (Table I).

Genotypes and Panton-Valentine Leukocidin

Genotypes of *S. aureus* isolates were compared using multilocus sequence typing (MLST) and typing of the hypervariable region of protein A (*spa* typing). In total, eight different *spa* types (t012, t021, t122, t186, t279, t701, t1855, t1877) were found in humans compared to nine different types in animals (t084, t224, t279, t304, t934, t1247, t2864, t7722, t7723). Noteworthy, a veterinarian and chimpanzee from the same sanctuary shared the same *spa* type (t279, Table I).

The predominant MLST sequence type (ST) was ST15 (30%, $N = 3$) in humans and ST2168 (44%, $N = 16$) in chimpanzees, both belonged to MLST clonal complex (CC) 15 but were clustered in different subgroups (Table I). Isolates belonging to the classical human ST15, the most prevalent ST in sub-Saharan Africa, were also found in chimpanzees (13.9%, $N = 5$). Other human-related STs from chimpanzees included ST80 (25%, $N = 9$) and ST101 (5.7%, $N = 2$).

The Panton-Valentine leukocidin (PVL) was markedly more frequent in isolates from chimpanzees than from humans (28% vs. 10%). In chimpanzees, PVL positive isolates were always associated with ST80 (90%, $N = 9$) and ST2178 (10%, $N = 1$). The human PVL-positive *S. aureus* isolate belonged to ST30.

DISCUSSION

We compared characteristics of *S. aureus* from sanctuary chimpanzees to strains isolated from sanctuary veterinarians and published data on the basis of resistance pattern to antibiotic agents, genotypes, and the presence of Panton-Valentine leukocidin. We found higher resistance rates for penicillin, oxacillin, trimethoprim/sulfamethoxazol, erythromycin, and clindamycin in the veterinarians (Table I). This is not surprising as these resistance rates are consistent with other reports of human *S. aureus* in Africa [Okon et al., 2009; Schaumburg et al., 2011]. In contrast, it was surprising that resistance rates were high in isolates from animals, as wild great apes and monkeys very rarely display appreciable antibiotic resistance [Schaumburg et al., 2012]. In a study of *S. aureus* in wild apes and monkeys in Côte d'Ivoire and Gabon, penicillin resistance was only found in one isolate

derived from a habituated chimpanzee that had received antibiotic treatment before (Côte d'Ivoire) and all isolates were susceptible to aminoglycosides, lincosamides, fluorquinolones glycopeptides, nitrofurantoin, fosfomycin, rifampicin, tetracycline, trimethoprim/sulfamethoxazol [Schaumburg et al., 2012]. The high rates of antibiotic resistance observed in our data set may be due to frequent treatment of sanctuary animals with antibiotics or transmission of resistant isolates from humans to animals during close contact associated with care-giving or a combination of both. Infant and juvenile sanctuary apes require extensive care and contact from human care-givers to provide the body warmth, socialization, and play a mother would provide in the wild (Fig. 1). In support of such linkages for transmission, we found that 45% of all *S. aureus* from sanctuary chimpanzees in both study sites (Uganda and Zambia) were colonized with isolates belonging to known human-related STs (ST15, ST80, ST101). These STs have never been detected in wild apes [Schaumburg et al., 2012]. In further support of transmission of *S. aureus* from humans to apes, direct transmission was evident in one case as a veterinarian and a chimpanzee from the same sanctuary shared the same *spa*-type t279 (Table I). Considering that sanctuary veterinarians represent a small proportion of all personnel caring for sanctuary apes, frequent exchange of *S. aureus* between humans and great apes is likely the norm.

The high proportion of PVL positive isolates from chimpanzees further supports human-to-animal transmission of human-adapted *S. aureus,* as PVL is frequent in *S. aureus* from humans but uncommon in wildlife in sub-Saharan Africa [Breurec et al., 2011; Schaumburg et al., 2012]. Here, we used PVL as a marker for human *S. aureus* of African origin. To the best of our knowledge, no data are available on the pathogenic potential of PVL in great apes; however, in humans, PVL is associated with severe skin and soft-tissue infection and necrotizing disease such as necrotizing pneumonia [Lina et al., 1999]. In addition, it is a string cytotoxic factor for human neutrophils [Löffler et al., 2010].

Given the high percentage of chimpanzees with classical human STs, which all have been associated with disease development, reintroductions of chimpanzees carrying human *S. aureus* into areas inhabited by conspecifics have a high probability of exposing naïve wild great apes to pathogenic strains of *S. aureus*. Transmission is theoretically also possible through human–ape overlap associated with activities such as tourism and research; however, *S. aureus* transmission requires direct contact or contact with contaminated items. Although such conditions historically occurred at research and tourist sites, they have become rare as increased awareness of disease risks has led to policy changes at all sites reducing human–ape interaction.

Once established in a population, *S. aureus* would be impossible to monitor and control since symptoms cannot be observed in unhabituated individuals and daily nasal application of mupirocin ointment and antibiotic treatment over several days is not feasible at the population level. Further, clearing infected groups of apes prior to reintroduction is unrealistic since apes cannot adhere to the required hygiene regime for *S. aureus* decolonization. Strict health management would be required to ensure "human-pathogen-free" great ape groups designated for reintroduction. In such a scenario, candidate reintroduction apes could be screened for human *S. aureus* strains as well as other human-related pathogens. A prerelease quarantine could then be established and individuals who repeatedly test negative over several months could be considered at a lower risk for reintroduction. Apes colonized with human pathogens could be isolated and treated repeatedly, e.g. with antibiotics (tetracycline, rifampicin, cotrimoxazol, vancomycin) and only returned to the general sanctuary population once the infection has cleared. Such individuals could later be reconsidered to join the prerelease quarantine to advance toward reintroduction. A spontaneous decolonization without medical intervention might also occur in animals that are only intermittently colonized. Such individuals could also join the prerelease quarantine group. Such protocols are needed to assure that reintroductions adhere to the primary mandate of the IUCN reintroduction guidelines: it is critical that reintroduction efforts do not endanger resident wildlife populations [Beck et al., 2007].

Implementing such a process would be challenging since great ape social structure is complex and isolating individuals and changing group composition may result in aggression, anxiety, depression, and stress, which in return may alter temporarily an individual's immune function.

Further data are needed to understand carriage and disease development in sanctuary great apes to pinpoint the effective risk for wild populations. Also, guidelines for the management of sanctuary great apes carrying human *S. aureus* strains will need to be developed. However, *S. aureus* is just one out of many human pathogens that may be carried chronically by sanctuary apes. Data on the range of human pathogens carried by sanctuary great apes, an assessment of their impact on ape health, and an assessment of their potential to spread into wild populations are needed.

To pinpoint the spread of human *S. aureus* into wild populations through re-introduction, it will be important to track re-introduced great apes, wild individuals of groups joined by re-introduced apes, and neighboring great ape communities since pathogens may spread through immigrating individuals for all great ape species. Noninvasive tools for such studies are available [Schaumburg et al., 2012].

This is an empirical demonstration that ape reintroduction programs may pose a risk to wild populations. In addition to its pathogenic potential, *S. aureus* serves as an indicator of transmission potential for other human-adapted pathogens. In light of these findings, preparatory measures for ape reintroductions must be enforced and a cost benefit analysis should be made for each project before initiating reintroduction programs. In a world of ever-growing human-wildlife overlap, it is critical that pathogen-related risks for endangered wildlife populations are better understood and that innovative approaches are adopted to avoid introduction of such pathogens into wild populations.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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