DIVERSITY OF STAPHYLOCOCCAL SPECIES CULTURED FROM CAPTIVE LIVINGSTONE'S FRUIT BATS (*PTEROPUS LIVINGSTONII*) AND THEIR ENVIRONMENT

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Abstract: Livingstone's fruit bats (*Pteropus livingstonii*) are Critically Endangered and a captive population has been established as part of the IUCN Species Action Plan. The largest colony, in Jersey Zoo, was sampled for staphylococcal carriage and at infection sites, as disease associated with staphylococci had previously been found. Staphylococci were cultured from swabs from 44 bats (skin, oropharynx, mouth ejecta, skin lesions) and from their enclosure. The isolates were identified by MALDI-TOF; antimicrobial susceptibility testing was performed by disc diffusion and screening for *mecA* and *mecC*. Seventeen species of coagulase-negative staphylococci including *Staphylococcus xylosus*, *S. kloosii*, *S. nepalensis* and *S. simiae* were isolated. *Staphylococcus aureus* was identified from both carriage and lesional sites. These findings suggest *S. nepalensis* may be part of the normal carriage flora of bats. Antimicrobial resistance rates were low and MRSA was not identified. Sampling of mouth ejecta for staphylococci may provide results representative for carriage sites.

Keywords: Livingstone's fruit bat, MALDI-TOF, skin lesion, Staphylococci, *S. simiae*.

BRIEF COMMUNICATION

Conservation interventions to assist endangered species of wildlife often involve moving individuals from one place to another, sometimes with a period of captivity which may be very brief or encompass many generations.[15] It is important for the success of the intervention that the individual remains healthy and fit for eventual release, but also that the normal suite of colonising microorganisms associated with that individual remains unchanged.[6]

At Jersey Zoo (JZ) (Jersey, CI) as part of an International Union for Conservation of Nature (IUCN) Species Action Plan, a breeding colony of the Critically Endangered Livingstone's fruit bats (*Pteropus livingstonii*) was established between 1993 and 1995 by the capture of 17 wild bats from their native Comoros Islands.[9,17] Since then this founder population has bred, descendants have been transferred to, and returned from other zoos, principally Bristol Zoological Gardens, UK (BZG), and a group of around 45 bats was present at JZ during the study period.

Anecdotal clinical reports from JZ and BZG for the years 1993 to 2013 suggested that *Staphylococcus aureus* and other staphylococci had been involved in skin, soft tissue, bone and internal infections, some of which had resulted in death or euthanasia. Staphylococci are opportunistic pathogens and, in humans and dogs, it has been shown that the majority of infections involve endogenous strains that are carried by the infected individual on skin or mucosae.[16,21] Staphylococcal carriage is well documented in domesticated animal species but only in a few wildlife hosts.[3,7]

This study characterises the species and antimicrobial resistance patterns of staphylococci isolated from healthy and lesional skin, pharynx and mouth ejecta of captive Livingstone's fruit bats, and from environmental surfaces in their enclosure.

This study was approved by the Royal Veterinary College Clinical Research Ethical Review Board (CRERB URN 2015 1332). Livingstone's fruit bats and their enclosure at JZ (one large flight tunnel with a separate small hospital enclosure), were sampled on three occasions between 2014 and 2016.[22] Bats were sampled opportunistically by swabbing healthy ventral wing skin, oropharyngeal mucosae

in bats anaesthetised for other purposes, mouth ejecta and skin lesions, using cotton swabs dipped in tryptic soy broth (Oxoid™, Thermo Fisher Scientific Ltd, Basingstoke, Hampshire, RG24 8PW, UK) with 10% salt (Sigma-Aldrich, Gillingham, Dorset, SP8 4XT, UK) (TSB+). Environmental sites (food cups, ropes, flooring) were sampled by rubbing a TSB+-moistened cotton swab over an area approximately 3 cm^2 just before daily enclosure cleaning.

Swabs were incubated in TSB+, plated onto 5% sheep blood agar and up to five morphologically distinct staphylococcal-type colonies were tested for coagulase production by the slide coagulase test using rabbit plasma (Pro-Lab Diagnostics, Wirral, CH62 3QL, UK). Coagulase-negative isolates (CoNS) were identified using matrix assisted laser desorption/ionisation time of flight mass spectrometry (MALDI-TOF) (Idexx, Wetherby, LS22 7DN, UK). Antimicrobial susceptibility was determined in all isolates by disc diffusion for oxytetracycline (30 mg), amoxicillin (10 mg), clindamycin (2 mg), cephalexin (30 mg), enrofloxacin (5 mg), trimethoprim-sulfamethoxazole (25 mg), and in the CoNS for cefoxitin (30 mg) as an indicator for methicillin susceptibility (all discs from Oxoid™). Clinical and Laboratory Standards Institute (CLSI) clinical breakpoints were used as for CoNS and *S. aureus* from animals where available, otherwise as described by Carson (2012).[3,5] *S. aureus* isolates were confirmed by PCR demonstration of the *nuc* gene, their resistance to methicillin was screened using agar containing 2 mg/l oxacillin (ORSAB, plus selective supplement, Oxoid™) and the presence of *mecA* and *mecC* was determined in their genome sequences which had been obtained using Illumina Miseq sequencing (Illumina, California, 92122, US) as part of another continuing study.

A total of 91 swabs was taken from 44 different bats and from 19 environmental sites. Samples from bats included 62 swabs from carriage sites (skin n=42, oropharynx n=20), six from mouth ejecta and four from skin lesions. Swabs yielded 213 presumed staphylococcal isolates; of these, 145 were confirmed as belonging to seventeen species of CoNS by MALDI-TOF (Table 1). Four isolates were not *Staphylococcus*, five failed to regrow, 29 were below the manufacturer's cut-off point for MALDI-TOF identification and were excluded, and 30 coagulase-positive isolates were confirmed as *S. aureus*.

The skin lesions sampled in four individuals were: i) superficial acute necrotic dermatitis involving both wings of one bat, ii) superficial crusting dermatitis of an ear pinna, iii) deep pyoderma of a rostral mandible including osteomyelitis, and iv) an infected wing wound.

None of the *S. aureus* isolates showed phenotypic resistance to methicillin (screening agar) and none was found to carry *mecA* or *mecC.* All staphylococci were broadly susceptible to antimicrobials with only one isolate showing resistance to four antimicrobial classes, three isolates to three classes, and the remaining isolates to one or two classes. Resistance occurred most commonly to amoxicillin (n = 68, 38.9%), and 11 CoNS isolates were resistant to cefoxitin (7.6%).

Studies using the 16s rRNA gene to compare the skin microbiome of captive fruit bats and free-ranging insectivorous bats in different sites, and captive and free-ranging Tasmanian devils (*Sarcophilus harrisii*) have found that both the host species and the environment are important factors in determining the composition of the skin flora.[2,4,12] However, these studies did not specifically address the impact of pathogens such as staphylococci in captive populations. Staphylococcal faecal carriage in free-ranging straw-coloured fruit bats, and pharyngeal carriage in several bat species in Gabon have been described but this is the first report of the carriage of staphylococci on the wing, in the oropharynx and mouth ejecta of captive Livingstone's fruit bats, and of the staphylococci found in their enclosure.[1,8]

The data from this study show that *S. xylosus*, *S. nepalensis, S. saprophyticus,* and *S. aureus* were commonly present, and two species not previously described on bat skin were found: *S. nepalensis*, and *S. simiae*. *S. aureus* isolates were recovered from each of the four lesional swabs, suggesting that, as in other host species, this species has a significant role in skin and soft tissue infections in bats.

Sampling six mouth ejecta as described here yielded a substantial number of staphylococci which suggests this may provide representative results non-invasively and warrants further study.

Of the three species not previously identified from bats, *S. nepalensis* has been described in pneumonia in goats, in human clinical material, fermented fish, and associated with mature bat guano.[13,18,20,23] It has rarely been reported in mammalian skin carriage, but it is possible that in

previous reports it may have been misidentified as *S. xylosus* which is phenotypically nearly identical.[13] *S. simiae* was first isolated from the gastrointestinal tract of South American squirrel monkeys (*Saimiri sciureus*) and genomic analysis revealed a close relationship to *S. aureus*.[14,19] It has been isolated from fish and has been found to produce virulence factors but is otherwise little described.[11] *S. succinus* was only found in the environment in this study, so it cannot be definitively associated with Livingstone's bats. However, it was the predominant species recovered from small wild mammals in Europe, and vancomycin resistant isolates were recovered from free-ranging songbirds in America.[7,10]

In this colony of captive Livingstone's bats, the overall level of antimicrobial resistance was surprisingly low and is in contrast to other wildlife studies.[3] Clavulanic acid potentiated amoxicillin is the first line antimicrobial for sick bats in the colony, which may explain the finding of some resistance to amoxicillin in this study.

As in other host species, many different staphylococcal species were found on carriage sites of captive Livingstone's bats but *S. aureus* dominated on lesional skin. This suggests a similar opportunistic aetiology of staphylococcal skin infections as in other hosts and warrants investigations into bacterial complications when injuries or disease occur. The unique epidemiological setting of these captive bats provides further opportunity to study the origin and evolution of staphylococci in the context of some contact with humans and other wild and domestic animals.

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