Human-associated *Staphylococcus aureus* strains within great ape populations in Central Africa (Gabon)

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Abstract

The risk of serious infections caused by *Staphylococcus aureus* is well-known. However, most studies regarding the distribution of (clinically relevant) *S. aureus* among humans and animals took place in the western hemisphere and only limited data are available from (Central) Africa. In this context, recent studies focused on *S. aureus* strains in humans and primates, but the question of whether humans and monkeys share related *S. aureus* strains or may interchange strains remained largely unsolved. In this study we aimed to evaluate the distribution and spread of human-like *S. aureus* strains among great apes living in captivity. Therefore, a primate facility at the International Centre for Medical Research of Franceville (Gabon) was screened. We detected among the primates a common human *S. aureus* strain, belonging to the *spa*-type t148. It was isolated from three different individuals of the western lowland gorilla (*Gorilla gorilla gorilla*), of which one individual showed a large necrotizing wound. This animal died, most probably of a staphylococcal sepsis. Additionally, we discovered the t148 type among chimpanzees (*Pan troglodytes*) that were settled in the immediate neighbourhood of the infected gorillas. A detailed analysis by pulsed field gel electrophoresis showed that the gorilla and chimpanzee isolates represented two closely related strains. To our knowledge, this is the first report of a human-associated *S. aureus* strain causing disease in great apes. The simultaneous detection in gorillas and chimpanzees indicated an interspecies transmission of this *S. aureus* strain. Our results recommend that protection of wild animals must not only be based on habitat conservation, but also on the assessment of the risk of contact with human pathogens.

Keywords: Chimpanzee, colonization, furunculosis, gorilla, great apes, interspecies transmission, sepsis, species barrier, *Staphylococcus aureus*

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Introduction

Staphylococcus aureus is a leading human and veterinary pathogen. Its pathogenicity is based on a variety of virulence factors such as toxins and tissue-destroying enzymes [1]. *Staphylococcus aureus* causes a number of diseases ranging from local to systemic infections, which often lead to sepsis. In the last three decades, evolution of resistance, e.g. to methicillin, has become an enormous problem for treatment of *S. aureus* infections, turning this bacterium into one of the most prominent pathogens in hospital-associated infections [2].

In recent years, several studies have focused on animal colonization/infection by *S. aureus*, especially in pets [3,4] and farm animals [5,6]. In this context, a high prevalence of methicillin-resistant *S. aureus* (MRSA) and methicillin-sensitive *S. aureus* (MSSA) strains in livestock species, e.g. cattle and pigs, became prominent [7]. These studies clearly demonstrated the occurrence of interspecies transmission, which in turn highlights the possibility of zoonotic infections of humans by livestock-associated *S. aureus*. On the other hand, this also

opens the possibility of epidemics in animal populations caused by human-associated strains [8].

Recently, Schaumburg *et al.* [9] demonstrated that human-associated *S. aureus* strains are widely distributed on wild great apes. In contrast, in the same study, monkeys were mainly colonized by strains belonging to new multilocus sequence-types or *spa*-types, which had only rarely been isolated from humans [9]. However, our knowledge about interspecies transmission of pathogens from humans to primates is scarce.

In this study, the transmission of a widely distributed human-associated *S. aureus* strain (*spa*-type t148) and its species barrier transgression are described. This strain was able to colonize three individuals of a gorilla population; one of these gorillas died of an infection that was most likely caused by this strain, starting with a furuncle and ending in sepsis. A closely related variant of the same *spa*-type was also isolated from chimpanzees living in the direct neighbourhood of the infected gorilla, so that an interspecies transmission of the strain between the two great apes populations seems to be likely.

To our knowledge, this is the first report clearly demonstrating a direct correlation between a lethal infection of a great ape caused by a well-known and widely distributed human *S. aureus* strain and interspecies transmission of the causative bacteria.

Materials and Methods

Sampling

All individuals at the Centre of Primatology were routinely checked by a general medical examination, including biological and clinical diagnostics in November and December 2011. Primate samples were obtained non-invasively during routine health surveys, as nasal, oral, vaginal and rectal swabs; this procedure is not considered to be an animal experiment. The samples were immediately frozen at −80°C and afterwards delivered for bacteriological analysis. All samples were collected in accordance with international guidelines applied at the International Centre for Medical Research of Franceville Centre of Primatology.

Microbiological analyses

Isolated bacteria were cultivated on Columbia agar plates (Becton and Dickinson, Heidelberg, Germany), Mueller Hinton II (Difco, Detroit, MI, USA) or in tryptic soy broth (Oxoid, Wesel, Germany) at 37°C. Gram-positive and catalase-positive cocci were identified as *S. aureus* using BD Aureus select agar (Becton and Dickinson). Bacterial species identification was confirmed by standard slide tests for the clumping factor and the 4- to 24-h tube test for free coagulase in rabbit–citrate–plasma (Becton and Dickinson). *Staphylococcus aureus* ATCC 33592 (MRSA; American Type Culture Collection, Wesel, Germany) and *Staphylococcus epidermidis* DSM 20044 (German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany) served as positive and negative controls.

Molecular typing

Staphylococcus aureus isolates were discriminated by multilocus sequence-typing (MLST) [10], pulsed field gel electrophoresis (PFGE) [11] and *spa*-typing [12].

For *spa*-typing, five colonies were resuspended in 100 μL sterile water (*Ampuwa*, Fresenius, Bad Homburg, Germany). The cell suspension was heated for 10 min at 95°C. Cell debris was pelleted by centrifugation (5 min, 20 000 *g*, at room temperature). An appropriate volume of the resulting supernatant served as template for PCR. The repeat region of the *spa* gene was amplified by PCR [13]. Resulting PCR products were sequenced (Seqlab, Göttingen, Germany) and *spa*-types were determined using the StaphType software and the Ridom SpaServer (www.spaserver.ridom.de).

The MLST was performed as described previously [10]. Chromosomal DNA was purified using the PrestoSpinD BUG kit (Molzym, Bremen, Germany) according to the supplier's instructions. Cell lysis was achieved by supplementation of 10 μL lysostaphin (5 mg/mL; Genmedics, Reutlingen, Germany). Sequencing was performed by Seqlab (Göttingen, Germany). Analyses were performed employing the *S. aureus* MLST site (http://saureus.mlst.net/).

Pulsed-field gel electrophoresis was performed as previously described [11]. Briefly, chromosomal DNA was purified and digested using the restriction enzyme *Sma*I (Roche, Mannheim, Germany) and analysed on the DRIII contour-clamped homogeneous electric field system (Bio-Rad, Munich, Germany) by using pulsed field gel electrophoresis (1%; Bio-Rad), 6 V/cm, a field angle of 120°, a switch time of 5–15 s for 7 h, and a switch time of 15–60 s for a further 19 h. *Staphylococcus aureus* NCTC 8325 served as standard.

All strains were analysed for antimicrobial susceptibility by the agar disk-diffusion method including cefoxitin, oxacillin, ampicillin, ampicillin + sulbactam, cefazolin and penicillin (Oxoid). The latex-agglutination-based test systems (Oxoid) were used to test for production of the staphylococcal enterotoxins A, B, C and D and the toxic shock syndrome toxin (TSS). Expression of the exfoliative toxins A and B was detected by specific polyclonal antibodies in Ouchterlony immunodiffusion tests. The strains were tested for the presence of the *mecA* and the Panton–Valentine leukocidin encoding genes (*lukSF*)