Characterization of *Staphylococcus aureus* isolates from faecal samples of the Straw-Coloured Fruit Bat (*Eidolon helvum*) in Obafemi Awolowo University (OAU), Nigeria

Babatunji Akobi¹, Oladipo Aboderin², Takashi Sasaki³ and Adebayo Shittu¹*

**Abstract**

**Background:** Bats (*Chiroptera*) are one of the most diverse groups of mammals which carry out important ecological and agricultural functions that are beneficial to humans. However, they are increasingly recognized as natural vectors for a number of zoonotic pathogens and favourable hosts for zoonotic infections. Large populations of the Straw-Coloured Fruit Bat (*Eidolon helvum*) colonize the main campus of the Obafemi Awolowo University (OAU), Ile-Ife, Nigeria, but the public health implications of faecal contamination and pollution by these flying mammals is unknown. This study characterized *S. aureus* obtained from faecal samples of these migratory mammals with a view to determining the clonal types of the isolates, and to investigate the possibility of these flying animals as potential reservoir for zoonotic *S. aureus* infections.

**Results:** One hundred and seven (107) *S. aureus* isolates were recovered from 560 faecal samples in eleven roosting sites from January 2008 to February 2010. A large proportion of the isolates were susceptible to antibiotics, and molecular characterization of 70 isolates showed that 65 (92.9%) were assigned in coagulase type VI, while accessory gene typing classified 69 isolates into the following: type I (12; 17.1%), type II (3; 4.3%), type III (1; 1.4%) and type IV (53; 75.7%). On the whole, the isolates were grouped in five (A-E) main genotypes. Of the ten representative isolates selected for multilocus sequence typing (MLST), nine isolates were assigned with new sequence types: ST1725, ST1726, ST1727, ST2463-ST2467 and ST2470. Phylogenetic analysis provided evidence that *S. aureus* isolates in group C were closely related with ST1822 and associated clones identified in African monkeys, and group D isolates with ST75, ST883 and ST1223. The two groups exhibited remarkable genetic diversity compared to the major *S. aureus* clade.

**Conclusions:** Antibiotic resistance in faecal *S. aureus* isolates of *E. helvum* is low and multiple unique *S. aureus* lineages co-existed with *E. helvum*. The Straw-Coloured Fruit Bat in Ile-Ife, Nigeria is colonized predominantly by ST1725, ST1726, ST2463 and ST2470 with distinct genotypic characteristics that are rarely found in humans. This study has demonstrated on the possible existence of a reservoir of indigenous and anciently-divergent *S. aureus* clones among mammals in Africa.

**Keywords:** *Staphylococcus aureus*, *Eidolon helvum*, ST1725, ST1726, ST2463, ST2470, Anciently-divergent *S. aureus*
**Background**

Bats (Order: Chiroptera) are the only mammals capable of true sustainable flight and one of the most diverse and species rich mammals on earth [1]. They assist in the regulation of insect populations in their habitats, pollination of flowers and dispersal of seeds of economically important trees, and these ecological roles support forest regeneration and maintenance [2]. However, they roost near human habitation and their association with emerging infections has increased attention on these flying mammals as vectors of zoonotic pathogens [3-5]. The bat species *Eidolon helvum* is grouped under the suborder Megachiroptera, and it is the most widely distributed Straw-Coloured Fruit Bat which is found in the forest and savannah zones of sub-Saharan Africa [6,7]. The prime habitats for *E. helvum* are the tropical forest and typically roost in colonies on tall trees like *Eucalyptus saligna* and *Cocos nucifera* [8].

*Staphylococcus aureus* is part of the normal flora of the skin and mucous membrane of a wide variety of mammals and birds, and recent studies have indicated that animals could be a source of *S. aureus* infections in humans [9-11]. The main campus of the Obafemi Awolowo University, Ile-Ife (OAU) Nigeria, is colonized by a large population of *E. helvum* [12,13], but faecal contamination and pollution of the environment by these migratory mammals is a problem, moreover, the public health implications of their activities are not known. This study characterized *S. aureus* obtained from faecal samples of bats that colonize the main campus of the institution, with a view to understanding the clonal nature and diversity of the isolates, and to determine the possible risk of dissemination of *S. aureus* from bats to humans in the community through faecal shedding.

**Results and Discussion**

A total of 107 *S. aureus* isolates were obtained from 560 faecal samples of *E. helvum* based on phenotypic identification. Moreover, they were all genotypically confirmed by *hsp60* partial sequencing, and there was excellent agreement between the phenotypic and molecular methods in
the identification of the isolates. The number of samples and S. aureus isolates in each sampling site are indicated in Figure 1. Antibiotic susceptibility testing is paramount for monitoring resistance in commensal bacteria and various pathogens of clinical importance. In this study, all the isolates were susceptible to oxacillin, cefoxitin, tetracycline, chloramphenicol, gentamicin and mupirocin. However, four (3.7%) isolates were resistant to penicillin, while six (5.6%) and eight (7.4%) isolates were resistant to ciprofloxacin and erythromycin, respectively. None of the isolates exhibited inducible resistance however, 3.7% were constitutively resistant to clindamycin (Table 1). Studies have reported faecal carriage of methicillin-resistant S. aureus (MRSA) in animals [14,15]. However, MRSA was not detected in this study which is similar to recent reports on analysis of faecal samples from swine and feedlot cattle [16,17]. The low rate of resistance to different classes of antibiotics observed among the isolates in this study suggests that these migratory mammals may not have been exposed to the selective pressure of antimicrobial agents.

Molecular typing has been useful in understanding the epidemiology of S. aureus from animal and human hosts [18]. S. aureus is highly clonal in nature and though some are exclusively adapted to specific hosts [19], others are able to colonize multiple hosts [20-22]. Of the 107 S. aureus isolates, 70 (representing isolates obtained from faecal samples in the various sites) were randomly selected and further characterized. All the isolates were PVL-negative and 65 (92.9%) were grouped with coagulase (coa) type VI, but 5 (7.1%) were non-typeable. The accessory gene regulator (agr) typing classified 69 of the 70 isolates into the following: type I (12; 17.1%), type II (3; 4.3%), type III (1; 1.4%) and type IV (53; 75.7%). Based on their genotypic characteristics, ten representative isolates were selected for MLST and nine new sequence types: ST1725, ST1726, ST1727, ST2463-ST2467 and ST2470 were identified, and the sequences for the

| Antibiotics (disk content in μg) | Number of isolates | Resistance rate (%) |
|----------------------------------|--------------------|---------------------|
| Penicillin (10 units)            | 103                | 4                   |
| Oxacillin (1 μg)                 | 107                | 0                   |
| Cefoxitin (30 μg)                | 107                | 0                   |
| Erythromycin (15 μg)             | 99                 | 8                   |
| Clindamycin (2 μg)               | 103                | 4                   |
| Tetracycline (30 μg)             | 107                | 0                   |
| Ciprofloxacin (5 μg)             | 101                | 6                   |
| Chloramphenicol (30 μg)          | 107                | 0                   |
| Fusidic Acid (10 μg)             | 104                | 3                   |
| Gentamicin (10 μg)               | 107                | 0                   |
| Mupirocin (5 μg and 200 μg)      | 107                | 0                   |

S= Susceptible; R= Resistant.

ciprofloxacin and erythromycin, respectively. None of the isolates exhibited inducible resistance however, 3.7% were constitutively resistant to clindamycin (Table 1). Studies have reported faecal carriage of methicillin-resistant S. aureus (MRSA) in animals [14,15]. However, MRSA was not detected in this study which is similar to recent reports on analysis of faecal samples from swine and feedlot cattle [16,17]. The low rate of resistance to different classes of antibiotics observed among the isolates in this study suggests that these migratory mammals may not have been exposed to the selective pressure of antimicrobial agents.

Molecular typing has been useful in understanding the epidemiology of S. aureus from animal and human hosts [18]. S. aureus is highly clonal in nature and though some are exclusively adapted to specific hosts [19], others are able to colonize multiple hosts [20-22]. Of the 107 S. aureus isolates, 70 (representing isolates obtained from faecal samples in the various sites) were randomly selected and further characterized. All the isolates were PVL-negative and 65 (92.9%) were grouped with coagulase (coa) type VI, but 5 (7.1%) were non-typeable. The accessory gene regulator (agr) typing classified 69 of the 70 isolates into the following: type I (12; 17.1%), type II (3; 4.3%), type III (1; 1.4%) and type IV (53; 75.7%). Based on their genotypic characteristics, ten representative isolates were selected for MLST and nine new sequence types: ST1725, ST1726, ST1727, ST2463-ST2467 and ST2470 were identified, and the sequences for the

| Allele | ArcC, aroE, gIpf, gmk, pta, tpi, yqiL | MLST (ST) |
|--------|--------------------------------------|-----------|
| 1-13-84-1-12-5-11 (ST1725) | 14 (20) |
| 1-13-84-1-184-5-11 (ST1726) | 21 (30) |
| 193-245-227-136-185-5-11 (ST1727) | 01 (1.4) |
| 211-305-248-189-266-202-186 (ST2464) | 01 (1.4) |
| 211-305-248-189-195-202-275 (ST2465) | 01 (1.4) |
| 270-307-304-143-195-202-276 (ST2466) | 01 (1.4) |
| 271-356-248-189-267-202-186 (ST2467) | 01 (1.4) |
| 09 (12.9) |
| 01 (1.4) |
| 01 (1.4) |
| 70 (100) |

NT: Non-typeable.
coa: coagulase gene.
agr: accessory gene regulator.
All the isolates were PVL negative.
Housekeeping genes have been deposited in the MLST database (http://www.mlst.net), while one representative isolate (Q22) was assigned with ST15. Overall, the 70 isolates were assigned into five main genotypes A to E (Table 2).

As shown in Figure 2, there was a clear phylogenetic out-group among the *S. aureus* taxon consisting of isolates in the *hsp60*-allele types C and D, which suggests that these genotypes diverged long before clones belonging to the major *S. aureus* clades exhibited the current size of genetic divergence. Moreover, based on concatenated sequences of seven genes used in MLST, isolates in *hsp60*-allele type C were closely related with *S. aureus* ST1822 and associated clones, and type D isolates with ST75, ST883 and ST1223 (Figure 3). We have tentatively designated these isolates as anciently-diverged *S. aureus*. Some studies had previously reported that divergent *S. aureus* ST75 (*agr* type I) and ST883 (*agr* type IV) originated in northern Australia, while ST1223-related clones were found in South East Asia [23-25]. Moreover, *S. aureus* isolates assigned with ST1822-related clones have been identified in African monkeys [26]. In this study, we identified divergent clones (ST2463-ST2467, ST2470) among Straw-Coloured Fruit Bats in Nigeria, which suggests that anciently-diverged *S. aureus* have not only been distributed in Australia and South East Asia, but also among mammals in Africa. These lineages evolved independently from major *S. aureus* populations over an extended period of time, and may be a new subspecies of *S. aureus*. A recent study had reported that chromosomal recombination had occurred at *coa* and *agr* loci at a uniform rate [27]. Therefore, it is difficult to identify the prototype of these genes. The *agr* type I or IV and the *coa* type VI, which were found most frequently in the anciently-diverged *S. aureus* isolates, may be the closest relation to the origin of *agr* and *coa* genes, respectively.

**Conclusions**

This study isolated *S. aureus* from faecal samples of *E. helvum*, a migratory mammal with an abundant population in OAU, Ile-Ife, Nigeria, and represents the first molecular study on *S. aureus* colonization of bats in Africa. The isolates were largely susceptible to a number of antibiotics. The combination of coagulase gene type VI and *agr* type IV are rare among *S. aureus* isolates associated with humans [28-31], and the evidence that
isolates in group C were closely related with divergent ST1822-related clones identified in African monkeys, and group D isolates with ST75, ST883 and ST1223 indicate that there is the possible existence of a reservoir of indigenous and anciently-diverged clones among mammals in Africa.

Methods

Sample sites
A total of eleven roosting sites located in the academic area and the students’ hostel in OAU, Ile-Ife were identified for the study (Figure 1), and the duration for sample collection was from January 2008 to September 2008, February to May 2009, and February 2010. The faecal samples were obtained once a month in a designated sampling site between 6-7am by a non-invasive method in which three sterilized piece (36 × 45 inches) of cotton material were spread under the roosting trees. Fresh faecal samples were collected with sterile swab sticks and conveyed promptly to the Department of Microbiology Laboratory (OAU) for microbiological analysis.

Isolation and identification of S. aureus isolates
The swab stick was inserted into a test tube containing 3 ml of sterile nutrient broth (Biolab, supplied by Merck, Johannesburg, South Africa), swirled briefly to discharge the contents into the medium, and the culture was incubated at 37°C overnight. Thereafter, a loopful was streaked on mannitol salt agar (MSA) (Biolab, supplied by Merck, Johannesburg, South Africa) and incubated at

Figure 3 Phylogenetic tree based on concatenated arcC, aroE, glpF, gmk, pta, tpi and yqiL sequences of representative S. aureus isolates (F10, AC19, R5, AC10, F9, P1, Q15, R3, F16 and Q22). This tree was constructed by the neighbor-joining method, using MEGA ver. 5.05.
37°C for 48 hours. Preliminary identification of *S. aureus* was based on positive Gram stain, and positive results for catalase, coagulase (tube method) and DNase tests. The procedure described previously [32] was employed for DNA isolation. In summary, a single colony was suspended to a McFarland 1.0 standard in 100 μl of TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) with 10 U of achromopeptidase (Wako Chemical, Co. Ltd.), and the suspension was incubated at 55°C for 10 min. The supernatant was used as crude DNA for PCR. Molecular identification and confirmation of the isolates was based on sequencing analysis of the *hsp60* gene as previously reported [33]. PCR products were sequenced by using a Big Dye Terminator (version 3.1) cycle sequencing kit (Applied Biosystems, Foster City, CA) with an ABI Prism 3100 genetic analyzer (Applied Biosystems).

**Antibiotic susceptibility testing**

The susceptibility testing of the isolates to 11 antibiotics was performed using the disk diffusion method and the following antibiotics were tested: penicillin (10 units), oxacillin (1 μg), cefoxitin (30 μg), erythromycin (15 μg), clindamycin (2 μg), tetracycline (30 μg), ciprofloxacin (5 μg), chloramphenicol (30 μg), fusidic acid (10 μg) gentamicin (10 μg) and mupirocin (5 μg and 200 μg). *S. aureus* ATCC 25923 was the control strain for the susceptibility testing. The result was interpreted as resistant or susceptible based on the interpretative standard according to the Clinical Laboratory Standards Institute (CLSI) manual for bacterial isolates from animals [34]. Interpretative zone diameter for resistance and susceptibility breakpoints to fusidic acid and mupirocin which are not stated in the CLSI guidelines were considered as described previously [35,36]. The D-test for determining inducible resistance of clindamycin using erythromycin was performed. A truncated or blunted clindamycin zone of inhibition (D-Shape) indicated inducible resistance. Constitutive resistance was recognized by a clindamycin zone diameter of ≤14 mm [37].

**Molecular characterization of the *S. aureus* isolates**

Characterization of 70 isolates was determined by detection of the Panton Valentine Leukocidin (PVL) gene [38], and or *aroE* and glpF genes using the standard MLST primers. Therefore degenerate primers CC75dege-aroE-F (5′-WTGCACTWTHGGGWRYYCC-3′), CC75dege-aroE-R (5′-GGWWTATAAAYAARTT CACT-3′), CC75aroEseq-F (5′-CCAATTTGAACATCCTTATC-3′), CC75dege-glPF-F (5′-GCGWGAATTYHT DGGWACWG-3′), CC75dege-glPF-R (5′-ATWGYGYY AAWTGHCAATGWGC′), and CC75glpF-seq-R (5′-GCAT GTGCAATTCTTGDDC′), were designed by multiple alignment of amino acid sequences of each gene with complete genomes of *S. aureus*, *S. epidermidis*, *S. haemolyticus* and *S. lugdunensis* from the KEGG database (http://www.genome.jp/kegg/). Sequences of *arcC*, *aroE*, glpF, gmk, pta, tpi and yqiL in *S. simiae*, which was used as an outgroup, were obtained from the draft genome sequence of *S. simiae* CCM7213 [43]. A phylogenetic tree was constructed based on concatenated *arcC*, *aroE*, glpF, gmk, pta, tpi and yqiL sequences using the neighbor-joining method, using MEGA ver. 5.05.

**Abbreviations**

OAU: Obafemi Awolowo University; PVL: Panton Valentine Leukocidin; Agr: Accessory gene regulator; Coag: Coagulase; MLST: Multilocus sequence typing; ST: Sequence type; *E. helvum*: Eidolon helvum; *S. aureus*: Staphylococcus aureus; MSA: Mannitol salt agar; DNase: Deoxyribonuclease; CLSI: Clinical Laboratory Standards Institute; MRSA: Methicillin resistant *Staphylococcus aureus*.

**Competing interests**

The authors declare that they have no competing interest.

**Authors’ contributions**

AS, OA, TS conceived the study, BA conducted the sample collection, AS, OA, TS carried out the molecular characterization. All authors read and approved the final version of the manuscript.

**Author details**

1. Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria.
2. Department of Medical Microbiology and Parasitology, Obafemi Awolowo University, Ile-Ife, Nigeria.
3. Laboratory of Bacterial Genomics, Pathogen Genomics Center, National Institute of Infectious Diseases, Tokyo, Japan.

**Received**: 26 June 2012 **Accepted**: 16 November 2012

**Published**: 26 November 2012

**References**

1. Eick GN, Jacobs DS, Matthee CA: A Nuclear DNA Phylogenetic Perspective on the Evolution of Echolocation and Historical Biogeography of Extant Bats (Chiroptera). *Mol Biol Evol* 2005, 22:1869–1886.
2. Mildenstein T, de Jong C: Natural history, ecology and socio-economic value of bats. In Investigating the Role of Bats in Emerging Zoonoses: Balancing Ecology, Conservation and Public Health. Interest. Edited by Newman SH, Field HE, de Jong CE, Epstein JH. Rome: FAO Animal Production and Health Manual No. 12; 2011:15–28.
3. Hayman DTS, Suu-Ire R, Breed AC, McEachern JA, Wang L, Wood JLN. Cunningham AA: Evidence of henipavirus infection in West Africa Fruit Bats. *PLoS One* 2008, 23:e2759.
4. Mühldorfer K, Wibbelt G, Haensel J, Riehm J, Speck S: Yersinia species isolated from Bats, Germany. Emerg Infect Dis 2010, 16:578–580.

5. Drexler JF, Corman VM, Müller MA, Maganga GD, Vallo P, Binger T, Giroz-Rausch F, Rasche A, Yordanov S, Seebens A, Oppong S, Adu Sarkodie Y, Pongombo C, Lukashev AN, Schmidt-Chanasit J, Stöcker A, Carneiro AJ, Erbar S, Maisner A, Fronhoffs F, Buettner R, Kalko EK, Krupa T, Frank CR, Kallies R, Vandeker EK, Henler G, Bausken C, Hassanin A, Krüger DH, Matthee S, Ulrich RG, Leroy EM, Drosten C: Bats host major mammalian paramyxoviruses. Nat Commun 2012, 3:396.

6. DeFrees SL, Wilson DE: Edidion helvum. Mammm Species 1988, 312:1–5.

7. Mickleburgh SP, Hutson AM, Racey PA: Old World fruit bats. An action plan for their conservation. Gland, Switzerland: IUCN. 1992.

8. Jones C: Comparative ecology of three pteropid bats in Rio Muni, West Africa. J Zool 1972, 167:353–370.

9. van Cleef BAGL, Monnet DL, Voss A, Krozinvak E, Allenger FB, Struelens M, Zemlickova H, Skov RL, Vuopio-Varkila J, Gland, Switzerland: IUCN; 1992.

10. van der Meer-Marquet N, François P, Dommeril-Valentin AS, Coulomb F, Decruex C, Hombrock-Aller C, Leihani O, Neivee C, Ratovohny D, Schrenzel J, Roland Q, Bloodstream Infection Study Group of Réseau des Hygiénistes

11. Jones C: Comparative ecology of three pteropid bats in Rio Muni, West Africa. J Zool 1972, 167:353–370.

12. van der Meer-Marquet N, François P, Dommeril-Valentin AS, Coulomb F, Decruex C, Hombrock-Aller C, Leihani O, Neivee C, Ratovohny D, Schrenzel J, Roland Q, Bloodstream Infection Study Group of Réseau des Hygiénistes

13. van der Meer-Marquet N, François P, Dommeril-Valentin AS, Coulomb F, Decruex C, Hombrock-Aller C, Leihani O, Neivee C, Ratovohny D, Schrenzel J, Roland Q, Bloodstream Infection Study Group of Réseau des Hygiénistes

14. van der Meer-Marquet N, François P, Dommeril-Valentin AS, Coulomb F, Decruex C, Hombrock-Aller C, Leihani O, Neivee C, Ratovohny D, Schrenzel J, Roland Q, Bloodstream Infection Study Group of Réseau des Hygiénistes

15. van der Meer-Marquet N, François P, Dommeril-Valentin AS, Coulomb F, Decruex C, Hombrock-Aller C, Leihani O, Neivee C, Ratovohny D, Schrenzel J, Roland Q, Bloodstream Infection Study Group of Réseau des Hygiénistes

16. van der Meer-Marquet N, François P, Dommeril-Valentin AS, Coulomb F, Decruex C, Hombrock-Aller C, Leihani O, Neivee C, Ratovohny D, Schrenzel J, Roland Q, Bloodstream Infection Study Group of Réseau des Hygiénistes

17. van der Meer-Marquet N, François P, Dommeril-Valentin AS, Coulomb F, Decruex C, Hombrock-Aller C, Leihani O, Neivee C, Ratovohny D, Schrenzel J, Roland Q, Bloodstream Infection Study Group of Réseau des Hygiénistes

18. van der Meer-Marquet N, François P, Dommeril-Valentin AS, Coulomb F, Decruex C, Hombrock-Aller C, Leihani O, Neivee C, Ratovohny D, Schrenzel J, Roland Q, Bloodstream Infection Study Group of Réseau des Hygiénistes

19. van der Meer-Marquet N, François P, Dommeril-Valentin AS, Coulomb F, Decruex C, Hombrock-Aller C, Leihani O, Neivee C, Ratovohny D, Schrenzel J, Roland Q, Bloodstream Infection Study Group of Réseau des Hygiénistes

20. van der Meer-Marquet N, François P, Dommeril-Valentin AS, Coulomb F, Decruex C, Hombrock-Aller C, Leihani O, Neivee C, Ratovohny D, Schrenzel J, Roland Q, Bloodstream Infection Study Group of Réseau des Hygiénistes

21. van der Meer-Marquet N, François P, Dommeril-Valentin AS, Coulomb F, Decruex C, Hombrock-Aller C, Leihani O, Neivee C, Ratovohny D, Schrenzel J, Roland Q, Bloodstream Infection Study Group of Réseau des Hygiénistes

22. van der Meer-Marquet N, François P, Dommeril-Valentin AS, Coulomb F, Decruex C, Hombrock-Aller C, Leihani O, Neivee C, Ratovohny D, Schrenzel J, Roland Q, Bloodstream Infection Study Group of Réseau des Hygiénistes
susceptible clones of Staphylococcus aureus. J Clin Microbiol 2000, 38:1008–1015.

43. Suzuki H, Lefèbure T, Bitar PP, Stanhope MJ: Comparative genomic analysis of the genus Staphylococcus including Staphylococcus aureus and its newly described sister species Staphylococcus simiae. BMC Genomics 2012, 13:38.

doi:10.1186/1471-2180-12-279

Cite this article as: Akobi et al.: Characterization of Staphylococcus aureus isolates from faecal samples of the Straw-Coloured Fruit Bat (Eidolon helvum) in Obafemi Awolowo University (OAU), Nigeria. BMC Microbiology 2012 12:279.