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Clonal diversity of methicillin-sensitive *Staphylococcus aureus* from South Australian wallabies

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ABSTRACT

Seven methicillin-sensitive *Staphylococcus aureus* nasal isolates from apparently healthy captive and wild wallabies were characterised by DNA microarray and antibiotic susceptibility assays. Isolates were found to belong to uncommon clonal complexes including those previously associated with birds, pigs and humans.

Staphylococcus aureus is a versatile bacterium which can infect or colonise a variety of mammals, including humans. With the use of molecular techniques, *S. aureus* strains can be differentiated assigning them to clonal complexes (CC) [1]. Clonal complexes group bacterial isolates from the same species based on the genetic variation present in seven housekeeping genes. This variation can be used to infer the relationship of all isolates within a particular CC to a common ancestor [2]. Some CCs have been identified predominantly in specific hosts, such as CC692 in birds whereas others, such as CC15, are less specific [3,4].

Here, anterior nasal swabs were collected from 68 captive and 30 free-ranging wallabies (*Petrogale lateralis*, *Petrogale xanthopus* and *Macropus eugenii*) at two locations in South Australia, the Monarto Zoo (a 1000 ha open range zoo) and the Anangu Pitjantjatjara Yankunytjatjara Lands (a lightly populated remote indigenous land), during routine health examinations between July 2009 and October 2010. From these 98 nasal swabs, a total of seven *S. aureus* isolates were identified from five captive animals and two free-ranging animals (Table 1). Isolates were identified with a combination of biochemical assays and 16S rRNA sequence analysis [5]. Antimicrobial susceptibility tests revealed that four strains were susceptible to every antimicrobial agent tested [5,6]. Antibiotics tested included β-lactams, aminoglycosides, macrolide, glycopeptide, cephalosporin, tetracycline and chloramphenicol. Three strains, A7, A8 and A78, exhibited ampicillin and penicillin resistance with A8 also demonstrating intermediate resistance towards cefotaxime (Table 1).

Molecular characterisation of the *S. aureus* isolates was performed by DNA microarray analysis (StaphyType, Alere Technologies, Jena, Germany) using previously described protocols and modified to include probes for *mecC* and the SCC*mec*-XI-associated *blaZ* allele [7]. The assignment of isolates to a CC was determined by an automated comparison of hybridisation profiles to reference profiles [1]. Analyses identified a single CC692 strain, three CC49 and three CC15 strains. All strains demonstrated the absence of methicillin-resistance genes and were thus classified as methicillin-sensitive *S. aureus* (MSSA). Microarray analysis also revealed that these strains possessed limited antimicrobial resistance determinants confirming the above-mentioned phenotypic analyses, with the *bla* operon being the only one identified (Table 1).

Virulence genes common in staphylococci including those encoding enterotoxins and exfoliative toxins (*eta*, *etb* and *etd*), epidermal cell differentiation inhibitors (*edinA*, *edinB* and *edinC*), toxic shock syndrome toxin (*tst*) and the Panton–Valentine leucocidin toxin (*lukF*-PV and *lukS-PV*), were not found. These data combined with veterinary records which indicated that these animals were in a non-diseased state support the *S. aureus* status as commensal organisms in this population. All isolates carried a variety of genes encoding proteins associated with adherence to host structures. The presence of these genes as well as their assignment to allelic variants depended on CC affiliation and not on host species as there was no difference to previously described isolates of the same CCs from other hosts [1,8,9].

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Table 1
Microbiological characteristics of commensal MSSA in the nasal passages of South Australian wallabies [b].

				Antibiotic susceptibility profile[a]											Microarray-based analysis
Isolate	Wallaby species	Captivity status	Clonal complex	AMP	OX	PEN	AMC	FOX	CN	S	VA	CTX	TE	E	C	Resistance genes
A7	BFRW	Free-ranging	CC15	R	-	R	-	-	-	-	-	-	-	-	-	*blaZ*, *blaI*, *blaR1*
A8	BFRW	Free-ranging	CC15	R	-	R	-	-	-	-	-	iR	-	-	-	*blaZ*, *blaI*, *blaR1*
A78	BFRW	Captive	CC15	R	-	R	-	-	-	-	-	-	-	-	-	*blaZ*, *blaI*, *blaR1*
A70	BFRW	Captive	CC49	-	-	-	-	-	-	-	-	-	-	-	-	None detected
A73	BFRW	Captive	CC49	-	-	-	-	-	-	-	-	-	-	-	-	None detected
M9	YFRW	Captive	CC49	-	-	-	-	-	-	-	-	-	-	-	-	None detected
M7	YFRW	Captive	CC692	-	-	-	-	-	-	-	-	-	-	-	-	*fosB*

Abbreviations: BFRW, black-flanked rock wallaby (*Petrogale lateralis*); YFRW, yellow-footed rock wallaby (*Petrogale xanthopus*); −, sensitive; R, resistant; iR, intermediate resistant; AMP, ampicillin; OX, oxacillin; PEN, penicillin; AMC, amoxicillin-clavulanic acid; FOX, cefoxitin; CN, gentamicin; S, streptomycin; VA, vancomycin; CTX, cefotaxime; TE, tetracycline; E, erythromycin; C, chloramphenicol.

[a] Disk diffusion breakpoints were determined as previously described [5].

[b] Data pertaining to antibiotic susceptibility profiles and isolate captivity status have been previously published [5] but have been included here to aid the reader in analysis.

To date, CC692 has been isolated exclusively from various birds and, as such, to the best of our knowledge, this represents the first report of CC692 from any mammalian species [4]. For CC49-MSSA, only a few human and veterinary cases have been reported from Western Europe. CC49-MRSA were observed in pigs and rats (the latter being *mecC*-positive) [9,10]. CC15-MRSA is very common in humans; studies from Europe showed it to be one of the most prevalent lineages [8]. Isolates of CC15 were an unexpected finding in two free-ranging wallabies and a single captive wallaby. These three CC15 strains had very similar hybridisation profiles to the 115 *S. aureus* CC15 strains examined from asymptomatic human carriers in Germany, including the presence of immune evasion genes *chp* and *scn* and the absence of staphylokinase gene *sak*, both considered typical qualities of this lineage [3]. This provides further support to the notion that these strains are commensal organisms and are not involved in disease. Since immune evasion cluster genes are apparently host-specific and their carriage might rapidly change in adaption to a new host, this observation might suggest a recent transmission of CC15 from humans to wallabies.

This study describes the first genotyping data on commensal *S. aureus* from South Australian native wallabies. Results indicate that wallabies did not harbor unique host-specific strains as the three identified CCs have been described in other species. Reassuringly, resistance genes were rare with no MRSA recovered amongst the seven isolates from apparently healthy wallabies. The absence of both common virulence genes in conjunction with resistance genes provides further confirmation to support these strains status as commensal organisms. Additionally, given the *S. aureus* genotypes are typically associated with humans and birds, interspecies transmission from non-macropod hosts cannot be ruled out. These findings also raises questions about which genotypes can be considered the indigenous flora of wallabies and there was no evidence for a zoonotic background of particular “Australian” clones of *S. aureus* such as ST93 and ST1850.

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