Infection, Genetics and Evolution 22 (2014) 208–215

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Global distribution and diversity of ovine-associated *Staphylococcus aureus* ☆

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ARTICLE INFO

Article history:
Available online 12 September 2013

Keywords:
S. aureus
Ovine
Caprine
MLST
spa typing
Global diversity

ABSTRACT

Staphylococcus aureus is an important pathogen of many species, including sheep, and impacts on both human and animal health, animal welfare, and farm productivity. Here we present the widest global diversity study of ovine-associated *S. aureus* to date. We analysed 97 *S. aureus* isolates from sheep and sheep products from the UK, Turkey, France, Norway, Australia, Canada and the USA using multilocus sequence typing (MLST) and *spa* typing. These were compared with 196 sheep isolates from Europe ($n = 153$), Africa ($n = 28$), South America ($n = 14$) and Australia ($n = 1$); 172 bovine, 68 caprine and 433 human *S. aureus* profiles. Overall there were 59 STs and 87 *spa* types in the 293 ovine isolates; in the 97 new ovine isolates there were 22 STs and 37 *spa* types, including three novel MLST alleles, four novel STs and eight novel *spa* types. Three main CCs (CC133, CC522 and CC700) were detected in sheep and these contained 61% of all isolates. Four *spa* types (t002, t1534, t2678 and t3576) contained 31% of all isolates and were associated with CC5, CC522, CC133 and CC522 respectively. *spa* types were consistent with MLST CCs, only one *spa* type (t1403) was present in multiple CCs. The three main ovine CCs have different but overlapping patterns of geographical dissemination that appear to match the location and timing of sheep domestication and selection for meat and wool production. CC133, CC522 and CC700 remained ovine-associated following the inclusion of additional host species. Ovine isolates clustered separately from human and bovine isolates and those from sheep cheeses, but closely with caprine isolates. As with cattle isolates, patterns of clonal diversification of sheep isolates differ from humans, indicative of their relatively recent host-jump.

1. Introduction

Staphylococcus aureus is widely recognised as a bacterial species that can colonise and infect a variety of hosts including humans, farmed and companion animals and exotic species (Cookson et al., 2007; Espinosa-Gongora et al., 2012; Porrero et al., 2012; Sasaki et al., 2012; Smith et al., 2005a). Multilocus sequence typing (MLST) has become the classical technique for analysis of bacterial population structure, and has been used extensively in the analysis of *S. aureus* populations from a variety of human and animal sources (Enright et al., 2000; Espinosa-Gongora et al., 2012; Sasaki et al., 2012; Smith et al., 2005b; Smyth et al., 2009). Animal isolates of *S. aureus* are commonly assigned to host-specific clonal complexes (CCs) including CC97 in cattle and CC133 in sheep (Smith et al., 2005b; Smyth et al., 2009). However, the discriminatory ability of MLST is low when compared with other techniques such as PFGE or *spa* typing. Whilst PFGE is laborious and can lack reproducibility, *spa* typing is based on sequence data (Harmsen et al., 2003; Shopsin et al., 1999) and is reproducible and comparable between studies. In addition, *spa* types associate with MLST CCs (Strommenger et al., 2006), hence *spa* typing is often used as an initial screen of study isolates (Eriksson et al., 2013; Porrero et al., 2012).

Notwithstanding the premise of host-specificity, the zoonotic transmission of *S. aureus* from livestock to humans has received a great deal of attention recently (Fitzgerald, 2012; Fluit, 2012; Lamamy et al., 2013; Pantosti, 2012; Price et al., 2012; Verkade and Kluytmans, 2013), and is being reported increasingly (Garcia-Alvarez et al., 2011; Petersen et al., 2013). Of particular concern is the zoonotic transmission of methicillin-resistant *S. aureus* (MRSA) that has occurred in Europe over the last decade, and the suggestion that some livestock associated strains might have the ability to colonise and infect humans (Garcia-Alvarez et al., 2011).

The ability of *S. aureus* to switch hosts has contributed to its ubiquity in human and veterinary medicine. Recently it has been estimated that individual *S. aureus* lineages switched from human to bovid hosts (cattle, sheep and goats) at different times. The earliest switch was approximately 5429 years ago and resulted in the

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http://dx.doi.org/10.1016/j.meegid.2013.09.008

bovine-associated CC151 and ovine-associated CC130 (Weinert et al., 2012). Additional lineages have arisen in both cattle and sheep since this initial host switch, the timing of which coincides with historical estimates of domestication events (Guinane et al., 2010; Weinert et al., 2012). Also two *S. aureus* host back-jumps are proposed; that is two lineages of *S. aureus* (ST59/966/754 and ST25 in CC151 and CC97 respectively) that switched from human to bovine hosts that have now independently switched back to human hosts (Weinert et al., 2012). It is therefore highly plausible that additional back-jumps from bovine and other species could occur in the future. This may be more likely in the developing world where there is more frequent contact between humans and their animals than in more developed regions. However, this may be influenced by animal numbers. In developed regions the time spent with individual animals may be less, but the pathogen load may be higher (because of higher animal numbers). Studies of farm personnel indicate that there is zoonotic transmission of *S. aureus*, because farm workers are often colonised by the same *S. aureus* MLST or *spa*-type as found in livestock (Cui et al., 2009; Spohr et al., 2011), although this is not always the case (Smith et al., 2005a).

In dairy and suckler ewes (ewes rearing lambs for meat production) *S. aureus* is a major cause of clinical mastitis, with a reported annual incidence rate of 0–6.6% (Arsenault et al., 2008). Both clinical and subclinical intramammary infections reduce farm profitability, and impact on ewe health and welfare, and lamb growth rates. Mastitis has been estimated to cost £8.40 per ewe in the UK (Conington et al., 2008). With an estimated national flock of 14.8 million breeding ewes (EBLEX, 2012), this results in a potential cost to the UK sheep industry in excess of £120 M/annum. Infections are treatable with antibiotics but the mammary gland rarely returns to full function, and infection will often result in the formation of intra-mammary abscesses. Asymptomatic carriage of *S. aureus* occurs in the nares, vagina and on skin, and these sites can act as potential reservoirs of infection (Mørk et al., 2012) making prevention of disease difficult. In addition, the related subspecies, *S. aureus* subsp. *anaerobius* causes Morel's disease in sheep, a condition that leads to the formation of abscesses close to, or within, superficial lymph nodes (de la Fuente et al., 2011; Elbir et al., 2010).

Until recently, few studies focused on the analysis of ovine strains of *S. aureus*, and those that have, characterised isolates from relatively restricted geographical regions. This provides detailed information about the strains circulating within a region, but little information on the spread and diversity of global populations of *S. aureus* that colonise and infect sheep. The aim of the current study was to characterise the diversity of *S. aureus* in sheep and sheep cheese by examining 97 new isolates from the UK, Turkey, France, Norway, Australia, Canada and the USA, together with 196 existing ovine profiles from Africa, Australasia, Europe and S. America, to investigate the diversity and spread of global ovine isolates, and how they compare to strains from other hosts.

2. Methods

2.1. The ovine dataset, source of isolates

A total of 97 *S. aureus* isolates from sheep/ovine cheese were analysed in this study, this included 24 isolates from clinical mastitis, subclinical intra-mammary infections (IMI) and intra-mammary abscesses of sheep in England, 11 from cases of clinical mastitis in Australia, one from a severe case of clinical mastitis in Canada, 12 from cases of clinical mastitis, subclinical IMI, gangrenous mastitis, intra-mammary abscesses and carriage in France, 13 from cases of clinical mastitis, subclinical IMI and carriage in Norway (Mørk et al., 2012; 2007), three from subclinical IMI in the USA (Spanu et al., 2011) and 33 isolates from sheep milk cheeses in Turkey (Ertas et al., 2010), The strains used are described in Supplementary dataset 1.

Cultures from England were isolated as described previously (Smith et al., 2011), and confirmed as *S. aureus* by positive tube coagulase test result and *nuc* gene amplification (Brakstad et al., 1992). All isolates supplied from elsewhere were checked for purity and confirmed as *S. aureus* as above; where only DNA was provided, positive *nuc* gene amplification was used to verify the isolate was *S. aureus*.

2.2. DNA extraction and multi-locus sequence typing from the ovine dataset

DNA was extracted using the NucleoSpin Tissue Kit (Machery-Nagel GmbH & Co. KG, Düren, Germany). MLST and *spa* typing were performed as described previously (Harmsen et al., 2003; Shopsin et al., 1999; Smith et al., 2005a; 2005b). Briefly, for MLST, the primers of Enright et al. (2000) were used to amplify seven gene fragments (*arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi* and *yqiL*), and raw PCR products were shipped to LGC genomics (Berlin, Germany) for purification and sequencing using forward and reverse primers. Sequence files were aligned and manually edited, with allele number and sequence types (STs) assigned using the *S. aureus* MLST website (http://saureus.mlst.net/, last accessed 27th March 2013). Novel allele trace files and allelic profiles of novel STs were sent to the database curator for allele or ST assignment and entry into the database. For *spa* typing, primers 1095F and 1517R (Harmsen et al., 2003; Shopsin et al., 1999) were used to amplify the polymorphic x region of the *spa* gene in all isolates. PCR products were sequenced as described above and sequence files manually aligned. *spa* types were assigned using DNAGear (AL-Tam et al., 2012), and novel types submitted to the Ridom SpaServer database (http://spa.ridom.de/submission.shtml, last accessed 7th August 2012) for *spa* type assignment.

2.3. Construction of the dataset of all S. aureus profiles

In addition to the MLST and *spa* profiles of the 97 *S. aureus* isolates characterised in the current study, further MLST and *spa* profiles of ovine isolates were obtained by searching the PubMed literature database (http://www.ncbi.nlm.nih.gov/pubmed, last accessed 28th March 2013) for articles describing characterised isolates using the terms 'ovine' or 'sheep', 'aureus' and 'MLST'. An additional search of the *S. aureus* MLST database using the keywords 'sheep' and 'ovine' was also carried out. Where information on farm of origin was available, only one example of each ST and/or *spa* type per farm was included in the dataset to minimise sampling bias. This produced an ovine *S. aureus* dataset that was used in the analyses described below (Supplementary dataset 1). Data on geographical origin and isolation site were retained in the dataset.

Example MLST and *spa* profiles of *S. aureus* strains from goats, cattle and humans formed a second dataset for comparison with the ovine dataset. Bovine and caprine profiles were obtained from the references identified in the ovine search, and human *S. aureus* profiles were obtained from descriptions of the analysis of large culture collections (Cookson et al., 2007; Feil et al., 2003). A full list of the isolates compared is presented in Supplementary dataset 2.

2.4. Data analysis

2.4.1. Population diversity

Simpson's indexes of diversity [*D*] were calculated for individual loci, ST and *spa* types using V-DICE (VNTR DIversity and Confidence Extractor; http://www.hpa-bioinformatics.org.uk/cgi-bin/