Veterinary Microbiology 170 (2014) 157–161

Contents lists available at ScienceDirect

Veterinary Microbiology

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

Short Communication

Clonal diversity, virulence-associated genes and antimicrobial resistance profile of *Staphylococcus aureus* isolates from nasal cavities and soft tissue infections in wild ruminants in Italian Alps

Camilla Luzzago [a,*], Clara Locatelli [b], Alessia Franco [c], Licia Scaccabarozzi [b], Valentina Gualdi [d], Roberto Viganò [e], Giuseppe Sironi [a], Martina Besozzi [a], Bianca Castiglioni [f], Paolo Lanfranchi [a], Paola Cremonesi [f], Antonio Battisti [c]

[a] *Department of Veterinary Science and Public Health, University of Milan, Milan, Italy*
[b] *Department of Health, Animal Science and Food Safety, University of Milan, Milan, Italy*
[c] *Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana, Rome, Italy*
[d] *Parco Tecnologico Padano, Lodi, Italy*
[e] *AlpVet, Crodo (VB), Italy*
[f] *Institute of Agricultural Biology and Biotechnology, Milan, Italy*

ARTICLE INFO

Article history:
Received 22 August 2013
Received in revised form 22 January 2014
Accepted 24 January 2014

Keywords:
Staphylococcus aureus
Virulence factor
MRSA
Genotyping
Chamois
Wild ruminants

ABSTRACT

Staphylococcus aureus is a commensal and a pathogenic bacterium that causes a wide variety of diseases in humans and animals with a high impact on public health and the livestock industry. *S. aureus* virulence pattern, antimicrobial resistance profile and host specialization are of great concern both in livestock and in companion animals. Concerning wild animals, *S. aureus* carriage and antimicrobial resistance profile has been recently investigated in free-ranging species both in aquatic and terrestrial environment. Here we report genotyping (*spa* typing, Multilocus Sequence Typing and SCC*mec* typing), virulence and antimicrobial resistance profile of four *S. aureus* isolated in Alpine chamois (*Rupicapra r. rupicapra*) and roe deer (*Capreolus capreolus*), euthanized due to walking impairment and signs of disorientation. *S. aureus* was isolated from nasal cavities in both wild ruminant species and in soft tissue infections in chamois. A marked *S. aureus* genetic heterogeneity was detected: *spa* type t1523, sequence type 45 (Clonal Complex 45), and *spa* type t1328, ST22 (CC22) from the nasal cavities and the liver of a chamois kid respectively, t1773, ST700 (CC130) from an adult chamois abscess, and a new sequence type, ST2712, belonging to CC97 from the roe deer nasal cavities.

One of the main findings was the confirmation that the t1328, ST22 isolate, obtained from the liver of the chamois kid, was a methicillin-resistant *S. aureus* (MRSA) harbouring a SCC*mec* cassette type IV. The set of virulence marker and toxin genes investigated showed profiles characteristic of the *S. aureus* lineages detected, including those of the human adapted ST (CC) 22 and ST (CC) 45 isolates.

* Corresponding author at: Department of Veterinary Science and Public Health, University of Milan, Via Celoria 10, 20133 Milan, Italy. Tel.: +39 2 50318068; fax: +39 2 50318079.
E-mail address: camilla.luzzago@unimi.it (C. Luzzago).

0378-1135/$ – see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.vetmic.2014.01.016

1. Introduction

Staphylococcus aureus is a commensal and a pathogenic bacterium that causes a wide variety of diseases in humans and animals with a high impact on public health and the livestock industry. *S. aureus* virulence pattern, antimicrobial resistance profile and host specialization are of great concern both in livestock and in companion animals that can act as transient carriers or as a reservoir of zoonotic (Huijsdens et al., 2006) and human strains (Sung et al., 2008). Concerning free-living wild ruminants, a low prevalence of methicillin-resistant *S. aureus* (MRSA) carriage was recently reported in healthy Iberian Ibex (*Capra pyrenaica hispanica*) and red deer (*Cervus elaphus*) (Porrero et al., 2013). Here we report genotyping (*spa* typing, Multilocus Sequence Typing and SCC*mec* typing), virulence and antimicrobial resistance profiles of *S. aureus* isolated from nasal cavities and infection cases in Alpine chamois (*Rupicapra r. rupicapra*) and roe deer (*Capreolus capreolus*).

2. Materials and methods

2.1. Sampling

S. aureus isolates were obtained from a convenience samples consisting of three animals that had been euthanized due to walking impairment and disorientation symptoms by gamekeepers, in compliance with the local ethical guidelines, in the north-western Italian Alps (Verbano Cusio Ossola province), in autumn 2011. In the area, a population of 3500 chamois and 2500 roe deer were estimated to be on an overall suitable area of 65.756 ha at the 2011 census. Moreover, wild ruminants are in close contact with goat and sheep flocks and cattle herds during summer in overlapping pastures. Afterwards and to date, no other wild ruminant with similar clinical signs has been culled by the gamekeepers. The three animals comprised two chamois, a three year old male and a kid, and a 1–2 year old female roe deer. The whole animals were stored at −20 °C and a *post-mortem* examination was performed within a range of 7–21 days. Samples of selected organs (spleen, lymph node, rumen, abomasum, liver, kidney, lung and heart in adult chamois and roe deer; spleen, liver and kidney in the kid chamois) were collected in 10% buffered formalin, embedded in paraffin-wax and stained with haematoxylin and eosin (HE) for histopathological examination. Samples for bacteriological analysis were (i) swabs collected from nasal cavities, (ii) organs (brain, lung, liver, spleen and kidney) and (iii) an abscess in an enlarged submandibular lymph node of the three year old chamois. The samples from nasal cavities were incubated at 37 °C for 18–20 h in Mueller–Hinton broth added with to 6.5% NaCl to enhance staphylococcal growth. Samples from the organs were cultured onto 5% sheep blood agar plates both with and without previous 24 h brain heart infusion enrichment. Bacterial colonies were identified as *S. aureus* by colony morphology, gram-stain, haemolytic patterns, catalase and coagulase reaction. Confirmation was obtained by PCR (Baron et al., 2004).

2.2. PCR of virulence factors

The DNA was extracted from the isolates with a commercial kit (QIAamp DNA Mini Kit; Qiagen GmbH, Hilden, Germany) and PCR reactions were performed targeting virulence-associated genes using primers and protocols described previously [*nuc, sea, sec, sed, seg, seh, sei, sej, sek*, and *sel* (Cremonesi et al., 2005); *coa*, *clf*A, *spa*, *tst*, *seb, see, eta*, and *etb* (Akineden et al., 2001); *luk*E (Fournier et al., 2008); *LukS*-PV-*lukF*-PV (PVL) and *mec*A (McClure et al., 2006); *blaZ* (Martineau et al., 2000), *sak*, *fmtB, scn*, and *chp* (Sung et al., 2008); *LukE–LukD* and *LukM–LukF*PV*(P83)* (Jarraud et al., 2002; Kaneko et al., 1997); *cna* (Zecconi et al., 2006)].

2.3. Genotyping

Spa typing was performed by DNA sequencing of the X-region of the *Staphylococcus* Protein A (*spa* typing, Harmsen et al., 2003), with repeats and *spa* types determined by Ridom StaphType software (Ridom GmbH, Würzburg, Germany). Seven housekeeping genes were used for MLST typing, according to standard protocol (http://saureus.mlst.net) as described by Enright et al. (2000). All PCR were performed in singleplex on previously extracted DNA and PCR products were analyzed by agarose gel electrophoresis. Unique single bands of the expected size were purified with a commercial kit (Wizard® SV Gel and PCR Clean-up System, Promega, Italy) and sequenced on both strands using an ABI 3730 automated DNA sequencer with the ABI PRISM® BigDye™ Terminator Sequencing Kits v 3.1 following manufacturer's protocol. The DNA sequences were analyzed and manipulated with BioEdit (http://www.mbio.ncsu.edu/BioEdit/bioedit.html) and consensus sequence (CAP–contig assembly program) was verified by comparison to the sequence databases at http://saureus.mlst.net/databases in order to obtain the allelic profile and the sequence type (ST) for each tested strain. The e-BURST algorithm was instead used to assign the MLST clonal complex (CC) (http://eburst.mlst.net).

Typing of the Staphylococcal Cassette Chromosome *mec* (SCC*mec*) on one *mecA*-positive isolate was obtained by means of a multiple-PCR approach, as previously described (Kondo et al., 2007).

2.4. Antimicrobial susceptibility testing

S. aureus antimicrobial susceptibility was tested by the disk diffusion method according to Clinical Laboratory Standards Institute recommendations (CLSI, 2008). Antimicrobial activities towards a wide spectrum of antimicrobials used in human and animal therapy were tested: penicillin G (10 IU), ampicillin (10 μg), amoxicillin/clavulanic acid (20/10 μg), cefoxitin (30 μg), ceftiofur (30 μg), and ceftriaxone (30 μg) to represent β-lactams; enrofloxacin (5 μg), ciprofloxacin (5 μg), tetracycline (30 μg), doxycycline (30 μg), trimethoprim–sulfamethoxazole (1.25/23.75 μg), erythromycin (15 μg), streptomycin (10 μg), kanamycin (30 μg), gentamicin (10 μg), tobramycin (10 μg). All the antibiotic disks were purchased from Oxoid (Basingstoke, UK). CLSI clinical breakpoints