Veterinary Microbiology 178 (2015) 105–113

Contents lists available at ScienceDirect

Veterinary Microbiology

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

Antimicrobial resistance and population structure of *Staphylococcus epidermidis* recovered from animals and humans

M. Angeles Argudín [a,1,*], Wannes Vanderhaeghen [b], Stien Vandendriessche [c], Ilse Vandecandelaere [d], François-Xavier André [a], Olivier Denis [c], Tom Coenye [d], Patrick Butaye [a,e,2]

[a] Department of Bacterial Diseases, Veterinary and Agrochemical Research Centre, Brussels, Groeselenbergstraat 99, B-1180 Ukkel, Belgium
[b] Department of Obstetrics, Reproduction and Herd Health, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium
[c] Laboratoire de Référence MRSA-Staphylocoques, Department of Microbiology, Hôpital Erasme, Université Libre de Bruxelles, Route de Lennik 808, 1070 Brussels, Belgium
[d] Laboratory of Pharmaceutical Microbiology, Department of Pharmaceutical analysis, Ghent University, Ottergemsesteenweg 460, B-9000 Ghent, Belgium
[e] Department of Pathology, Bacteriology, and Avian Diseases, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium

ARTICLE INFO

Article history:
Received 2 March 2015
Received in revised form 17 April 2015
Accepted 19 April 2015

Keywords:
PFGE
MLST
Methicillin resistance

ABSTRACT

While *Staphylococcus epidermidis*, as part of the commensal flora, is a well-known human opportunistic pathogen, only little is known about the genetic relatedness of *S. epidermidis* carriage isolates from animal and human origin. This study aimed to compare *S. epidermidis* recovered from livestock, livestock-farmers and humans associated with the hospital environment. A total of 193 *S. epidermidis* isolates from three populations [animals (n = 33), farmers (n = 86) and hospital-associated (n = 74)] were characterized by broth microdilution antimicrobial susceptibility testing, staphylococcal cassette chromosome *mec* (SCC*mec*) typing, pulsed field gel electrophoresis (PFGE) and multilocus sequence typing (MLST). The overall *S. epidermidis* nasal colonization rate was low in animals (1–9%) but high among farmers (75%). High levels of multi-resistance were found in all populations. Tetracycline resistance was high in animal and farmer isolates; resistance to erythromycin, clindamycin and trimethoprim was high in animal and hospital-associated isolates. Methicillin-resistant *S. epidermidis* – MRSE isolates were found in all collections, with 22 (67%) MRSE in animals, 44 (51%) MRSE in farmers and 42 (57%) MRSE associated with the hospital-setting. Known SCC*mec* types and variants were detected in 79% of MRSE; the rest were non-typeable cassettes. In total 79 PFGE-types were found, of which 22 were shared between livestock, farmers and the hospital settings. Clonal complex 2 was predominant in all three populations and most STs corresponded to types previously observed in community and nosocomial *S. epidermidis* populations. *S. epidermidis* isolates from livestock, farmers and hospital-setting showed a high level of diversity, but some clones can be found in humans as well as in animals.

* Corresponding author. Tel.: +32 2 555 69 71; fax: +32 2 555 31 10.
E-mail addresses: maria.argudin@erasme.ulb.ac.be, argudinmaria@gmail.com (M.A. Argudín).
[1] Present address: Laboratoire de Référence MRSA—Staphylocoques, Department of Microbiology, Hôpital Erasme, Université Libre de Bruxelles, Route de Lennik 808, 1070 Brussels, Belgium.
[2] Present address: Ross University School of Veterinary Medicine, Department of Biomedical Sciences, Basseterre, PO Box 334, St Kitts and Nevis, West Indies and Ghent University, Faculty of Veterinary Medicine, Department of Pathology, Bacteriology and Poultry Diseases, Salisburylaan 133, 9820 Merelbeke, Belgium.

http://dx.doi.org/10.1016/j.vetmic.2015.04.019
0378-1135/

1. Introduction

Staphylococci are common bacterial colonizers of the skin and mucous membranes of humans and other mammals. *Staphylococcus epidermidis* in particular, is the most prevalent staphylococcal species found in humans. It is nowadays considered to be an important opportunistic pathogen, representing the most common source of device-related infections (Otto, 2009, 2012). About 70–95% of *S. epidermidis* strains circulating in the human hospital environment have been estimated to be methicillin-resistant; most of them display high resistance rates to other antimicrobial classes too (Otto, 2012). In veterinary medicine, *S. epidermidis* is one of the main etiological agents of ruminant intramammary infections (Feßler et al., 2010; Onni et al., 2011) and it is also implicated in diverse infections in companion animals (Kern and Perreten, 2013; Weiß et al., 2013). Moreover, *S. epidermidis* carriage has been reported in livestock (Zhang et al., 2009; Huber et al., 2011; Bhargava and Zhang, 2012; Vanderhaeghen et al., 2012) and domestic animals (Bagcigil et al., 2007). It has been suggested that the *S. epidermidis* strains that cause mastitis emanate from humans (Watts and Owens, 1989; Thorberg et al., 2006; Jaglic et al., 2010). Yet, in general, little is known about transmission of *S. epidermidis* between humans and animals, nor about the genetic relatedness between *S. epidermidis* strains found in animals and humans.

The present study aimed at investigating the molecular epidemiology between *S. epidermidis* isolates from humans and animals, by comparing the population structure of *S. epidermidis* isolates recovered from livestock, livestock farmers, hospitalized patients and laboratory personnel.

2. Materials and methods

2.1. Sample isolation from animals and farmers

Farms with veal calves (n = 15), dairy cattle (n = 10), beef cattle (n = 10) and broilers (n = 10) were selected as previously described (Vanderhaeghen et al., 2013; Vandendriessche et al., 2013). Samples were collected from 150 veal calves, 100 beef cows, 100 dairy cows and 200 broilers. Nasal swabs from farmers working on the same bovine and broiler farms were collected from 17 veal farmers, 21 dairy farmers, 20 beef farmers and 14 broiler farmers, as previously described (Vandendriessche et al., 2013). Bacterial strains were isolated as previously described (Vanderhaeghen et al., 2013; Vandendriessche et al., 2013). Colonies were subcultured on blood agar plates and visually inspected after 48–72 h at 37 °C. Each colony showing a unique *S. epidermidis* morphotype was further included in the study as previously described (Vanderhaeghen et al., 2013). This study was approved by the Medical Ethics Commission of the ULB-Erasme Hospital in Brussels (reference P2009/065); all participants signed an informed consent form.

2.2. Bacterial isolates from humans associated with the hospital environment

A total of 74 *S. epidermidis* non-duplicate isolates recovered from human patients and laboratory staff, and therefore considered as hospital-associated, were included. Twenty-four *S. epidermidis* isolates were obtained from blood cultures of patients attending the Erasmus hospital (Brussels, Belgium). Thirty-four *S. epidermidis* strains were isolated from biofilms on endotracheal tubes and throat and nose swabs of mechanically ventilated patients at the Intensive Care Unit of Ghent University hospital (Gent, Belgium) (Vandecandelaere et al., 2012). The remaining 16 isolates were recovered from the nares and skin of healthy personnel working in the Laboratory of Pharmaceutical Microbiology of the Ghent University. These personnel works with hospital samples from the Ghent University hospital.

2.3. Identification, mecA detection and SCCmec typing

Genomic DNA was purified as previously described (Vanderhaeghen et al., 2013). Species identification by tDNA intergenic spacer analysis, the detection of *mecA* and the SCC*mec* typing by determination of *ccr* and *mec* complexes were performed as previously described (Vanderhaeghen et al., 2013).

2.4. Antimicrobial susceptibility testing

Minimal inhibitory concentrations (MICs) of 18 antimicrobials (penicillin, cefoxitin, kanamycin, streptomycin, erythromycin, clindamycin, quinupristin/dalfopristin, linezolid, tiamulin, chloramphenicol, rifampicin, ciprofloxacin, fusidic acid, tetracycline, trimethoprim, sulfamethoxazole, vancomycin and mupirocin) were determined for all *S. epidermidis* isolates using custom veterinary international Sensititre staphylococci plates EUST (Trek Diagnostics System, United Kingdom) according to the manufacturer's instructions. The interpretation of MIC values and determination of wild type (WT) and non-wild type (NWT) (Schwarz et al., 2010) was according to the epidemiological cut-off (ECOFF) values of the European Committee for Antimicrobial Susceptibility Testing (EUCAST) for coagulase negative staphylococci (CoNS) (http://www.eucast.org). Methicillin resistant *S. epidermidis* (MRSE, defined as *mecA*-positive isolates) classified as cefoxitin-susceptible were also tested for oxacillin susceptibility according to CLSI (CLSI, 2014) by the disc diffusion method, using Mueller-Hinton agar and commercially available oxacillin discs (Oxoid, Belgium).

2.5. Macrorestriction-PFGE analysis and MLST typing

Whole cell DNA from each *S. epidermidis* isolate was analyzed by SmaI macrorestriction and pulsed field gel electrophoresis (PFGE) using a CHEF Mapper system (Bio-Rad Laboratories, United Kingdom). Isolates resistant to SmaI restriction were typed using the SmaI neoschizomer Cfr9I, as described by Argudín et al. (2010). Plugs were prepared according to the protocol of Argudín et al. (2015). PFGE profiles were compared using BioNumerics software version 6.6 (Applied Maths). A dendrogram with one of each different SmaI/Cfr9I pattern was constructed using the unweighted pair group method with arithmetic averages. The Dice similarity coefficient was used, with optimization and position tolerance settings of 0.8 and 1.3, respectively (Miragaia et al., 2008). According to the criteria established by Miragaia et al. (2008), a cutoff similarity value of 79% was used to establish PFGE types.

Isolates from the most frequent PFGE types were further analyzed by multilocus sequence typing (MLST). Briefly, amplicons of the multilocus sequence typing genes were purified with the Nucleospin Extract II kit (Macherey-Nagel, Düren, Germany) and sequenced by Macrogen (Amsterdam, The Netherlands). The *S. epidermidis* multilocus sequence typing website (http://sepidermidis.mlst.net/) was used to assign sequence types (STs). The BURST algorithm (http://eburst.mlst.net/) was used to define clonal complexes (CCs).

2.6. Data analysis

To assess the discriminatory power of the PFGE method, genotypic diversity was calculated using the Simpson's index of diversity (SID) and its confidence intervals (CIs) as previously