Veterinary Microbiology 162 (2013) 806–812

Contents lists available at SciVerse ScienceDirect

Veterinary Microbiology

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

Genotyping of *Staphylococcus aureus* isolates from diseased poultry

Stefan Monecke [a,b,*], Antje Ruppelt [a], Sarah Wendlandt [c], Stefan Schwarz [c], Peter Slickers [b], Ralf Ehricht [b], Sonia Cortez de Jäckel [d]

[a] Institute for Medical Microbiology and Hygiene, Technical University of Dresden, Dresden, Germany
[b] Alere Technologies GmbH, Jena, Germany
[c] Institute of Farm Animal Genetics, Friedrich-Loeffler-Institut (FLI), Neustadt-Mariensee, Germany
[d] Poultry Clinics and Laboratory Dr. Pöppel, Delbrück, Germany

ARTICLE INFO

Article history:
Received 10 February 2012
Received in revised form 6 August 2012
Accepted 15 October 2012

Keywords:
Staphylococcus aureus
MRSA
MSSA
CC398
Microarray analysis
Minimum inhibitory concentrations
spa typing
dru typing

ABSTRACT

To gain insight into the genomic diversity of *Staphylococcus aureus* associated with diseases in domestic poultry, 131 isolates from clinically ill turkeys ($n = 80$) and chickens ($n = 51$) were collected and genotyped using microarray hybridisations. MRSA isolates were subjected to *spa* and *dru* typing and their antimicrobial resistance geno- and phenotypes were determined. Most (68 out of 80) turkey isolates belonged to the clonal complex (CC) 398. Seventeen of the 80 isolates (21.2%) were MRSA. The most common MRSA type among turkeys was CC398-MRSA-V ($n = 8$), but CC5-MRSA-III ($n = 4$), CC9-MRSA-IV ($n = 2$), CC398-MRSA-IV ($n = 2$) and a single CC398-MRSA with an unidentified/truncated SCC*mec* element were also found. Among the chicken isolates, CC5 predominated (44 out of 51). Five of the chicken isolates were MRSA (9.8%), all belonging to CC398-MRSA-V. These data show that the current dissemination of livestock-associated MRSA also engulfs chickens and turkeys, and that MRSA surveillance among these species is warranted.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

Staphylococcus aureus is a versatile bacterium which can infect or colonise a variety of different host species. While it is found in various mammals including humans, there are also several reports about its presence in both, domestic and wild birds. While multilocus sequence types (STs), such as ST692, have exclusively been observed in birds (see MLST database at http://saureus.mlst.net/), other STs are less host-specific. For instance, the most widespread and dominant *S. aureus* strains in poultry belong to ST5 within clonal complex (CC) 5, and appear to originate most likely from a single transmission from humans, where this lineage is also common, into fowl (Lowder et al., 2009). Strains that belong to CC398, especially the methicillin-resistant *S. aureus* CC398 that harbour a type V SCC*mec* element (CC398-MRSA-V), proved to be present in a wide variety of host species. They have been found not only in humans (Huijsdens et al., 2006; Krziwanek et al., 2009; Schijffelen et al., 2010), but also in pigs (Huijsdens et al., 2006; de Neeling et al., 2007), dairy cows and veal calves (Feßler et al., 2010; Vanderhaeghen et al., 2010), horses (Walther et al., 2009) and dogs (Nienhoff et al., 2009) and also in chickens (Nemati et al., 2008), chicken meat (Nemati et al., 2008; Feßler et al., 2011) as well as turkey meat (Feßler et al., 2011).

To gain insight into the genomic relationships of *S. aureus* strains associated with diseases in domestic poultry, consecutive isolates from clinically ill chickens and turkeys were collected and genotyped using hybridisations to a previously described microarray (Monecke et al., 2011). This comprehensive genotyping approach of a

* Corresponding author at: Institute for Medical Microbiology and Hygiene, Technical University of Dresden, Dresden, Germany.
Tel.: +49 351 6200; fax: +49 351 458 6311.
E-mail address: monecke@rocketmail.com (S. Monecke).

0378-1135/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.vetmic.2012.10.018

large number of independent methicillin-susceptible and methicillin-resistant *S. aureus* provides for the first time insight into the genomic relationships of such strains from diseased poultry. For a better comparison with MRSA from diseased pigs and cattle, but also with MRSA from poultry meat and poultry meat products, the MRSA isolates were subjected to *spa* and *dru* typing and their antimicrobial resistance geno- and phenotypes were determined.

2. Material and methods

2.1. Bacterial isolates

A total of 131 *S. aureus* isolates were obtained from routine diagnostic work at the Poultry Clinic and Laboratory Dr. Pöppel, Delbrück, Germany, between January 2009 and July 2011. This unit serves numerous large poultry farms mainly in the German federal state of North Rhine-Westphalia (although eight turkey isolates came from Baden-Wuerttemberg, and five chicken isolates from Lower Saxony). Fifty-one isolates originated from domestic chickens (38 broilers including four chicks, 13 layers) and 80 from domestic turkeys. Turkey isolates originated from 26 different farms, broilers from 18 and layers from 10 farms. The isolates were obtained from necropsy material from cases of invasive infections (joints, liver, heart, lungs), and only one isolate per animal was chosen for further analysis.

Staphylococci were identified by Gram staining, catalase and coagulase test (rabbit plasma tube test) and by the ID 32 STAPH system (bioMérieux, Craponne, France). All strains were tested phenotypically for antibiotic resistance by the disc diffusion method using DIN breakpoints. Detection of oxacillin resistance was performed using oxacillin and cefoxitin disks and by screening for growth on the Brilliance MRSA-Agar (Oxoid, Wesel, Germany).

2.2. DNA microarray-based typing

All isolates were characterised using the Alere StaphyType DNA microarray (Alere Technologies GmbH, Jena, Germany) covering 333 target sequences which correspond to approximately 170 distinct genes and their allelic variants. These include species markers, SCC*mec*, capsule and *agr* group typing markers, resistance genes and genes encoding exotoxins as well as adhesion factors (see Supplement 1). A full list including primer/probe sequences has been provided previously (Monecke et al., 2011).

The assay was performed according to the instructions given by the manufacturer and to previous descriptions (Monecke et al., 2011). In short, *S. aureus* were grown on Columbia blood agar, harvested and lysed. DNA was prepared utilising spin columns or the automated EZ1 system (both by Qiagen, Hilden, Germany). DNA samples were subjected to a linear primer elongation using only one primer per target. During this step, biotin-16-dUTP was incorporated into the resulting amplicons. In a later step, these amplicons were hybridised to the microarray. After washing, horseradish-peroxidase-streptavidin was added which subsequently triggered the precipitation of a dye. An image of the microarray was taken and analysed using reader and software provided by Alere Technologies GmbH (Jena, Germany). The automated comparison of the hybridisation patterns of the actual isolate to a reference database allowed determining its affiliation to clonal complexes as defined by MLST (Enright et al., 2000) and, in case of MRSA, to epidemic strains defined by MLST and SCC*mec* carriage (Monecke et al., 2011). PSM-*mec* was not covered by the array; it was detected by PCR as previously described (Monecke et al., 2012a).

2.3. Susceptibility testing and detection of resistance genes in MRSA

Additional susceptibility testing of the MRSA isolates was performed by broth microdilution using custom-made microtitre plates (MCS Diagnostics, Swalmen, the Netherlands) which contained the following antimicrobial agents (with test range in parentheses) in two-fold dilution series: ampicillin (0.03–64 μg/ml), apramycin (0.03–64 μg/ml), ceftiofur (0.03–64 μg/ml), cefotaxime (0.015–32 μg/ml), cefquinome (0.015–32 μg/ml), cephalothin (0.06–128 μg/ml), chloramphenicol (0.5–256 μg/ml), clindamycin (0.03–64 μg/ml), enrofloxacin (0.008–16 μg/ml), erythromycin (0.015–32 μg/ml), florfenicol (0.12–256 μg/ml), gentamicin (0.12–256 μg/ml), nalidixic acid (0.06–128 μg/ml), oxacillin + 2% NaCl (0.03–16 μg/ml), penicillin G (0.015–32 μg/ml), quinupristin/dalfopristin (0.008–16 μg/ml), spectinomycin (0.12–256 μg/ml), tetracycline (0.12–256 μg/ml), tiamulin (0.03–64 μg/ml), trimethoprim (0.06–128 μg/ml), trimethoprim/sulfamethoxazole (0.015/0.03–32/608 μg/ml), and vancomycin (0.008–16 μg/ml). In addition, susceptibility to kanamycin (2–128 μg/ml) was tested by broth macrodilution. Susceptibility testing followed the recommendations given in the CLSI document M31-A3 (CLSI, 2008); classification of the isolates as resistant or susceptible based on the breakpoints given in the CLSI documents M31-A3 (CLSI, 2008) and M100-S21 (CLSI, 2011). *S. aureus* ATCC®29213 served as quality control strain.

As the aforementioned DNA microarray already covers a considerable number of resistance genes, specific PCR assays were only conducted for resistance genes not yet included in the DNA microarray. These are *tet*(L) (tetracycline resistance), *dfrK* (trimethoprim resistance), the linkage of *tet*(L)-*dfrK*, *dfrK* as part of Tn*559*; *erm*(T) (macrolide-lincosamide-streptogramin B resistance), *spc* (spectinomycin resistance), the linkage of *erm*(A)-*spc*, as well as *vga*(C), *vga*(D), *vga*(E) and *lsa*(C) (pleuromutilin-lincosamide-streptogramin A resistance), and *vga*(E) as part of Tn*6133*. These PCR assays followed previously described protocols (Feßler et al., 2010, 2011; Hauschild et al., 2012).

2.4. spa and dru typing

The *spa* typing was performed in accordance with the Ridom StaphType standard protocol (http://spaserver.ridom.de). A relatively novel typing method for methicillin-resistant staphylococci, *dru* typing (Goering et al., 2008),

Download English Version:

https://daneshyari.com/en/article/5801085

Download Persian Version:

https://daneshyari.com/article/5801085

Daneshyari.com