Veterinary Microbiology 167 (2013) 680–685

Contents lists available at ScienceDirect

Veterinary Microbiology

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

Short communication

Identification and characterization of methicillin-resistant *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus* and *Staphylococcus pettenkoferi* from a small animal clinic

Sonja Weiß, Kristina Kadlec, Andrea T. Feßler, Stefan Schwarz *

Institute of Farm Animal Genetics, Friedrich-Loeffler-Institut (FLI), Neustadt-Mariensee, Germany

ARTICLE INFO

Article history:
Received 19 June 2013
Received in revised form 21 July 2013
Accepted 24 July 2013

Keywords:
MRSE
MRSA
Molecular typing
Multiresistance

ABSTRACT

The aim of this study was to isolate and characterize methicillin-resistant staphylococci (MRS) in a small animal clinic and to investigate their distribution and possible transmission. Swabs ($n = 72$) were taken from hospitalized pets, the environment and employees of a small animal clinic and screened for the presence of MRS. The staphylococcal species was confirmed biochemically or by 16S rDNA sequencing. Susceptibility to antimicrobial agents was tested by broth dilution. The presence of *mecA* and other resistance genes was confirmed by PCR. Molecular typing of the isolates followed standard procedures. In total, 34 MRS belonging to the four species *Staphylococcus aureus* ($n = 5$), *Staphylococcus epidermidis* ($n = 21$), *Staphylococcus haemolyticus* ($n = 6$) or *Staphylococcus pettenkoferi* ($n = 2$) were isolated. All isolates were multidrug-resistant with resistance to at least three classes of antimicrobial agents. Among the five methicillin-resistant *S. aureus* (MRSA) isolates, four belonged to the clonal complex CC398; two of them were isolated from cats, the remaining two from pet cages. Overall, the MRS isolates differed in their characteristics, except for one *S. epidermidis* clone ($n = 9$) isolated from hospitalized cats without clinical staphylococcal infections, pet cages, the clinic environment as well as from a healthy employee. This MRSE clone was resistant to 10 classes of antimicrobial agents, including aminocyclitols, β-lactams, fluoroquinolones, lincosamides, macrolides, phenicols, pleuromutilins, sulfonamides, tetracyclines and trimethoprim. These findings suggest a possible transmission of specific MRS isolates between animal patients, employees and the clinic environment.

1. Introduction

Among methicillin-resistant staphylococci (MRS), methicillin-resistant *Staphylococcus aureus* (MRSA) isolates – especially those of clonal complex (CC) 398 – have been found at different prevalences among different farm animal species (Feßler et al., 2012; Monecke et al., 2013). In pets, methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) isolates have been found frequently (Weese and van Duijkeren, 2010). MRSA, but especially MRSP, often show expanded resistance to antimicrobial agents and the treatment of infections caused by these bacteria represents a real challenge for veterinary practitioners (Perreten et al., 2010; Kadlec et al., 2010). Comparatively little is known about the presence and antimicrobial resistance of methicillin-resistant members of other staphylococcal

* Corresponding author. Tel.: +49 5034 871 241; fax: +49 5034 871 143.
E-mail address: stefan.schwarz@fli.bund.de (S. Schwarz).

0378-1135/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.vetmic.2013.07.036

species. Animal clinics in which sick animals, that might shed pathogenic bacteria, are present and antimicrobial agents are frequently applied may constitute a suitable environment for the development and spread of multi-resistant staphylococci including MRS. A screening for carriage of MRSA and MRSP of canine and feline patients before entering a small animal clinic confirmed that dogs and cats can act as vehicles and bring these bacteria into the clinic environment (Nienhoff et al., 2011a,b). The presence of MRS in animals does not only bear the risk of difficult-to-treat infections of the animals, but also constitutes a risk for the clinic employees who are in close contact with the animals and may become occupationally colonized (Guardabassi et al., 2004). Furthermore, contaminated clinic facilities can serve as a source of infections (Aksoy et al., 2010). In this regard, the stationary area within a clinic is a critical place, as animals that are vulnerable for infections are present and the selective pressure due to the use of antimicrobial agents is relatively high.

The aim of this study was to characterize MRS isolated from a small animal clinic in order to gain information about their distribution within the clinic and to investigate a possible transmission between animal patients, clinic environment and employees.

2. Materials and methods

2.1. Isolation and identification of methicillin-resistant staphylococci

In total, 72 swabs were taken from pets ($n = 10$, nasopharyngeal swabs), the clinic environment ($n = 58$, surface swabs) and employees ($n = 4$, nasal swabs). Pet swabs were obtained from hospitalized animals (nine cats, one dog) exclusively, environmental swabs comprised clinic facilities in the stationary area ($n = 34$), the operating room ($n = 15$) and the consulting rooms ($n = 9$). After overnight enrichment in Mueller-Hinton (MH)-broth with 6.5% sodium chloride, the samples were cultured on MH-agar plates with 6.5% sodium chloride and 0.25 μg oxacillin/mL with subsequent passage onto blood agar plates. Oxacillin resistance was confirmed by broth macrodilution according to the recommendations given by the Clinical and Laboratory Standards Institute (CLSI, 2008) and by PCR detection of the resistance gene *mecA* (Strommenger et al., 2006). The staphylococcal species were confirmed biochemically by using the ID 32 STAPH system (bioMérieux, Nürtingen, Germany). When the numeric profile did not allow an unambiguous species assignment, PCR amplification of a 16S rDNA sequence using the primers 16S fwd (5′-GGTGAGTAACACGTGGA-TAA-3′) and 16S rv (5′-ATGTCAAGATTTGGTAAGGTT-3′) was performed (Herbert et al., 2001). The amplicon of 895 bp size was generated using the following PCR protocol: initial denaturation at 94 °C for 2 min, followed by 30 cycles (denaturation at 94 °C for 1 min, annealing at 54 °C for 1 min, extension at 72 °C for 2 min), and a final extension step at 72 °C for 7 min. Sequence comparison was carried out by using the nucleotide basic local alignment search tool (http://blast.ncbi.nlm.nih.gov/).

2.2. Susceptibility testing and detection of resistance genes

MIC values were determined by broth microdilution according to CLSI standard M31-A3 (CLSI, 2008). The antimicrobial agents tested and the test ranges corresponded to those previously described by Feßler et al. (2010). *S. aureus* ATCC®29213 served as quality control strain. With respect to the resistance phenotype, resistance genes were detected by previously described specific PCR assays (Feßler et al., 2010, 2011; Wendlandt et al., 2013a,b).

2.3. Genotypic characterization of MRS

Two previously described multiplex PCRs were applied for SCC*mec* typing (Kondo et al., 2007). For all MRS, *dru* typing was performed according to http://dru-typing.org (Goering et al., 2008). The MRSA isolates were *spa* typed according to http://www.spaserver.ridom.de (Harmsen et al., 2003). All MRSA isolates were subjected to the CC398-specific PCR assays A07 and C01 as described by van Wamel and colleagues (2010). Multilocus sequence typing (MLST) for non-CC398 MRSA isolates (Enright et al., 2000) and for methicillin-resistant *Staphylococcus epidermidis* (MRSE) isolates (Thomas et al., 2007) was performed according to www.mlst.net. The MRSE isolates were subjected to macrorestriction analysis with *Sma*I and subsequent pulsed-field gel electrophoresis (PFGE) as previously described (Strommenger et al., 2006).

3. Results and discussion

3.1. Identification and characterization of MRS isolates

From 72 swabs taken, 34 MRS [*S. aureus* ($n = 5$), *S. epidermidis* ($n = 21$), *Staphylococcus haemolyticus* ($n = 6$), *Staphylococcus pettenkoferi* ($n = 2$)] could be isolated. These included six MRS from hospitalized cats without clinical staphylococcal infections, four MRS from healthy employees and 24 MRS from the clinic environment (Table 1). Most MRS were detected in the stationary area of the clinic whereas all samples taken from the operating room and the consulting rooms, except one from a stethoscope, were MRS-free. This may underline the effectiveness of the cleaning and disinfection procedures undertaken in that clinic. However, despite all these efforts, it is unrealistic to expect a pathogen-free clinic environment, especially in the stationary area of veterinary clinics. Diseased animals (but also their owners), who may carry and shed MRS, can bring these bacteria into the clinic environment and employees may be occupationally colonized.

MIC values for oxacillin varied among the MRS from 1 μg/mL to ≥32 μg/mL. All MRS harbored the resistance gene *mecA*. The MRS had SCC*mec* types IV ($n = 22$) or V ($n = 8$), or were non-typeable ($n = 4$) by the multiplex PCR assays used (Table 1). The *dru* typing revealed five novel *dru* types (dt5i, dt9bb, dt9bc, dt10cb, and dt12w) in addition to five known *dru* types (dt10a, dt10g, dt10r, dt11a, and dt11c) with dt10a being predominant ($n = 17$) (Table 1). All isolates were multidrug-resistant (Schwarz et al., 2010) and showed resistance to at least three classes