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Short communication

Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from small and exotic animals at a university hospital during routine microbiological examinations

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Abstract

Clinical specimens of small animals ($n = 869$) were screened for the occurrence of methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* (MSSA; MRSA) during routine microbiological examinations, and results were confirmed by a multiplex PCR strategy. The genetic relatedness of all *mec*A-positive *S. aureus* isolates was further investigated by pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), PCR for Panton-Valentine leukocidine genes (PVL) and staphylococcal cassette chromosome *mec*-typing (SCC*mec*).

A total of 61 *S. aureus* isolates were found during a 20-month period of investigation, 27 (44.3%) of them harbouring the *mec*A gene for methicillin-resistance. The majority of MRSA were isolated in specimens from dogs ($n = 18$) and cats ($n = 4$). One guinea pig and one rabbit were found to be positive for an MRSA infected site. Similarly, three exotic animals, a turtle, a bat and a parrot, were found to be infected with MRSA. PFGE and MLST analysis revealed a certain genotype (“A” and “A-1”) dominating the isolate collection (23 of 27). Furthermore, one isolate showed homologous PFGE pattern to the German epidemic strain Barnim (“BE”) and another one (“BE-1”) was considered to be closely related. A third genotype (“B”) was detected in two cases. Two different sequence types (ST) were identified among the 27 MRSA isolates. PFGE type “A” and both strains related to the Barnim epidemic strain were assigned to ST22, whereas ST239 was associated to PFGE profile “B”.

The present data show that certain MRSA genotypes are capable of infecting a wide spectrum of small and exotic animals, especially in clinical facilities.

Keywords: MRSA; Small animal; Wound infection

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1. Introduction

Among pathogens causing infectious diseases, *Staphylococcus aureus*, especially methicillin-resistant *S. aureus* (MRSA), are an important cause of human infectious diseases. In Germany, the MRSA situation in hospitals and other community health settings varies regionally. A validated surveillance study (SARI) reported about cumulative MRSA percentages ranging from 0 to 64.4% in German intensive care units (Meyer et al., 2006).

Since the mid-1970s, cases of infections due to MRSA were reported in different animal species (Devriese and Hommez, 1975; Hartmann et al., 1997; Pak et al., 1999; Shimizu et al., 1997). Meanwhile, MRSA infections in domestic animals have been reported among horses, pigs, cattle, sheep, cats, dogs and rabbits. In the last years however, there has been an increase in reports on MRSA infections in small animals and horses (Middleton et al., 2005; Rich and Roberts, 2006; Seguin et al., 1999; Walther et al., 2006). Moreover, occurrence of MRSA has been reported as a problem in veterinary (teaching) facilities (O'Mahony et al., 2005; Strommenger et al., 2006; Weese et al., 2006).

In April 2003, a dog suffering from delayed wound healing after surgery due to MRSA attracted our attention. The animal was a patient of the small animal hospital at the Free University Berlin. We decided to perform a screening for MRSA in addition to routine diagnostic procedures on all microbiological specimens originating from small animal patients of that facility. A second aim was to analyze the genetic relatedness of collected MRSA isolates using common typing techniques, including pulsed field gel electrophoresis (PFGE), multilocus sequence typing (MLST), Staphylococcal cassette chromosome *mec*- (SCC*mec*) typing and PCR detection of the genes encoding for the Panton-Valentine leukocidine (PVL) factor.

2. Materials and methods

2.1. Setting

All microbiological specimens reported on here were delivered from Small Animal Clinic of the Veterinary Faculty at the Free University Berlin. This clinical facility has a case load of about 30,000 small and exotic animals per year. Animals with all kinds of diseases are admitted to this veterinary hospital, most of them being out patients.

2.2. Bacterial isolation and identification

The Institute for Microbiology and Epizootics (IMT), part of the Free University Berlin, provides microbiological diagnostic services for the different veterinary clinical teaching departments of the university and for private veterinary hospitals or smaller settings.

A total of 869 clinical specimens, which were routinely sent in for diagnostic purposes between May 2003 and December 2004, were investigated in this study. All clinical specimens originated from small animal patients of the veterinary teaching hospital. The whole spectrum of animal species and diagnostic material (origin of specimens) investigated is given in Table 1.

All specimens were routinely inoculated onto the following media: Standard nutrient agar I (Roth GmbH, Karlsruhe, Germany) with 5% defibrinated sheep blood, Chrom agar orientation (Mast Diagnostica, Reinfeld, Germany) and Gassner agar (Sifin, Berlin, Germany). All plates were investigated twice, first after 18 h and a second time after 36 h of incubation at 37 °C. Staphylococcal isolates were identified by morphology on agar plates, Gram stain appearance, catalase test, the tube coagulase reaction and their ability to ferment mannitol anaerobic ally (Mossel, 1962; Songer and Post, 2005).

All coagulase-positive *Staphylococcus* spp. isolates were routinely investigated by PCR, regardless of other phenotypic test results. Genotypic confirmation of *S. aureus* was implemented by amplification of a species-specific sequence of the thermostable nuclease (*nuc*) and methicillin-resistance was diagnosed by detecting the *mec*A- gene responsible for broad spectrum β-lactam-resistance in staphylococci (Merlino et al., 2002).

2.3. Typing of MRSA strains

2.3.1. PFGE

PFGE analysis was used to provide a DNA fingerprint profile from all MRSA isolates which were collected during this study ($n = 27$). Briefly,