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Methicillin-resistant *Staphylococcus aureus* from infections in horses in Germany are frequent colonizers of veterinarians but rare among MRSA from infections in humans

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ABSTRACT

A total of 272 methicillin-resistant *Staphylococcus aureus* (MRSA) from equine infections originating from 17 equine hospitals and 39 veterinary practices in Germany as well as 67 isolates from personnel working at equine clinics were subjected to molecular typing. The majority of isolates from horses was attributed to clonal complex (CC) 398 (82.7%). Within CC398, 66% of isolates belonged to a subpopulation (clade) of CC398, which is associated with equine clinics.

MRSA attributed to CC8 (ST254, t009, t036, SCC*mec*IV; ST8, t064, SCC*mec*IV) were less frequent (16.5%). Single isolates were attributed to ST1, CC22, ST130, and ST1660. The emergence of MRSA CC22 and ST130 in horses was not reported so far. Nasal MRSA colonization was found in 19.5% of veterinary personnel with occupational exposure to horses. The typing characteristics of these isolates corresponded to isolates from equine infections. Comparing typing characteristics of equine isolates with those of a substantial number of isolates from human infections typed at the German Reference Center for Staphylococci and Enterococci (2006–2014; n = 10864) yielded that the proportion of isolates exhibiting characteristics of MRSA from equine medicine is very low (<0.5%). As this low proportion was also found among MRSA originating from nasal screenings of human carriers not suffering from a staphylococcal infection (n = 5546) transmission of MRSA from equine clinics to the community seems to be rare so far.

Introduction

Staphylococcus aureus becomes methicillin resistant by acquisition of the *mec* genes (*mec*A and homologues) contained by staphylococcal cassette chromosome *mec* (SCC*mec*) elements from which at least 11 basic types are known so far. *S. aureus* shows a rather clonal population structure; typing of isolates by relevant methods reveals allocation to certain clonal types, in particular multilocus-sequence typing (MLST) and *spa*-typing are used as standard methodologies [1,2]. MRSA is globally prevalent in nosocomial settings as hospital-associated MRSA (HA-MRSA), which is mainly due to intra- and interhospital spread of epidemic clonal lineages [3–5]. In addition, MRSA emerged in the community without any relation to healthcare facilities (CA-MRSA, [6]). The first MRSA in animals was reported from cases of mastitis in dairy cattle in 1972, followed by sporadic observations of infections in various animals including postsurgical wound infections in horses [7]. Since 2006 MRSA attributed to clonal complex CC398 received specific attention since these so-called livestock-associated MRSA (LA-MRSA) is widely disseminated among various livestock animals mainly as an asymptomatic nasal colonizer [8,9]. Because of its capacity to cause a variety of infections in humans such as skin and soft-tissue infections, surgical wound and joint infections, invasive device infections (catheter, endoprostheses), ventilator-associated pneumonia, and septicemia [10–12] MRSA CC398 became a public health issue.

Furthermore, MRSA raised attention as nosocomial pathogens in companion animals and equine medicine. For companion animals, such as cats and dogs, clusters of MRSA infections in veterinary facilities were observed [13–15]. Several studies have provided evidence of hospital-associated (HA)-MRSA HA-MRSA transmission from humans to small animals in veterinary facilities and vice versa. Molecular typing

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of the isolates suggested an origin in human hospitals [16,17]. The first report of an outbreak of MRSA infections in horses in a veterinary hospital came from the United States in 1999 [18], and was followed by descriptions of clusters of MRSA infections in equine hospitals in Canada [19,20] and in Central Europe a few years later [21,22]. The majority of the Canadian MRSA isolates from horses and staff, as reported by previous studies, has typically been identified as Canadian epidemic MRSA-5, equivalent to "USA500", a putatively equine clinic associated strain, which accounted for nearly 10% of MRSA in Canadian hospitals by the end of the 1990 [23]. It exhibits MLST ST8, *spa* type t064 (corresponds to *spa* type 7 according to the Kreiswirth nomenclature), and contains SCC*mec*IV [20]. This strain type was also reported for MRSA isolates from horses from the United States and from Ireland [24,25]. In a Canadian veterinary hospital a cluster of skin and soft tissue infections in humans working there was also observed [26]. At this time the central European MRSA isolates from nosocomial infections in horses exhibited ST254, t036, and SCC*mec*IVh [21,22]. Meanwhile, MRSA CC398 is prevalent as a nosocomial pathogen in veterinary clinics, particularly in those for horses in Austria [22,27], Belgium [28,29], Germany [30], the Netherlands [31], Switzerland [32], and the United Kingdom [17]. Furthermore, nasal colonization of veterinary personnel attending horses was reported [22,31–33]. The majority of MRSA CC398 isolates from horse clinics exhibited a typical pattern of characteristics when subjected to typing: *spa* type t011, more rarely t6867, SCC*mec*IVa, and phenotypic resistance to gentamicin based on the *aac*A-*aph*D gene [22,31,32]. A more detailed analysis of the population-structure through mutation discovery at 97 loci revealed that MRSA CC398 from horse clinics exhibiting the above mentioned characteristics represent a particular subpopulation (clade) of LA-MRSA CC398 [34].

The significance of MRSA from colonization and infections in horses for infections in humans has not been assessed in more detail so far. Therefore, the objective of this study was to determine the proportion of typical equine MRSA clones among the MRSA from human infections based on a comparative analysis of typing characteristics.

Materials and methods

MRSA from infections in horses

The 272 isolates included originated from infections like soft-tissue and joint infections, pneumonia, sinusitis, metritis, omphalophlebitis or postoperative wound infections and were derived from horses treated in 17 veterinary hospitals as well as in 39 large animal practices in all regions of Germany (predominantly in the federal states of North Rhine-Westphalia and Lower Saxony) between January 2011 and February 2015.

MRSA from nasal swabs from veterinary personal and veterinarians ttending horses

We prospectively collected nasal swabs of employees (veterinarians and other staff; n = 349) in five equine clinics and three large practices from which also MRSA from infections in horses were derived between 2012 and 2015. This resulted in 67 MRSA isolates. Swabs were taken from both nostrils and processed as described previously [35].

MRSA CC398 from different types of human infections

Isolates included in the analysis comprised (i) a sample of MRSA isolates (n = 8912) which were sent to the German Reference Center for Staphylococci and Enterococci between 2006 and 2013 for strain characterization and typing in line with routine diagnostic procedures or in case of outbreak investigations., and (ii) MRSA isolates from blood cultures (n = 1952) which were prospectively (2011–2013) collected in North Rhine-Westphalia and *spa*-typed [36].

MRSA isolates from nasal swabs from humans: These isolates (n = 5546) originated from 150 different hospitals all over Germany. They were collected both from patients with no staphylococcal infection at admission to hospitals and from inpatients between 2006 and 2014 and sent to the German Reference Centre for Staphylococci and Enterococci for molecular typing.

Primary diagnostics, species identification of *S. aureus*, and further characterization by means of *spa*-typing, attribution to clonal lineages (complexes) and demonstration of *mec*A and of *mec*C as well as phenotypic antibiotic susceptibility testing were performed as described previously [35,37]. Minimum inhibitory concentrations (MICs) were determined for 18 antibiotics belonging to 15 antibiotic classes (including anti-staphylococcal β-lactams (penicillin, oxacillin), aminoglycosides (gentamicin), macrolides (erythromycin), lincosamides (clindamycin), tetracyclines (tetracycline), fluoroquinolones (ciprofloxacin, moxifloxacin), phosphonic acids (fosfomycin), glycopeptides (vancomycin,teicoplanin), oxazolidinones (linezolid), rifamycins (rifampicin), steroid antibiotics (fusidic acid) lipopeptides (daptomycin), glycylcyclines (tigecycline), pseudomonic acids (mupirocin), and cotrimoxazol). SCC*mec* elements were characterized by using a PCR approach including a combination of different PCRs as described (www.staphylococcus.net). PCR for *luk*-PV and for the genes of the immune evasion cluster (IEC) was performed as described previously [38]. PCR for IEC included *int*3 as a marker for integrase group 3 phages which usually contain the IEC. MRSA CC398 disseminated in equine clinics can be discriminated from LA-MRSA CC398 of other origin by means of a canonical SNPs (canSNPs [34]). By aligning whole genome sequences of three isolates of equine origin attributed to the equine clinic clade, one isolate of porcine origin (http://www.digibib.tu-bs.de/?docid=00058391) and 20 genomic sequences of isolates of different hosts attributed to CC398 published by Price et al. [39], we identified a further canSNP in position 1837869 in SAPIG1748 (AM990992.1,[40]), which can be easily identified by ordinary PCR and use of degenerated forward primers (SNP at the very 3′end, degenerated nucleotide in the second last position in bold): for the horse-specific clade of CC398, forward primer 1748 h1 5′ATGCTTTTTGGCCAGCT**I**T, (canSNP1748T), and for the general LA-MRSA CC398 subpopulation forward primer 1748u2 5′ ATGCTTTTTGGCCAGCT**I**G (canSNP1748G). As a reverse primer 1748r 5′ ATTACTCAAGGAACTGAA was used. PCR conditions (using PuReTaq Ready-To-Go PCR Beads (GE Healthcare)) were: 95 °C$^{5.00}$ [95 °C$^{0.30}$; 45 °C^{30}; 72 °C^{30}]x35; 72 °C$^{4.00}$. Correct amplification was confirmed by sequencing of the amplicons for reference strains SO385 (LA-MRSA subpopulation, [40]), and 71193 (human ancestral clade [41]), and 07-00334, (equine clinic subpopulation [34]).

This PCR was validated by application to a set of 195 isolates attributed to CC398 which was used for a phylogenetic analysis [34]. It comprised of 195 isolates from different host species (horse n = 53, human n = 80, pig n = 35, chicken n = 7, cattle n = 6, dog n = 5, turkey n = 4, goose n = 2, goat n = 1, cat n = 1, environment n = 1). PCR for antibiotic resistance genes and PCR conditions were performed as described previously [42]. For PCR detection of *dfr* genes we followed the protocols according to McDougal et al. [43] for *dfrA* and to Argudin et al. [44] for *dfr*G and *dfr*K.

Results

MRSA isolates from horses

As shown in Table 1 the majority of MRSA isolates from horses was attributed to CC398 (84.5%). Among the 135 isolates exhibiting *spa* type t011 and gentamicin resistance, n = 127 contained SCC*mec*IV and were attributed to the equine clinic specific clade by means of the canSNP in ORF1748. The latter also applied to 40 isolates exhibiting *spa* type t6867 and to isolates exhibiting the rare *spa* types t588, t779, t1255, t4628, t4872, t10643 and t13788.