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Comparison of livestock-associated and health care–associated MRSA—genes, virulence, and resistance☆

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ABSTRACT

Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) may colonize and infect humans with close contact to pigs. We compared phenotypic and genotypic differences in resistance and virulence of LA-MRSA isolates from farms and farmers with hospital-acquired methicillin-resistant *S. aureus* (HA-MRSA) and assessed carriage rates. Samples from pigs (n = 330), occupationally exposed personnel (n = 63), the farm environment (n = 134), and hospital patients (n = 220) were obtained. Approximately 50% (166/330) of pigs were MRSA positive. All LA-MRSA were resistant to tetracycline, compared to only 8% of HA-MRSA ($P < 0.001$). In contrast, HA-MRSA isolates showed significantly higher resistance rates to quinolones (81% versus 7%; $P < 0.001$). All strains isolated from occupationally exposed personnel (61.9%; 39/63) belonged to CC398. HA-MRSA isolates were diversely distributed, with predominance of CC5 (62.7%). Human strains carried significantly more virulence genes than porcine strains, especially exotoxins ($P < 0.001$) and immune-evasion cluster genes ($P < 0.001$). There were significant differences in resistance patterns and recognized genotypic virulence loci between LA-MRSA and HA-MRSA.

1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) remains a major health care issue and still causes many health care–associated infections associated with prolonged hospital stays and increased mortality (Borg et al., 2014; De Angelis et al., 2015). In 2003, an MRSA clonal complex (CC) emerged in farm animals in Europe, the CC398 (Garcia-Graells et al., 2012; Kock et al., 2013). These livestock-associated MRSA (LA-MRSA) appear to colonize and possibly infect humans when in close contact to colonized animals (Layer et al., 2012; Nair et al., 2016). Veterinarians, veterinary students, and agriculture workers are at particular risk for occupational acquisition of these LA-MRSA strains (Bos et al., 2014; Frana et al., 2013; Verstappen et al., 2014). The data on LA-MRSA transmission, however, are somewhat controversial. Epidemiological research suggests that these organisms are less virulent and less transmissible than health care–associated MRSA (HA-MRSA) strains (Bootsma et al., 2011; Garcia-Graells et al., 2012; van de Sande-Bruinsma et al., 2015; van Rijen et al., 2008; Wassenberg et al., 2011). However, LA-MRSA carriage with a higher risk of infection in LA-MRSA carriers and thus invasive potential has been reported for sublineages as well (Ballhausen et al., 2014; Denis et al., 2009; Lewis et al., 2008). Differences in the ability to cause infections in humans of different MRSA clones might be based on the genetic repertoire. Adaption to human or porcine hosts might affect bacterial microevolution and might result in differences in virulence gene patterns. Thus, the molecular epidemiology of MRSA is characterized by the occurrence of pandemic clones (Bootsma et al., 2011), which also differ phenotypically and genotypically in susceptibility patterns. Differences in antibiotic usage in livestock and in the human sector have been discussed as relevant factors of development of antimicrobial resistance among different MRSA clones resulting in phenotypic and genotypic differences in antimicrobial resistance.

The primary objectives of our study were to compare the genes of MRSA strains from hospital patients, pig farms, and humans working in pig livestock and to assess the differences in resistance and virulence genes. Second, we tried to determine phenotypic differences in susceptibility patterns between human and animal strains, and third, to assess prevalence rates of MRSA in pig farms.

2. Materials and methods

2.1. Study design and population

A cross-sectional study was conducted from June 2012 to October 2012 in 27 pig farms located in four federal states of Germany (Hesse, North Rhine-Westphalia, Baden-Württemberg and Lower Saxony). We collected nasal swabs from pigs (n = 330), farmers, veterinarians, veterinary students (n = 63), and the farm environment (dust samples) (n = 134). Participation was offered to all occupationally exposed individuals. Occupationally exposed individuals were defined as all persons (farm workers, veterinarians, veterinary students) who had at least

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1 week of continuous contact to pigs before sampling. After informed consent, participating individuals were screened for MRSA carriage (nasal and rectal swabs) and handed out a short questionnaire to evaluate risk factors and possible signs for MRSA infection (e.g., skin lesions, chronic wounds, etc.). Animal samples were equally distributed among pig farms; per farm, approximately 10 healthy animals were sampled.

In addition, 220 MRSA isolates were collected from inpatients of the Heidelberg University Hospital from December 2011 to October 2012. All bacterial isolates were collected by active screening using nasal swabs and rectal swabs or were collected from clinical specimen. If one site was found positive, the individual was classified as MRSA positive. The patients were screened at admission. Only high-risk patients were screened (search and destroy policy). High-risk patients were defined as patients admitted to intensive care units, patients admitted to hematology-oncology and bone marrow/solid organ transplant units, patients admitted to neonatology units, patients admitted to the dialysis unit, patients previously known to be a MRSA carrier, and patients who had received antibiotic therapy within the last 3 months before admission.

2.2. Isolation and identification of bacteria

Swabs were inoculated on Columbia 5% sheep blood agar plates (BD) and chromogenic plates for MRSA detection (ChromAgar MRSA II; BD) and incubated aerobically for 48 h at 36 °C. If growth on chromogenic plates was detected, identification by matrix-assisted laser desorption ionization–time-of-flight mass spectrometry (Bruker, Bremen, Germany), as described elsewhere, was performed (Eigner et al., 2009). Agglutination with Pastorex® StaphPlus (Alere, Jena, Germany) was performed to confirm early *S. aureus* growth. Dust samples were collected dry; briefly, sterilized brushes were used to collect dust samples, which immediately were transferred into collection tubes (Cellstar; Greiner Bio-One, Frickenhausen, Germany) and transported at room temperature to the laboratory. Environmental samples were collected in all stables from 3 specific locations: window ledges, partition walls between stables, and pipes under the ceiling. The collected dust samples were spiked into trypticase soy bouillon (CASO-Bouillon, Heipha, Germany) and incubated for 24 h at 36 °C. Samples were then inoculated on Columbia 5% sheep blood agar plate (BD) and chromogenic plates for MRSA detection (ChromAgar MRSA II; BD) and incubated aerobically for 48 h at 36 °C and further processed as described above.

2.3. Molecular methods

The Microarray system IdentiBac (Alere) was used for genotyping of MRSA strains according to manufacturer's instructions. IdentiBac was used to identify resistance genes and assess genetic relatedness between strains as well as genetic differences between porcine MRSA and MRSA strains from hospital patients.

We performed molecular typing by multilocus sequence typing for 323 MRSA isolates and stratified the strains by sequence types and subtypes. Furthermore, we performed SCC*mec* typing. Because of transmission dynamics in pig farms, animals in 1 farm are often colonized with the same strain; therefore, we contented ourselves with typing a representative sample of more than 80% of all LA isolates. Precisely, 133 of 166 porcine MRSA isolates, 32 of 39 MRSA isolates from exposed personnel, and 126 of 220 MRSA isolates from hospital patients were genotyped.

2.4. Antimicrobial susceptibility testing

Susceptibility testing was performed by VITEK2 (bioMérieux, Marcy l'E´toile, France) using the AST-P576 gram-positive card for all strains. Results were interpreted according to EUCAST clinical breakpoints. Disk diffusion test according to EUCAST criteria was used for mupirocin susceptibility testing; only the strains isolated from humans were tested for mupirocin resistance.

2.5. Statistical analysis

For descriptive purposes, arithmetic mean value, SD, median, interquartile range, and cumulative frequencies were calculated as appropriate. Categorical and continuous variables were analyzed using χ^2 tests and either the Student *t* tests or nonparametric tests, whenever appropriate. $P \leq 0.05$ was regarded as statistically significant. Statistical analysis was performed using the SPSS version 21.0 statistical package (SPSS, Chicago, IL, USA).

3. Results

3.1. MRSA in pig livestock

MRSA was found in 23 (85.0%) of the 27 investigated farms. Of the 330 animal nasal swabs, 166 (50.3%) were positive. The sampled pig cohort was divided into piglets (47.0%; n = 155/330), porkers (a pig that is fattened for meat production) (40.0%; n = 132/330), and sows (13.0%; n = 43/330). Porkers revealed the highest positivity rate (59.8%), followed by piglets (49.0%) and sows (25.6%). No animal was suffering from infection. Additionally, MRSA was detected in 77/134 (57.4%) of the environmental samples. Among the 63 occupationally exposed individuals, 61.9% (39/63) carried MRSA (24/38 farm workers, 12/16 veterinary students, 3/9 veterinarians). There were no statistically significant differences between the human categories. No occupationally exposed person was infected with MRSA.

3.2. MRSA in human sector

Of the 220 isolated HA-MRSA strains, 114 patients were collected by active screening of hospitalized patients. The remaining 106 strains were collected form clinical specimen for the purpose of microbiological diagnostic testing. The ratio of rectal to nasal MRSA carriage was 1:2.4.

3.3. Antimicrobial susceptibility testing

All porcine MRSA were resistant to tetracyclines, antibiotics frequently used in animal husbandry and animal health management. Tetracycline resistance was significantly ($P < 0.001$) more frequent in porcine MRSA strains than in hospital strains (8%) (Fig. 1). The resistance of porcine MRSA to gentamicin and trimethoprim was also significantly (17% and 50%, respectively) ($P < 0.001$) higher compared to hospital MRSA strains (3% each). Additionally, 73% of porcine MRSA isolates were resistant to fosfomycin, compared to 1% of hospital MRSA ($P < 0.001$). In contrast, hospital strains were significantly more resistant to quinolones than porcine strains (81% versus 7% to moxifloxacin and 81% versus 11% to ciprofloxacin; $P < 0.001$). Resistance to mupirocin was found in only 1 HA-MRSA isolate.

3.4. Genotyping results—resistance genes

The analyzed MRSA isolates from hospitalized patients, in comparison to porcine strains, contained significantly fewer resistance genes *tet*K and *tet*M (7.9% and 3.2%, respectively) ($P < 0.001$), which are mediating tetracycline resistance (Table 1). In comparison, the *fos*B gene, mediating fosfomycin resistance, was found significantly more often in hospital strains compared to porcine strains (74.6% versus 2.3%, respectively; $P < 0.001$). In the present study, no strain carried the genes *van*A/*van*B, mediating glycopeptide resistance. With the exception of a single strain, MRSA CC5 ST5/ST225 SCC*mec* II Rhine-Hesse, no other isolate carried the *mup*A gene for mupirocin resistance.

3.5. Genotyping results—virulence genes

The human strains showed significant differences compared to the porcine strains in presence of virulence genes (Table 2). Human strains