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Transmission of methicillin-resistant *Staphylococcus aureus* isolates on broiler farms

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ABSTRACT

The aim of the present study was to investigate the resistance pheno- and genotypes and the molecular typing characteristics of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from broiler farms in order to explore transmission between the different reservoirs. Thirty-seven MRSA CC398 isolates (11 from broilers, 15 from the broiler houses, 5 from farm residences and 6 from humans living and/or working on the farms) cultured from samples at four different farms during a previous study, were included. In addition to the previously determined *spa* types, the isolates were characterized by *dru* typing, *SCCmec* typing, pulsed-field gel electrophoresis and DNA microarray. Resistance phenotypes were determined by broth microdilution. Resistance genes were detected by DNA microarray or specific PCR assays. Selected isolates from broilers and humans ($n = 7$) were analysed by whole genome mapping. On the same farm, isolates from chickens, broiler houses, the farm residences and humans were often closely related or indistinguishable. On three of the four farms, however, MRSA isolates with different characteristics were present. On the one hand, the apparent similarity of MRSA isolates from the same farm indicates transmission between broilers, humans and their environment. On the other hand, different MRSA isolates were present on the same farm, indicating introduction from different sources or diversification over time. This study shows that different typing methods should be used to investigate epidemiological links between isolates and that whole genome mapping can be a useful tool to establish these links.

1. Introduction

Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA), isolates of the clonal complex (CC) 398 are often found as colonizers, but also as causative agents of infections in food-producing animals (primarily pigs, but also cattle and poultry) (de Neeling et al., 2007; van Duijkeren et al., 2007; Nemati et al., 2008; Kadlec et al., 2009; Feßler et al., 2010; Graveland et al., 2011; Geenen et al., 2013). LA-MRSA can be transmitted from food producing animals to humans, especially in persons that have close contact with the animals (Catry et al., 2010; Weese and van Duijkeren, 2010; Graveland et al., 2011). Several studies identified LA-MRSA with indistinguishable characteristics among food-producing animals and farm

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personnel (Khanna et al., 2008; Feßler et al., 2010, 2012; Graveland et al., 2011). Although the characteristics of LA-MRSA has been extensively studied on pig, cattle and veal calf farms, data on MRSA isolates from broilers and broiler farms are scarce. LA-MRSA isolates of the CC398 and CC9, have been identified in healthy and diseased poultry and in poultry meat (Nemati et al., 2008; Feßler et al., 2011; Monecke et al., 2013). LA-MRSA CC398 can colonize fattening turkeys and persons living on such farms (Richter et al., 2012). Personnel working at broiler slaughterhouses had an increased risk of MRSA carriage and this risk was significantly higher for personnel having contact with living animals (5.2%) than for all other personnel (1.9%) (Mulders et al., 2010). Molecular analysis identified MRSA isolates with indistinguishable characteristics from slaughterhouse personnel and poultry at slaughter, which might suggest an exchange of MRSA (Wendlandt et al., 2013c).

A previous study investigated the prevalence of LA-MRSA on broiler farms and identified risk factors for humans living and/or working on these farms. The authors concluded that living and/or working on MRSA-positive farms was a risk for MRSA-carriage, in particular in persons with contact to living animals (Geenen et al., 2013). There is only few data on transmission pathways of LA-MRSA on broiler farms. The aim of the present study was to analyse MRSA isolates from broilers, the farm environment and persons living/working on the broiler farms from the aforementioned study (Geenen et al., 2013). The characteristics, including the antimicrobial resistance pheno- and genotypes of the isolates were studied with different methods, to determine their genetic relatedness in order to gain hints towards transmission between the different reservoirs.

2. Materials and methods

2.1. Bacterial isolates and susceptibility testing

A total of 37 MRSA isolates, 11 from broilers, 15 from dust in the broiler houses, 6 from humans, and 5 from the farm residence were included in this study. These isolates originated from four farms investigated in a previous study (Geenen et al., 2013). All isolates were tested for their susceptibility to 30 antimicrobial agents by broth microdilution using custom-made microtitre plates (MCS Diagnostics, Swalmen, The Netherlands). The antimicrobial agents and test ranges were the same as previously described (Feßler et al., 2010, 2012). In addition, kanamycin susceptibility was tested by broth macrodilution in the range previously described (Monecke et al., 2013). Performance and evaluation followed the recommendations given in the document M31-A3 of the Clinical and Laboratory Standards Institute (CLSI, 2008). *S. aureus* ATCC®29213 served as quality control strain in the minimal inhibitory concentration (MIC) determinations.

2.2. Molecular analyses

While *spa* types of all MRSA had been determined previously (Geenen et al., 2013), all isolates were subjected to SCC*mec* typing, *dru* typing, and pulsed-field gel electrophoresis (PFGE) using the enzyme ApaI as described earlier (Kadlec et al., 2009; Feßler et al., 2010, 2012; Monecke et al., 2013). For the comparison of the PFGE patterns, the criteria published by Tenover et al. (1995) were applied. The unrelated main patterns (≥7 fragments difference) were numbered A-E, while patterns that were closely related (2–3 fragments difference) or possibly related (4–6 fragments difference) to one of the main patterns were indicated by additional numbers 1 and 2. All MRSA isolates were analysed by a *S. aureus*-specific DNA microarray (StaphyType, Alere, Jena, Germany) (Monecke et al., 2008a,b). This microarray detects more than 300 sequences, including species-specific genes, virulence genes and antimicrobial resistance genes. Arrays were mounted in microtitre stripes (ArrayStrip/ArrayMate/StaphyType system by Alere) and processed according to the manufacturer's protocols. The isolates were screened by specific PCR assays for recently detected resistance genes, which are not part of the microarray (Feßler et al., 2010, 2011; Wendlandt et al., 2013a,b).

A subset of seven isolates from humans or broilers from three farms, selected on the basis of their apparent similarity by other typing methods, were analysed by whole genome mapping (WGM). This method creates high-resolution, ordered whole genome restriction maps and was recently introduced as a typing tool for LA-MRSA (Bosch et al., 2013). High molecular weight DNA, with an average molecule size of approximately 250,000 bp, is required for WGM which was isolated using the Argus™ HMW DNA isolation kit (OpGen, Gaithersburg, USA). Thereafter, DNA was applied to Mapcards containing micro channels in which DNA molecules were stretched, immobilized to a glass surface, digested with *Afl*II and stained with a fluorescent agent in a micro fluids system. The resulting restriction fragments were sized in the whole genome mapper and assembled into a whole genome map in which the restriction sites are mapped in the order in which they occur in the chromosome. Bionumerics software version 7.0 (Applied Maths, Sint-Martens-Latem, Belgium) was used for the analysis and clustering of the whole genome maps. The WGM cut-off value for isolates with indistinguishable whole genome maps was >98%. Isolates with 95–98% resemblance were considered as closely related. Isolates with <95% resemblance were classified as unrelated (Bosch et al., 2013).

3. Results and discussion

3.1. Resistance pheno- and genotypes of the MRSA isolates

Table 1 shows the resistance phenotype and genotype of each of the 37 MRSA isolates. All isolates were resistant to at least three classes of antimicrobial agents. All isolates were resistant to β-lactams and tetracyclines, and all but one isolate were resistant to trimethoprim and all but two were resistant to macrolides/lincosamides (ML). Non-susceptibility to gentamicin ($n = 18$) was also common. The genes *mecA*, *blaZ/I/R* (β-lactam resistance) and *tet*(M) (tetracycline resistance) were detected in all isolates. The tetracycline resistance genes *tet*(K) ($n = 15$) and *tet*(L)