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Prevalence of types of methicillin-resistant *Staphylococcus aureus* in turkey flocks and personnel attending the animals

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SUMMARY

Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) have been isolated from a number of livestock species and persons involved in animal production. We investigated the prevalence of LA-MRSA in fattening turkeys and people living on farms that house fattening turkeys. Eighteen (90%) of 20 investigated flocks were positive for MRSA, and on 12 of the farms 22 (37·3%) of 59 persons sampled were positive for MRSA. People with frequent access to the stables were more likely to be positive for MRSA. In most flocks MRSA that could be assigned to clonal complex (CC) 398 were detected. In five flocks MRSA of *spa*-type t002 that is not related to CC398 were identified. Moreover, other methicillin-resistant *Staphylococcus* spp. were detected on 11 farms and in eight people working on the farms.

Key words: Livestock, methicillin resistance, occupational health, turkeys, zoonoses.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) have been isolated from a number of farm animal species including pigs [1, 2], broilers [3], veal calves [4], and dairy cattle [5, 6]. While it has been pointed out, that meat from turkeys is quite often contaminated with MRSA [7], only limited information is available regarding the situation in turkey flocks with respect to prevalence, site of colonization or infection and involved strains.

For pigs, cattle and broilers colonization of humans with occupational exposure to colonized livestock is also well documented [3, 5, 8], while corresponding information for turkeys and people handling turkeys is scarce. The objective of this study was to analyse the prevalence of MRSA in turkeys on farms, to characterize the isolates, to evaluate potential sampling material and to analyse the risk of colonization for people working on the farms.

MATERIAL AND METHODS

A total of 500 swab samples were taken in a random sample of 20/90 meat turkey farms located in three districts in Baden-Württemberg in the southwest of Germany, from June to October 2009. Farmers were

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informed on the aims of the study prior to agreeing to participate. One tracheal and one cloacal swab from each of 10 turkeys per flock and five dust samples per turkey house were collected 1 day to 2 weeks prior to slaughter. The number of turkeys sampled was determined with regard to the need for acceptable sensitivity for the investigation, and to limit the stress to the flock, thereby assuring acceptance by the herdsmen. Limitations to the sensitivity of animal samples were considered acceptable as additionally dust samples were collected. Half of the animals sampled were females aged 14–16 weeks, the other half were males aged 18–20 weeks. The animals were of the turkey breed Big 6 (18 flocks) or Big 9 (two flocks).

In the examined turkey flocks an average of 7927 (range 3000–20 000) animals were fattened. Male meat turkeys reached an average weight of 18·8 kg, and females 10·3 kg shortly before slaughter. The average feed conversion ratio was 1:2·6, i.e. 2·6 kg of feed were needed for 1 kg of body weight gain. The mortality rate of the male turkeys averaged at 10%, and that of females 4%. The meat turkeys originated from three different breeder companies, supplying 12, seven and one of the investigated flocks. During the fattening period antimicrobials had been administered to the turkeys as group treatment in all of the flocks in cases of disease. Most of the meat turkeys were kept on wood shavings and/or straw in an open building construction.

Five dust swab samples were taken from areas (500 cm^2) of different localizations in the turkey houses. In all turkey houses, sampling sites included windowsills of the right and left side of the house, the surface of a feed trough, the surface of a food distribution system and the wall of the separation area for sick animals. All samples were collected with sterile swabs while wearing sterile gloves. The samples were transported in a cool box to the laboratory and analysis commenced on the same day.

Furthermore, nasal swabs were taken from three persons per farm, except for one farm where only two persons could be included in this study. People were informed about the nature of the study and agreed to participate. Two groups of people were tested. One group consisted of persons who had been in the turkey houses at least once a week ($n=39$, two persons per farm, one person missing), the other group included persons who had been in the turkey houses never or less than once per week ($n=20$, one person per farm).

Isolation of MRSA

All samples were processed according to the protocol for a study in the EU, described in detail in the Commission Decision 2008/55/EC [9]. This method utilizes a combination of pre-enrichment, selective enrichment and detection of MRSA by cultivation on a chromogenic selective agar. The tracheal and cloacal swabs as well as the human samples were examined individually, the five dust swabs were pooled per turkey house.

The single swabs were inoculated in 10 ml and the five dust samples pooled in 100 ml Mueller–Hinton broth (Merck, Germany) enriched with 6·5% NaCl (Merck). The Mueller–Hinton broth was incubated at 37 °C for 16–24 h and subsequently 1 ml was transferred into 9 ml tryptone soy broth (TSB) supplemented with 3·5 mg/l cefoxitin and 75 mg/l aztreonam (CM0129, Oxoid, Germany). TSB was incubated for a further 16–24 h at 37 °C and in a final step 20 μl broth were plated onto chromogenic selective MRSA agar (MRSA Ident agar 1348e, Heipha, Germany). After another incubation period of 24 h at 37C, 2–5 of the colonies showing a wine-red colour (MRSA) or a white, translucent appearance (methicillin-resistant *Staphylococcus* spp. other than *S. aureus*) were subcultured for species identification.

Detection of the *mec*A gene by real-time PCR

The selected colonies grown on the chromogenic agar were submitted to a SybrGreen real-time PCR assay for detection of the *mec*A resistance gene. The real-time PCR assay was designed on the basis of a common gene sequence obtained by an alignment of the *mec*A gene sequences EU790489, AB221123, AB221124, AB221120, and EO9771 derived from the NCBI Gene Bank. Using Primer Express 2.0 software (Applied Biosystems, Germany) the oligonucleotides TGA AAA ATG ATT ATG GCT CAG GTA CT (forward primer) and CAT AT GAA GGT GTG CTT ACA AGT GC (reverse primer) were designed as primers generating a PCR product of 81 bp. The use of reagents, execution and evaluation of the PCR corresponded to the method of Sting & Stermann [10]. Amplification and melting-curves matching those of the MRSA reference strain DSM 11729 were considered positive. The melting curves showed a peak at 73·5 $\pm$ 1 °C.

Before the PCR was applied for investigation of field samples validation studies were performed using *mec*A gene-positive (DSM 11729) and *mec*A

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gene-negative (ATCC 25923) reference strains of *S. aureus* and further 40 *S. aureus* field isolates. The field isolates had been previously tested as *mec*A gene-positive (20 isolates) or *mec*A gene-negative (20 isolates) using the Light Cycler System (Roche Diagnostics, Germany) [11].

To confirm nucleotide sequences of the *mec*A gene as specific binding sites for the primers, DNA sequencing was performed on MRSA strain DSM 11729 using primers CAA TAC AAT CGC ACA TACA TTA ATA GAG AA (forward primer) and TCG AGT GCT ACT CTA GCA AAG AAA AT (reverse primer) resulting in an amplicon of 487 bp that embraced the relevant sequence of the amplicon generated by the *mec*A real-time PCR. The PCR reaction mixture consisted of 20·0 μl Mastermix (PCR MasterMix, Germany), 0·1 μM of each primer (final concentration) and 5 μl template, filled with aqua dest to a final volume of 50 μl.

The following run conditions of the real-time PCR were used: initial pre-denaturation (94 °C, 120 s) followed by an initial denaturation step at 94 °C for 30 s, 35 cycles of 55·8 °C for 30 s, 72 °C for 60 s, and a final extension step at 72 °C for 300 s. The PCR products were visualized by agarose gel electrophoresis and the band cut out for subsequent purification (QIAquick PCR Purification kit, Qiagen, Germany) and DNA sequencing on demand (Microsynth, Switzerland).

Fourier transform infrared (FT-IR) spectroscopy

Pure cultures of *mec*A-bearing isolates grown on non-selective agar supplemented with 2% sheep blood (blood agar base CM55 and sheep blood SR0051C, Oxoid) were prepared to dried bacteria films for FT-IR as described previously [12]. The infrared transmission spectra were recorded for each sample from 500 to 4000 cm^{-1} using FT-IR spectrometer (IFS 28/B, BrukerOptics, Germany). The acquisition and first analysis of data were performed using OPUS software (v. 4.2, BrukerOptics). The differentiation methods were constructed with NeuroDeveloper software (v. 2.5b, Synthon, Germany) which is based on an artificial neural network concept (ANN) [12].

The hierarchical classification on genus and species levels was integrated in a database containing spectra of 550 Gram-positive isolates. As previously described, the identification was performed by a procedure of several discriminating steps by FT-IR/ANN analysis [12]. In a first step staphylococci could be clearly separated from other Gram-positive bacteria, e.g. Actinomycetales, bacilli, *Listeria* or streptococci. The second level separated strains of *S. aureus* from other staphylococci, which were differentiated at the species level in further steps. According to internal validation results, the species of the selected isolates could be identified with a probability for a correct result of 96·4% ($n=312$ isolates) for repeated determination. According to validation results of this set of isolates the error rate is 0·4% (data not shown).

Molecular typing of MRSA

Chromosomal DNA of the staphylococcal isolates identified as MRSA was extracted using the RTP bacteria DNA Mini kit (Invitek, Germany) and isolates were confirmed as MRSA by multiplex PCR [13]. Subsequently, SCC*mec*-typing [14] and *spa*-typing [15] were performed. In addition, the multi-locus sequence type (MLST) was determined in one isolate per identified *spa* type [16]. DNA sequencing of the PCR products was performed by Qiagen (Germany). *spa*type and MLST were determined using Ridom Staphtype software (Ridom GmbH, Germany) and the *S. aureus* MLST database (http://www.saureus.mlst.net), respectively. Extensively characterized MRSA field isolates taken out of the strain collection of the NRL-Staph [SCC*mec*-typing: MRSA 9 (type II), MurSA-S-143 (type III), MurSa-S-66 (type IVa), MRSA 2 (type V)] and the *S. aureus* reference strains DSM 1104 (*spa*-typing, species identification) and DSM 13661 (species identification) were used as control strains.

For the analysis strains were compared based on their *spa*-type/SCC*mec*-type combinations. Odds ratios and their confidence intervals and Cohen's κ were calculated according to the method of Thrusfield [17].

Testing for antimicrobial resistance

Susceptibility testing and evaluation of resistance were performed as described previously [2]. A selection of 95 isolates from humans ($n=21$), tracheal swabs ($n=35$), cloacal swabs ($n=24$), and dust ($n=15$) were tested. The selection was based on coverage of the different *spa* types and isolates from the different farms. All isolates were tested against the following antimicrobials using commercial discs (Oxoid): ciprofloxacin (5 μg), clindamycin (2 μg), erythromycin (15 μg), fusidic acid (10 μg), gentamicin (10 μg), kanamycin (30 μg), linezolid (30 μg), mupirocin (20 μg),

oxacillin (1 μg), quinupristin/dalfopristin (15 μg), rifampicin (5 μg), sulfamethoxazole/trimethoprim (25 μg), teicoplanin (30 μg), and tetracycline (30 μg). The reference strain *S. aureus* ATCC 25923 was used as a quality control.

RESULTS

MRSA in turkey flocks

MRSA was detected in 18 (90%) of the 20 turkey flocks investigated (Table 1). Two flocks (farms 4 and 5) were MRSA negative in all animal and environmental samples taken. All female and eight male flocks were positive. In 16 (89%) of the 18 positive flocks, MRSA could be found in all types of samples, i.e. tracheal, cloacal and dust swabs. In one flock (farm 14), only the environmental swab and in another flock (farm 12) only one tracheal swab and the environmental swab were positive, whereas all cloacal swabs were negative. Agreement of animal samples and environmental samples for the classification of the flocks was $\kappa = 0{\cdot}90$ for cloacal swabs and $\kappa = 0{\cdot}94$ for tracheal swabs.

In two farms (farms 12 and 18) the *spa*-type/SCC*mec*-type combination obtained from MRSA isolated from environmental swab differed from that of the animal samples. When considering *spa*-type/SCC*mec*-type combinations, κ fell to 0·85 for tracheal swabs and dust and to 0·69 for cloacal swabs and dust.

MRSA in individual turkeys

Of the total 200 turkeys examined 143 (71·5%) animals proved to be MRSA positive. In 84 (58·7%) of these turkeys MRSA was isolated from tracheal and cloacal swabs, 45 (31·5%) animals harboured MRSA organisms only in the trachea and 14 (9·8%) animals only in the cloaca. Agreement of the samples was $\kappa = 0{\cdot}70$. Of the animals that harboured MRSA in trachea and cloaca, 70 (83·3%) carried MRSA of the same *spa*-type and SCC*mec*-type combination in both locations and 14 (16·7%) were colonized by different strains in trachea and cloaca. Considering the differences in types agreement fell to $\kappa = 0{\cdot}62$.

MRSA in people working on turkey farms

Of the 59 persons sampled by nasal swabs, 22 (37·3%) were MRSA-positive. None of these samples showed clinical symptoms indicative of an MRSA infection. The people lived on 12 farms, of which 10 housed MRSA-positive turkey flocks. Four positive individuals lived on two farms with negative flocks. In one of these two negative flocks (farm 4) only other methicillin-resistant *Staphylococcus* spp., and no MRSA were detected in livestock. Thirteen people working on the farms harboured isolates with the same *spa*-type/SCC*mec*-type combination of MRSA that was also detected in the animals or in the dust sample of the same farm. Five people carried a MRSA type different from those of the animals and the dust samples from the same farm. People with daily or at least one contact per week with turkeys were found to be more likely [odds ratio (OR) 3·43, 95% (CI) 1·00–11·71] a carrier of MRSA (18/39 people with frequent contact, 46·2%) than persons being rarely (less than once a week) or never in the turkey houses (4/20 people with infrequent contact, 20·0%).

Other methicillin-resistant *Staphylococcus* spp.

Other methicillin-resistant *Staphylococcus* spp. were detected in 11 (55%) of the 20 flocks. In 10 of these flocks these species were only detected in animals (tracheal swab and cloacal swab). In one flock, *S. saprophyticus* was detected in a tracheal swab and in the dust sample (Table 2).

Eight (13·6%) persons from seven farms harboured other methicillin-resistant *Staphylococcus* spp. and one of these carried MRSA concurrently in their nasal mucosa. Six (15·4%) of these persons were among the 39 people with regular access to the turkey flocks, while one person with rare contact and one person without contact with the turkey flocks was colonized by other methicillin-resistant *Staphylococcus* spp. The difference between the two groups in the prevalence of these species was not significant (OR 1·6, 95% CI 0·3–8·8).

Characterization and typing of staphylococcal isolates

The *mec*A gene was detected in 317 staphylococcal isolates from animals, the environment and people. Of these, 267 were identified as *S. aureus* (129 tracheal, 98 cloacal, 18 dust, 22 human swabs). These MRSA were from five different *spa* types and carried a variety of SCC*mec* (Table 3). The most common *spa* types were t011 followed by t002, t1456, t034 and t2330. Details of *spa* types and SCC*mec* types are given in

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Table 1. *MRSA types* (spa *types*/*SCC*mec *types*) *detected in swabs from tracheae, cloacae and dust and in humans on 20 turkey meat production farms*

	Number of positive samples (*n*) per *spa*-type/ SCC*mec*-type combination (numbers in parentheses represent number of isolates if >1)			
Farm no. (sex of animals)	Tracheal swabs	Cloacal swabs	Dust swabs	Human swabs
1 (male)	t011/V (8) ST398 (1)	t011/V (5)	t011/V	t011/V (3) ST398 (1)
2 (male)	t011/IVa (8)	t011/IVa (7) t1456/IVa	t011/IVa	
	t1456/V t2330/V ST398			
				t011/V (2)
3 (male)	t002/nt (4) ST1791 (1)	t002/nt	t002/nt	
4 (male)				t011/V (3)
5 (male)				t011/IVa
6 (male)	t011/V (10)	t011/V (6)	t011/V	t011/V (2)
7 (female)	t011/IVa (8) t002/nt (2)	t011/IVa t002/nt (3)	t011/IVa	t011/IVa (2)
8 (male)	t1456/V (9) ST398 (1)	t1456/V (5)	t1456/V	
9 (female)	t011/IVa (6)	t011/IVa (3) t011/nt		
	t002/nt (3)	t002/nt (2)	t002/nt	
				t034/V ST398 t1456/V ST398
10 (male)	t011/V (8)	t011/V (9)		t011/V t011/IVa
	t002/nt (2)		t002/nt	
11 (female)	t011/IVa (5) t011/V	t011/IVa	t011/V	
12 (female)	t011/IVa			
			t011/V	
13 (male)	t011/V (10)	t011/V (10)	t011/V	t011/V (2)
14 (male)			t011/IVa	
15 (female)	t011/IVa (3) t011/nt (6)	t011/IVa t011/nt (7)	t011/IVa	t011/IVa
16 (female)	t011/V (3)	t011/V (3)	t011/V	
17 (female)	t011/V (6) t034/V ST398	t011/V (5)	t034/V	t011/V
18 (female)	t011/V (4)	t011/V (6) t011/IVa t002/nt		
			t011/nt	
19 (female)	t011/ V (4) t011/IVa (3) t011/nt (2)	t011/ V (5) t011/IVa (4)	t011/IVa	
20 (female)	t011/V t011/IVa (9)	t011/IVa (10)	t011/IVa	t011/ V
Total	129	98	18	22

nt, Not typable with the SCC*mec* method used.

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Table 2. *Methicillin-resistant* Staphylococcus *spp. other than* S. aureus *in 15 study farms** (*numbers in parentheses represent number of isolates if* >1)

Farm no.	Tracheal swabs	Cloacal swabs	Dust swabs	Human swabs
1	*S. saprophyticus*	*S. epidermidis*		
		S. haemolyticus		
		S. hyicus		
2	*S. haemolyticus*	*S. haemolyticus*		
4		*S. hyicus*		
		S. intermedius		
5				*S. haemolyticus*
6	*S. saprophyticus*			
	S. haemolyticus			
7		*S. saprophyticus*		
8	*S. saprophyticus* (2)	*S. saprophyticus*		*S. epidermidis*
	S. haemolyticus (4)	*S. haemolyticus* (3)		
9	*S. saprophyticus* (2)	*S. saprophyticus* (3)		
10				*S. haemolyticus*
11	*S. haemolyticus* (2)	*S. haemolyticus* (2)		
		S. intermedius		
		S. hyicus (2)		
12				*S. epidermidis*
13	*S. saprophyticus*		*S. saprophyticus*	
16	*S. saprophyticus*	*S. saprophyticus*		
		S. haemolyticus (3)		
				S. epidermidis (2)
17				*S. haemolyticus*
20		*S. haemolyticus* (2)		
				S. epidermidis
Total	16	25	1	8

* No isolates of methicillin-resistant *Staphylococcus* spp. other than *S. aureus* were obtained from farms 3, 14, 15, 18 and 19.

Table 3. *Summary statistics of MRSA types in different samples*

	Number of positive samples (*n*)				
MRSA type (*spa*-type/SCC*mec*-type combination)	Tracheal swabs	Cloacal swabs	Dust swabs	Human swabs	Total
Number of samples examined	200	200	20 pools	59	479
MRSA t011/SCC*mec* V	55	49	6	15	125
MRSA t011/SCC*mec* IVa	43	28	6	5	82
MRSA t011/SCC*mec* nt	8	8	1		17
Total: MRSA t011 (%)	82·2	86·7	72·2	90·9	83·9
MRSA t034/SCC*mec* V	1		1	1	3
MRSA t1456/SCC*mec* V	10	5	1	1	17
MRSA t1456/SCC*mec* IVa		1			1
MRSA t002/SCC*mec* nt	11	7	3		21
MRSA t2330/SCC*mec* V	1				1
Total	129	89	18	22	267
Total (%)	48·3	36·7	6·8	8·2	100

nt, Not typable with the SCC*mec* method used.

Table 1. Eight MRSA isolates covering the full spectrum of *spa* types detected in the turkeys (t011, t002, t034, t1456, t2330) and persons (t011, t034, t1456) were subjected to MLST. Of these seven could be assigned to ST398 and one (t002) originating form a turkey tracheal swab to ST1791.

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Fifty isolates were from other *Staphylococcus* spp. By means of FT-IR, 23 isolates were identified as *S. haemolyticus* (10 flocks: 8 tracheal, 12 cloacal, 3 human swabs), 15 as *S. saprophyticus* (7 flocks: 8 tracheal, 6 cloacal, 1 dust sample), six as *S. epidermidis* (5 flocks: 1 cloacal, 5 human swabs), four as *S. hyicus* (3 flocks: 4 cloacal swabs) and two as *S. intermedius* (2 flocks: 2 cloacal swabs).

Antimicrobial resistance in MRSA

Overall, 95 *mec*A-positive isolates of *S. aureus* were tested for their phenotypic resistance to 15 antimicrobials. During the fattening period antimicrobials were administered to the turkeys in all of the flocks in cases of disease. The used drugs were enrofloxacin, benzylpenicillin, amoxicillin, colistin, oxytetracycline, neomycin and tiamulin. Nearly all isolates were resistant to tetracycline (93/95, 97·9%) and oxacillin (86/95, 90·5%). The majority of isolates were resistant to erythromycin and clindamycin (79/95, 83·2%). A number of isolates were also resistant to kanamycin (40/95, 42·1%), gentamicin (26/95, 27·4%) and ciprofloxacin (30/95, 31·2%). Few isolates were resistant to chloramphenicol (7/95, 7·4%) and trimethoprim/sulfamethoxazole (1/95, 1·1%). All isolates were susceptible to teicoplanin, fusidic acid, mupirocin, quinupristin/dalfopristin and linezolid.

Resistance patterns differed between *spa* types. All (12/12) t002 were resistant to ciprofloxacin. Similarly, the t2330 (1/1) and most (9/10, 90%) t1456 isolates were resistant to ciprofloxacin. On the other hand, only a small number (8/70, 11·4%) of t011 isolates were resistant to this substance and none of the two t034 tested. Moreover, t002 and t1456 were more often (100%) resistant to erythromycin and clindamycin than t011 (77·1%). On the other hand, they were less often resistant to gentamicin and kanamycin (data not shown).

DISCUSSION

MRSA in turkey flocks and people working in turkey farms

The results of the present study show that 18 (90%) of the 20 investigated turkey flocks harboured MRSA and 71·5% of the animal carried MRSA at least in one body site. This is in line with reports on other livestock [18] and on turkey meat [7, 19]. The prevalence of MRSA in the examined turkey flocks was higher than in other investigations on livestock in Germany that also examined dust samples.

In all MRSA-positive flocks the dust swab sample was positive for MRSA. This indicates that dust sampling should be sufficient to monitor the presence of MRSA in turkey houses. However, only ten individual animals per flock were tested and a low level intra-flock prevalence could have been overlooked. However, in most (15/18) flocks more than half of the tracheal swabs were positive for MRSA, indicating that the presence of MRSA within a flock is often associated with a high intra-flock prevalence.

Dust samples do not allow the assessment of the intra-flock prevalence and the diversity of MRSA types within a flock. Our data show that there may be considerable diversity of types, which may best be detected by using tracheal swabs, as these were more often positive than cloacal swabs.

As reported for people in contact with pigs or veal calves [4, 20], frequent contact with turkeys increased the odds of being colonized with MRSA. Consequently, people in contact with turkeys are considered a risk group for nasal colonization with MRSA. Screening of all relevant professional groups at admission to healthcare facilities is useful to minimize the possible entry of resistant bacteria in hospitals and thus any potential risks to patients and staff. Further studies on the epidemiology of MRSA and its spread in poultry flocks and routes of transfer to humans are necessary and important.

Other methicillin-resistant staphylococci

Other methicillin-resistant staphylococci have been considered as a potential reservoir for SCC*mec* elements to be shared with *S. aureus* on pig farms [21]. No such information was available for turkey farms so far. Other methicillin-resistant staphylococci were detected in 11 (55%) of the 20 turkey flocks and eight (14%) of the 59 people examined. The selective media used are designed to select for MRSA. The detection of other methicillin-resistant *Staphylococcus* spp. can therefore only be regarded as a suggestion that these bacteria exist in the investigated population and may serve as a reservoir for SCC*mec* elements. However, the study protocol did not allow for valid prevalence estimation for these *Staphylococcus* spp. Further research is needed to estimate the prevalence of methicillin-resistant staphylococci other than *S. aureus* and to assess their clinical and potential public health relevance.

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Typing results

MLST, *spa*-typing and SCC*mec*-typing have been used individually or in combinations to investigate the evolution and clustering of the MRSA clones and their worldwide distribution [22, 23]. Our typing results show that in the majority of cases most birds within MRSA-positive flocks carry isolates that are characterized by the same combination of *spa* types and SCC*mec* types. This is suggestive of horizontal spread within the flock. In the present investigation *spa* types t011, t002, t1456, t034 and t2330 were detected. The most common *spa* type was t011 representing 83·2% of the avian and 90·2% of the human isolates. The *spa* types t002, t1456, and t034 occurred considerably less, and reached altogether a proportion of only 16% among all types. All t011, t1456, t034, and t2330 isolates subjected to MLST were characterized as CC398. The MLST CC398 comprising *spa* types t011, t1456, and t034 was also found by other authors in poultry and poultry meat [24–27]. In addition, it is known that mainly in people having contact with pigs, *spa* types t011 and t034 belonging to MLST CC398 can be isolated [24, 25]. The *spa* type t2330 isolated from a tracheal swab has been described for pigs, but not for turkeys [26, 27]. One tracheal isolate of *spa* type t002 was identified as MLST ST1791. In contrast to our findings, in animals *spa* type t002 is commonly reported in combination with the MLST ST5. It has also been identified in samples of turkey meat [19]. In contrast to LA-MRSA, t002 is one of the most frequently isolated types in humans in Germany [28]. However, in our study, it was only detected in animals and dust and the source of the pathogen is not clear. Further investigations into the isolates, comparing them to the strains prevalent in human medicine are required.

About half of the strains belong to SCC*mec* V, about a third to SCC*mec* IVa, leaving the remainder as untypable. Taking combinations of *spa*-typing and SCC*mec*-typing into account, the discriminatory power could be increased. Based on this information, all *spa*-type/SCC*mec*-type associations isolated from people could also be found in turkeys, suggesting that those strains can be transmitted from production animals to humans. The partial disagreement in the types of MRSA and *Staphylococcus* spp. between livestock and people and the presence of MRSA in people attending apparently negative flocks calls for further investigations. Potential explanations include exposure to different methicillin-resistant bacteria spread during previous fattening periods or by other fattening groups and contact with resistant bacteria from other origins that have not been identified so far. Since all MRSA isolates detected in people were from *spa* types associated with CC398, a livestock origin of the colonization is the most likely explanation. None of the positive individuals showed clinical signs indicative of an MRSA infection. This is in line with reports on the absence of genes for many virulence factors of *S. aureus* in MRSA CC398 [29]. Colonization with MRSA has been shown to increase the risk of infection fourfold [30]. However, LA-MRSA were not included in that study. Taking these aspects into account the prevalence and epidemiology of MRSA from food and animals should be investigated further.

Antimicrobial resistance

Monitoring of resistance patterns of multidrug-resistant pathogens is pivotal for treatment regimens and strategies. Overall the resistance patterns were similar to those reported for LA-MRSA previously [1, 2]. A number of isolates carrying the *mec*A gene did not express resistance to oxacillin. This has previously been reported and may in part be explained by heteroresistance [31]. To address this observation properly, genetic investigations will be required, which were not part of the present study.

However, the resistance patterns differed between *spa* types, with t002 and t1456 being frequently resistant to ciprofloxacin. This is in contrast to reports on pigs, and the results for t011 from turkeys which were less frequently resistant to ciprofloxacin. Fluoroquinolones are licensed for oral medication in poultry in Germany but not in pigs. Therefore resistance to this group of drugs may be an advantage in the poultry population. This is in line with a higher frequency of ciprofloxacin resistance in other zoonotic pathogens in poultry compared to pigs [32, 33]. These results call for further monitoring studies considering the types of pathogens as well as the animal species they originate from.

CONCLUSIONS

The prevalence of MRSA in the investigated turkey flocks was high and the predominant *spa* types were typical LA-MRSA. However, *spa* types from other clonal complexes were also detected. Dust from different locations in the barn and tracheal swabs proved

to be suitable sampling material for the dectection of MRSA in turkey flocks, while cloacal swabs were less frequently positive. People working on turkey farms, especially those working in the barn on a regular basis have an increased risk of being colonized with MRSA compared to the general public and should therefore be considered risk patients with respect to introduction of LA-MRSA into healthcare facilities.

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DECLARATION OF INTEREST

None.

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