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Resistance phenotypes and genotypes of methicillin-resistant *Staphylococcus aureus* isolates from broiler chickens at slaughter and abattoir workers

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Objectives: To comparatively investigate the resistance phenotypes and genotypes of various methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from broilers at slaughter and workers at the respective poultry slaughterhouses.

Methods: Forty-six MRSA isolates (28 from broilers and 18 from humans) obtained at four different slaughterhouses were included. In addition to previously determined sequence types (STs) and *spa* types, the isolates were characterized by *dru* typing, SCC*mec* typing and PFGE. Resistance phenotypes were determined by broth microdilution. Resistance genes and clonal complexes (CCs) were detected by DNA microarray or specific PCR assays.

Results: MRSA of CC398, *spa* type t011 and varying *dru* types represented 23/28 broiler isolates and 12/18 human isolates. Three ST9/t1430/dt10a isolates were each seen among the isolates from the abattoir workers and the broilers. In addition, two human CC398/ST1453/t4652/dt3c isolates, a single human CC398/t034/dt6j isolate and two chicken CC398/t108/dt11a isolates were detected. All CC398 isolates (including ST1453) and some of the ST9 isolates from chickens and humans showed resistance to four to nine classes of antimicrobial agents and carried a wide range of resistance genes. While the resistance phenotypes and genotypes of the chicken isolates of the same flock were closely related, they usually differed from the resistance phenotypes and genotypes of the isolates from the workers at the respective slaughterhouse.

Conclusions: The apparent homogeneity of MRSA isolates from the same flock suggests exchange of isolates between the respective animals. The apparent heterogeneity of MRSA isolates from abattoir workers might reflect their occupational contact with animals from numerous chicken flocks.

Keywords: *spa* typing, *dru* typing, DNA microarrays, MLST, resistance genes

Introduction

Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) has been shown to readily cross species barriers and to colonize or cause infections in a wide variety of animal species, but also in humans with exposure to livestock.[1–3] Several studies identified LA-MRSA with indistinguishable characteristics among food-producing animals and farm personnel. While these observations were mainly made on swine, veal calf or dairy farms,[4–10] comparatively little is known about poultry.[11] LA-MRSA, in particular isolates of clonal complexes (CCs) 398 and/or 9, have been identified in healthy chickens,[12] diseased chickens and turkeys,[13] and also in food of chicken and turkey origin.[14] Based on these observations, it is likely that LA-MRSA are present in commercially reared poultry and may also be transferred to humans occupationally exposed to poultry. Such occupational exposure may occur on the farm level, but also at slaughterhouses. A previous study investigated the prevalence of LA-MRSA in broiler chicken flocks and identified risk factors for slaughterhouse personnel in the Netherlands.[15] The authors concluded that there is an increased risk of MRSA carriage in personnel working at broiler slaughterhouses, in particular when the workers have contact with live animals.[15]

The aim of the present study was to comparatively analyse MRSA isolates of broilers and slaughterhouse personnel from the aforementioned study[15] for their molecular characteristics and their antimicrobial resistance phenotypes and genotypes.

Materials and methods

Bacterial isolates and susceptibility testing

A total of 46 MRSA isolates, which comprised 28 from broilers and 18 from humans, were included in this study. These isolates originated from a previous study[15] and isolates from all slaughterhouses ($n=4$), in which MRSA had been identified in both broiler chickens and abattoir workers, were included. The chicken isolates from each slaughterhouse originated from one to four different flocks. Although the isolates had been tested for their susceptibility to 12 antimicrobial agents using the Vitek system (bioMérieux, Craponne, France),[15] all isolates were reinvestigated for their susceptibility to 30 antimicrobial agents. The antimicrobial agents and test ranges were the same as previously described.[7,8,13] Susceptibility testing was conducted by broth microdilution using custom-made microtitre plates (MCS Diagnostics, Swalmen, the Netherlands). Performance and evaluation followed the recommendations given in CLSI document M31-A3.[16] *S. aureus* ATCC 29213 served as a quality control strain in the MIC determinations.

Molecular analyses

While *spa* types of all isolates and multilocus sequence types (STs) of the isolates with *spa* types t4652 and t1430 had been determined previously,[15] all isolates were subjected to SCC*mec* typing and *dru* typing as described previously.[13,14] To see more subtle variations that usually cannot be detected with the sequence-based typing methods, PFGE using the enzyme ApaI was conducted for all isolates. The preparation of DNA-containing agarose plugs and the performance of PFGE experiments were conducted as described previously.[8]

All MRSA isolates were also subjected to a previously described *S. aureus*-specific DNA microarray (StaphyType, Alere Technologies GmbH, Jena, Germany).[17] This microarray detects >300 sequences, including species-specific genes, virulence genes and antimicrobial resistance genes. This microarray was also used to confirm that isolates of *spa* types t011, t108 and t034 belonged to CC398. Arrays were mounted in microtitre strips (ArrayStrip/ArrayMate/StaphyType system, Alere Technologies GmbH) 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 current version of the microarray.[18,19]

Results and discussion

Molecular typing of the MRSA isolates

Among the 18 human isolates, 13 represented CC398, another 2 isolates ST1453 and 3 isolates ST9 (Table 1). It should be noted that ST1453 is a single locus variant of ST398, which has the *tpi* (triosephosphate isomerase) allele 175 in contrast to 26 for ST398. A similar distribution was seen among the MRSA from broiler chickens, with 25 of the 28 isolates representing CC398 and the remaining 3 isolates ST9. All CC398 isolates had the *spa* type t011, except a single t034 isolate from an abattoir worker and two t108 isolates from chickens. More variation was seen when *dru* typing was applied. Eight of the 13 CC398 isolates of human origin and 8 of the isolates from broiler chickens had dt11a, while a single isolate from an abattoir worker and 12 CC398 isolates from broiler chickens had dt10q. Additional *dru* types among the CC398 isolates were dt11c, dt11af and dt6j observed in single isolates from abattoir workers and dt11aw in five isolates from broiler chickens. A single isolate from an abattoir worker was *dru* non-typeable. SCC*mec* typing showed that all CC398 isolates carried an SCC*mec* type V element, except those 13 isolates that had *dru* type dt10q. These isolates harboured an SCC*mec* type IV element. The three human and the three chicken ST9 isolates shared the same characteristics: *spa* type t1430, *dru* type dt10a and SCC*mec* element of type IV. The two ST1453 isolates from abattoir workers were also indistinguishable; they shared *spa* type t4652, *dru* type dt3c and SCC*mec* element of type V.

As expected, PFGE analysis was occasionally able to further discriminate between isolates that otherwise showed indistinguishable characteristics. All six human MRSA CC398/t011/dt11a isolates from Slaughterhouse I showed slightly variable PFGE patterns A2 and A6–A10, while the corresponding chicken isolates from Slaughterhouse I showed the same ApaI fragment pattern A. In contrast, the human CC398/t011/dt10q and CC398/t011/dt11c isolates from Slaughterhouse I showed individual patterns B2 and E, respectively (Table 1). All chicken CC398/t011/dt11a and CC398/t011/dt11aw isolates from Slaughterhouse II showed pattern A1. The 12 chicken CC398/t011/dt10q isolates from Slaughterhouse III showed patterns B or B1, whereas the 2 human CC398/t011/dt11a or CC398/t011/dt11af isolates from the same slaughterhouse exhibited patterns C and C1, respectively. The two chicken CC398/t108/dt11a isolates from Slaughterhouse IV had pattern A5, whereas the two human CC398/t011/dt11a or CC398/t034/dt6j isolates from the same slaughterhouse exhibited the related patterns A3 and A11, respectively. Regardless of whether obtained from humans or chickens and from which slaughterhouse, the ST9/t1430/dt10a isolates showed closely related PFGE patterns D, D1 or D2. The two ST1453/t4652/dt3c isolates from workers at Slaughterhouse I presented the individual PFGE pattern F.

DNA microarray analysis revealed that all MRSA isolates were negative for the Panton–Valentine leucocidin genes *lukF-PV* and *lukS-PV*, the toxic shock syndrome toxin 1 gene *tst* as well as the exfoliative toxin genes *etA*, *etB* and *etD*. The MRSA ST9 isolates carried the enterotoxin gene cluster (*egc*), which comprised the staphylococcal enterotoxin genes G, I, M, N, O and U. The CC398 isolates did not carry enterotoxin genes.

Resistance phenotypes and genotypes of the MRSA isolates

Table 1 shows the resistance phenotype and genotype of each of the 46 MRSA isolates. A comparison of the resistance patterns revealed that all CC398, ST1453 and some of the ST9 isolates from chickens and humans showed resistance to four to nine classes of antimicrobial agents and carried a wide range of resistance genes known to occur in staphylococci.[20] Solely the three chicken and human MRSA ST9/t1430/dt10a isolates from Slaughterhouse I were only resistant to β-lactams and fluoroquinolones. The analysis of the resistance genes showed that for most resistance properties, different resistance genes were detectable. Occasionally, also more than one gene conferring the same resistance property was detected in the same MRSA isolate. In this regard, all isolates carried the β-lactamase operon *blaZ/I/R* in addition to the *mecA* gene. The tetracycline resistance genes *tet*(M) and

Table 1. Characteristics of the 46 MRSA isolates from broiler chickens at slaughter and abattoir workers

Isolate	Number of isolates	Slaughterhouse	Host	CC/MLST type	*spa* type	SCC*mec* type	*dru* type[a]	PFGE	Resistance phenotype[b]	Resistance genes
1	1	I	chicken	CC398	t011	V	dt11a	A	BLA-TET-ML-TMP-(TIA)-(ENR)-(KAN)	*mecA*, *blaZ/I/R*, *tet*(M), *tet*(K), *tet*(L)-*dfrK*, *erm*(T), *vga*(A), *aadD*
2–5	4	I	chicken	CC398	t011	V	dt11a	A	BLA-TET-ML-TMP-(TIA)-(ENR)-KAN	*mecA*, *blaZ/I/R*, *tet*(M), *tet*(K), *tet*(L)-*dfrK*, *erm*(T), *vga*(A), *aadD*
6	1	I	chicken	CC398	t011	V	dt11aw	A1	BLA-TET-ML-TMP-(TIA)-(ENR)-SPT-KAN	*mecA*, *blaZ/I/R*, *tet*(M), *tet*(K), *tet*(L)-*dfrK*, *erm*(C), *erm*(T), *vga*(A), *aadD*
7	1	I	human	CC398	t011	V	dt11a	A2	BLA-TET-ML-(TIA)	*mecA*, *blaZ/I/R*, *tet*(M), *tet*(K), *erm*(C), *vga*(A)
8	1	I	human	CC398	t011	V	dt11a	A6	BLA-TET-(TIA)-(ENR)	*mecA*, *blaZ/I/R*, *tet*(M), *tet*(K), *vga*(A)
9	1	I	human	CC398	t011	V	dt11a	A7	BLA-TET-ML-(TIA)-(ENR)	*mecA*, *blaZ/I/R*, *tet*(M), *tet*(K), *erm*(C), *vga*(A), *aadD*
10	1	I	human	CC398	t011	V	dt11a	A8	BLA-TET-ML-TMP-(TIA)	*mecA*, *blaZ/I/R*, *tet*(M), *tet*(K), *tet*(L)-*dfrK*, *erm*(C), *erm*(T), *vga*(A), *aadD*
11	1	I	human	CC398	t011	V	dt11a	A9	BLA-TET-ML-(ENR)	*mecA*, *blaZ/I/R*, *tet*(M), *tet*(K), *erm*(C)
12	1	I	human	CC398	t011	V	dt11a	A10	BLA-TET-ML-(ENR)	*mecA*, *blaZ/I/R*, *tet*(M), *tet*(K), *erm*(C)
13	1	I	human	CC398	t011	V	dt11c	E	BLA-TET-ML-TMP-GEN-(ENR)	*mecA*, *blaZ/I/R*, *tet*(M), *tet*(K), *tet*(L)-*dfrK*, *erm*(B), aacA/aphD, *aadD*
14	1	I	human	CC398	t011	IV	dt10q	B2	BLA-TET-ML-TMP-GEN-KAN	*mecA*, *blaZ/I/R*, *tet*(M), *erm*(C), *erm*(T), *dfrK* (Tn*559*), aacA/aphD, *aadD*
15, 16	2	I	human	ST1453	t4652	V	dt3c	F	BLA-TET-ML-TMP-TIA-SPT	*mecA*, *blaZ/I/R*, *tet*(M), *tet*(K), *spc-erm*(A)-*vga*(E) (Tn*6133*), *dfrK*
17	1	I	chicken	ST9	t1430	IV	dt10a	NT	BLA-ENR	*mecA*, *blaZ/I/R*, *qacC*
18, 19	2	I	human	ST9	t1430	IV	dt10a	D	BLA-ENR	*mecA*, *blaZ/I/R*, *qacC*
20	1	II	chicken	CC398	t011	V	dt11a	A1	BLA-TET-ML-TMP-(TIA)-ENR-KAN	*mecA*, *blaZ/I/R*, *tet*(M), *tet*(K), *tet*(L)-*dfrK*, *erm*(T), *vga*(A), *aadD*
21	1	II	chicken	CC398	t011	V	dt11aw	A1	BLA-TET-ML-TMP-(TIA)-ENR-SPT-KAN	*mecA*, *blaZ/I/R*, *tet*(M), *tet*(K), *tet*(L)-*dfrK*, *erm*(C), *erm*(T), *vga*(A), *aadD*
22	1	II	chicken	CC398	t011	V	dt11aw	A1	BLA-TET-ML-TMP-(TIA)-(ENR)-SPT-KAN	*mecA*, *blaZ/I/R*, *tet*(M), *tet*(K), *tet*(L)-*dfrK*, *erm*(C), *erm*(T), *vga*(A), *aadD*
23	1	II	chicken	CC398	t011	V	dt11aw	A1	BLA-TET-ML-TMP-(TIA)-(ENR)-KAN	*mecA*, *blaZ/I/R*, *tet*(M), *tet*(K), *tet*(L)-*dfrK*, *erm*(C), *vga*(A), *aadD*
24	1	II	chicken	CC398	t011	V	dt11aw	A1	BLA-TET-ML-TMP-(TIA)-(ENR)-KAN	*mecA*, *blaZ/I/R*, *tet*(M), *tet*(K), *tet*(L)-*dfrK*, *erm*(C), *erm*(T), *vga*(A), *aadD*
25	1	II	human	CC398	t011	V	NT	A4	BLA-TET-ML-(ENR)-TIA-SPT	*mecA*, *blaZ/I/R*, *tet*(M), *tet*(K), *erm*(C), *vga*(A)

26	1	II	chicken	ST9	t1430	IV	dt10a	D1	BLA-TET-ML-TMP-ENR-(KAN)	*mecA*, *blaZ/I/R*, *tet*(L)-*dfrK*, *erm*(B), *erm*(T), *aadD*, *qacC*
27	1	II	chicken	ST9	t1430	IV	dt10a	D1	BLA-TET-ML-TMP-ENR-KAN	*mecA*, *blaZ/I/R*, *tet*(L)-*dfrK*, *erm*(B), *aadD*, *qacC*
28, 29	2	III	chicken	CC398	t011	IV	dt10q	B	BLA-TET-TMP-SXT-GEN	*mecA*, *blaZ/I/R*, *tet*(M), *dfrK* (Tn*559*), aacA/aphD
30–38	9	III	chicken	CC398	t011	IV	dt10q	B	BLA-TET-TMP-GEN	*mecA*, *blaZ/I/R*, *tet*(M), *dfrK* (Tn*559*), aacA/aphD
39	1	III	chicken	CC398	t011	IV	dt10q	B1	BLA-TET-TMP-GEN	*mecA*, *blaZ/I/R*, *tet*(M), *dfrK* (Tn*559*), aacA/aphD
40	1	III	human	CC398	t011	V	dt11a	C	BLA-TET-ML-TIA-(ENR)-SPT	*mecA*, *blaZ/I/R*, *tet*(M), *tet*(K), *spc-erm*(A)-*vga*(E) (Tn*6133*), *erm*(C), *vga*(A)
41	1	III	human	CC398	t011	V	dt11af	C1	BLA-TET-ML-TIA	*mecA*, *blaZ/I/R*, *tet*(M), *tet*(K), *erm*(C), *vga*(A), *aadD*
42, 43	2	IV	chicken	CC398	t108	V	dt11a	A5	BLA-TET-ML	*mecA*, *blaZ/I/R*, *tet*(M), *tet*(K), *erm*(C)
44	1	IV	human	CC398	t011	V	dt11a	A3	BLA-TET-ML-TIA-SPT	*mecA*, *blaZ/I/R*, *tet*(M), *tet*(K), *erm*(C), *spc*
45	1	IV	human	CC398	t034	V	dt6j	A11	BLA-TET-ML-TMP-TIA-SPT	*mecA*, *blaZ/I/R*, *tet*(M), *tet*(K), *spc-erm*(A)-*vga*(E) (Tn*6133*), *erm*(C), *dfrK*
46	1	IV	human	ST9	t1430	IV	dt10a	D2	BLA-TET-ML-TMP-ENR-(KAN)	*mecA*, *blaZ/I/R*, *tet*(L)-*dfrK*, *erm*(B), *aadD*, *qacC*

[a]NT, non-typeable.
[b]Parentheses indicate that these isolates were classified as intermediate to the antimicrobial agent. BLA, β-lactam antibiotics; ENR, enrofloxacin; GEN, gentamicin; KAN, kanamycin; ML, macrolides-lincosamides; SPT, spectinomycin; TET, tetracyclines; TIA, tiamulin; TMP, trimethoprim; SXT, sulfamethoxazole/trimethoprim (19:1).

tet(L) were present either alone or together with *tet*(K) in the combinations *tet*(M)+*tet*(K) or *tet*(M)+*tet*(K)+*tet*(L). Moreover, the macrolide-lincosamide-streptogramin B resistance genes *erm*(B), *erm*(C) and *erm*(T) were present either alone or in the combinations *erm*(A)+*erm*(C), *erm*(B)+*erm*(T) or *erm*(C)+*erm*(T). Kanamycin resistance was due to the genes *aacA/aphD* or *aadD*, with both genes being present in only two isolates from workers in Slaughterhouse I. With the exception of the two ST1453/t4652/dt3c isolates, the trimethoprim resistance gene *dfrK* was either linked to the tetracycline resistance gene *tet*(L) or part of transposon Tn*559*.[20] Combined resistance to pleuromutilins, lincosamides and streptogramin A was based on the presence of either the *vga*(A) or *vga*(E) gene. The simultaneous presence of the genes *spc*-*erm*(A)-*vga*(E) in two human CC398 and the two ST1453 isolates suggests that these isolates harbour the transposon Tn*6133*. While the resistance genes detected in the MRSA isolates usually explain the resistance phenotypes observed, there were only a few cases in which no gene for a specific resistance property could be detected. This included one human CC398/t011/dt11a isolate, which exhibited a high tiamulin MIC but was negative for all pleuromutilin resistance genes, including the recently described gene *lsa*(E).[18] The same was true for three chicken CC398/t011/dt11aw isolates and the human CC398/t011/*dru*-non-typeable isolate, which had high spectinomycin MICs, but were negative for *spc* and the most recently described novel spectinomycin resistance gene *spw*.[19]

Conclusions

While the resistance phenotypes and genotypes of the chicken isolates from the same flock were closely related, they usually differed from the resistance phenotypes and genotypes of the isolates from the workers at the respective slaughterhouse. A similar variation was also detected in the molecular typing characteristics of the isolates from chickens and from abattoir workers. The apparent homogeneity of the chicken MRSA isolates from the same slaughterhouse suggests exchange of these isolates between the respective animals either at the farm or the slaughterhouse. The apparent heterogeneity of MRSA isolates from abattoir workers might reflect their occupational contact with animals from numerous chicken flocks.

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Transparency declarations

R. E. and S. M. are employees of Alere Technologies GmbH, the company that manufactures the microarrays used for this study, and have no competing interests. All other authors: none to declare.

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