

MRSA in bulk tank milk of dairy herds in Germany – changes over time

MRSA in Tankmilchproben von Milchviehbetrieben – Änderungen über die Jahre



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ABSTRACT

Objective Methicillin-resistant *Staphylococcus aureus* (MRSA) have repeatedly been isolated from dairy herds. It was the purpose of this study to compare the results of 3 subsequent national scale cross-sectional investigations in dairy herds in Germany on the prevalence of MRSA in bulk tank milk and the characteristics of the isolates.

Material and Methods The investigations were carried out in 2010, 2014 and 2019, respectively. MRSA were isolated from 25 ml of bulk tank milk using a double selective enrichment protocol. Samples were distributed across the country according to the regional dairy cattle population.

Results The prevalence of MRSA in bulk tank milk samples was lower in 2010 than in 2014 and tended to decrease until 2019. Prevalence was higher in samples from conventional than from organic herds and increased with herd size. Most isolates (75/78) were assigned to the clonal complex 398 and the *spa*-types t011 and t034. Resistance of the isolates to other antimicrobials than beta-lactams decreased over time.

Conclusions MRSA remain present in the German dairy population and are found more frequently in larger vs. smaller herds and in conventional vs. organic herds.

Clinical relevance MRSA should be considered in biosecurity protocols and with respect to occupational health of farm staff. Presence of MRSA in raw milk supports the recommendation not to drink unpasteurized raw milk.

ZUSAMMENFASSUNG

Ziel Methicillin-resistente *Staphylococcus aureus* (MRSA) wurden wiederholt in Milchviehherden nachgewiesen. Es war das Ziel dieser Studie, die Ergebnisse dreier Querschnittsstudien in Deutschland zu MRSA in Tankmilchproben in deutschen Milchviehbetrieben zu vergleichen im Hinblick auf die Prävalenz der Erreger und Erregereigenschaften.

Material und Methoden Die Untersuchungen wurden 2010, 2014 und 2019 durchgeführt. Je 25 ml Milch aus Tankmilchproben wurden mit einer zweistufigen selektiven Anreicherung

auf MRSA untersucht. Die Probenahme wurde proportional zur regionalen Milchviehdichte über das Land verteilt.

Ergebnisse Die Prävalenz von MRSA in den Tankmilchproben war 2010 niedriger als 2014, ging aber bis 2019 wieder leicht zurück. Die Prävalenz war höher in Proben von konventionellen Betrieben als in Proben von ökologisch wirtschaftenden Betrieben und stieg mit der Herdengröße. Die meisten (75/78) Isolate konnten dem klonalen Komplex 398 zugeordnet werden und den *spa*-Typen t011 und t034. Die Häufigkeit der Resistenz gegenüber weiteren Substanzen nahm im Laufe der Jahre leicht ab.

Schlussfolgerung MRSA werden nach wie vor in Tankmilchproben deutscher Milchviehbetriebe nachgewiesen und sind häufiger in größeren und konventionellen Betrieben als in kleineren und ökologisch wirtschaftenden Betrieben.

Klinische Relevanz Mitarbeitende in Milchviehbetrieben sollten auf das Risiko der MRSA-Besiedlung hinweisen, wenn sie als Patienten Kontakt zu Einrichtungen des Gesundheitswesens haben. Das Vorhandensein von MRSA in Rohmilch unterstreicht die Empfehlung, Rohmilch nicht ohne vorherige Erhitzung zu verzehren.

Introduction

Methicillin resistant *Staphylococcus aureus* (MRSA) are opportunistic pathogens that are associated with a substantial burden of disease especially in the health care system through nosocomial infections. MRSA have been identified in a variety of livestock, with highest prevalence being observed in pigs, veal calves and turkeys [1–4]. However, MRSA have also been detected in dairy and beef herds, where they pose an additional animal health threat by causing subclinical and clinical mastitis [5–7]. In Germany, MRSA in livestock and food are routinely investigated in the framework of a national monitoring program targeting zoonotic microorganisms and antimicrobial resistant bacteria in the food chain. Dairy herds were investigated in this framework targeting MRSA in bulk tank milk in 2010 and 2014 with prevalence in bulk tank milk samples being higher in 2014 than in 2010 and higher in larger herds and conventional farms than in smaller herds and organic farms in 2014 [6, 8]. In 2019 samples of bulk tank milk from dairy herds were again investigated for the occurrence of MRSA with the purpose of detecting further changes in prevalence and characteristics of obtained isolates over the years [9].

Material and Methods

Bulk tank milk samples of dairy herds were collected in Germany in 2010, 2014 and 2019. Sampling was distributed nationally according to the number of dairy cows in the respective federal states. In 2014 separate sampling frames were defined for conventional and organic dairy herds [8], while in 2010 the production type had not been recorded and in 2019 both production forms were included in the same sampling frame. On account of differences in the structure of the dairy herds in different regions in Germany, differences in regions were also investigated as previously described [8]. Briefly, The North-West (Schleswig-Holstein, Lower Saxony, Northrhine-Westphalia) is characterized by a variable herd size and regionally high animal populations. The South-West (SW) (Rhineland-Palatinate, Hesse, Saarland, Baden-Württemberg, Bavaria) is characterized by overall smaller herd sizes and an overall smaller regional animal density, albeit with some exceptions. The East (E) (Mecklenburg Western Pomerania, Brandenburg, Saxony Anhalt, Thuringia, Saxony) is characterized by very large herds but an overall limited animal density due to a limited number of herds.

Samples of 25 mL of bulk tank milk were collected on each farm and transported to the regional state laboratory at 4 °C. A harmo-

nized double selective enrichment protocol was implemented as described previously [6] within 48 h of arrival at the laboratory. Presumptive MRSA (one randomly chosen colony from the selective agar) were submitted to the National Reference Laboratory for coagulase-positive staphylococci, including *S. aureus* (NRL Staph; Berlin, Germany) for confirmation by an in-house multiplex real-time PCR based on [10], simultaneously targeting among others the *tuf* gene encoding the elongation factor EF-Tu and being specific for *Staphylococcus* species, the nuclease gene *nuc*, which is specific for *S. aureus*, and the resistance gene *mecA*. Isolates of *S. aureus* resistant to ceftioxin but negative for *mecA* are routinely tested for *mecC*. Furthermore, MRSA isolates were typed according to the repeat pattern of their *spa* gene [11]. Assignment of *spa* types to multilocus sequence type (MLST) clonal complexes (CC) was based on previously confirmed associations in the NRL Staph database or literature review. MLST [12] was performed on isolates that either could not be assigned to a *spa* type or showed *spa* types that did not occur previously in the NRL Staph database. The antimicrobial susceptibility of *S. aureus* was examined by broth microdilution according to the guidelines of Clinical and Laboratory Standards Institute [13, 14] at the NRL for Antimicrobial Resistance (NRL AR) and included 19 different antimicrobial substances (benzylpenicillin, ceftioxin, gentamicin, kanamycin, streptomycin, ciprofloxacin, erythromycin, clindamycin, fusidic acid, linezolid, mupirocin, rifampin, quinupristin/dalfopristin, sulfamethoxazole, trimethoprim, tetracycline, choramphenicol, tiamulin, vancomycin). Minimum inhibitory concentrations were evaluated using epidemiological cut off values provided by EUCAST as previously described [8]. Epidemiological cut off values were chosen as foreseen in the European legislation that is the legal background of the monitoring [15] and by EFSA [14].

Resistance to each antimicrobial was only descriptively compared on account of the limited number of isolates. However, a summary indicator was determined by calculating the number of tests with the outcome “resistant” divided by the total number of tests (i. e. no. of tests with the outcome “resistant”/no. of isolates x number of tested substances). This summary indicator was compared between years using Chi-square test.

Logistic regression was applied to analyze regional prevalence using only data from conventional herds and from herds where the production type had not been recorded including year and region as explanatory factors (► **Table 2**). The latter applied mostly to herds included in 2010 when this kind of information was not recorded.

► **Table 1** Number of tested herds (% positive samples) by year and production type.

► **Tab. 1** Anzahl untersuchter Herden und Prozentsatz positiver Proben nach Jahren und Produktionstyp.

Year	2010	2014		2019		
Conv/org	unknown	Conv.	Org.	Conv.	Unknown	Org.
Number of herds (% positive samples)						
Germany	297 (4.7)	372 (9.7)	303 (1.7)	307 (8.5)	12 (8.3)	45 (0)
North West	117 (8.5)	141 (14.9)	51 (0)	180 (9.4)	0 (0)	20 (0)
East	51 (3.9)	75 (12.0)	45 (0)	34 (20.6)	12 (8.3)	7 (0)
Southwest	129 (1.6)	156 (3.8)	207 (2.4)	93 (2.2)	0 (0)	18 (0)
Median herd size (no. of herds)*						
North West	n.d.	80 (133)	60 (49)	91.5 (180)		80 (20)
East	n.d.	252 (75)	70 (45)	545 (34)	n.d.	115 (7)
Southwest	n.d.	57 (141)	50 (204)	54 (72)		67.5 (18)

Conv: conventional, Org: organic, *information on herd size was not available for all herds, nd: not determined

► **Table 2** Association of positive samples with region and year for conventional herds and herds with unknown production type.

► **Tab. 2** Assoziation von positiven Proben mit Region und Jahr für konventionelle Herden und solche mit unbekanntem Produktionstyp.

	Coefficient of regression	Standard error	Wald	df ¹	p-value	Odds ratio	95% confidence interval for odds ratio	
							Lower	Upper
Region (Ref: South-West)				18.313	2	0.000		
North-West	1.473	0.357	17.000	1	0.000	4.361	2.165	8.782
East	1.511	0.403	14.089	1	0.000	4.531	2.059	9.974
Year	0.034	0.034	1.022	1	0.312	1.035	0.968	1.105
Constant	-72.295	67.950	1.132	1	0.287	0.000		

¹df: degrees of freedom.

Based on the small share of organic herds among the dairy herds at that time, it seemed adequate to consider these herds conventional. An additional analysis included herd size and production type (conventional vs. organic) (► **Table 3**). The latter approach included only datasets with information on herd size, i. e. data from 2014 and 2019. All calculations were carried out using IBM SPSS Statistics 26. Associations with $p < 0.05$ were considered as significant.

Results

Overall, 1336 bulk tank milk samples and 78 isolates were included in the analysis. All isolates that were confirmed as MRSA were positive for *mecA*. Herd size was only recorded in 2014 and 2019 and increased for conventional and for organic herds between the years (► **Table 1**).

► **Table 2** and ► **Table 3** display the results of logistic regression of factors associated with positive bulk tank milk samples. Prevalence of MRSA in bulk tank milk samples increased between 2010 and 2014. However, between 2014 and 2019 it tended to decrease in conventional as well as in organic herds. When considering regions in the model, including only samples from conventional herds or herds without information on their production type, year was not significantly associated with prevalence anymore (► **Table 2**). However, positive samples were regionally associated with herds in the North-West (OR 4.4), and in the East (OR 4.5) being more likely to be positive than herds in the South-West (► **Table 2**).

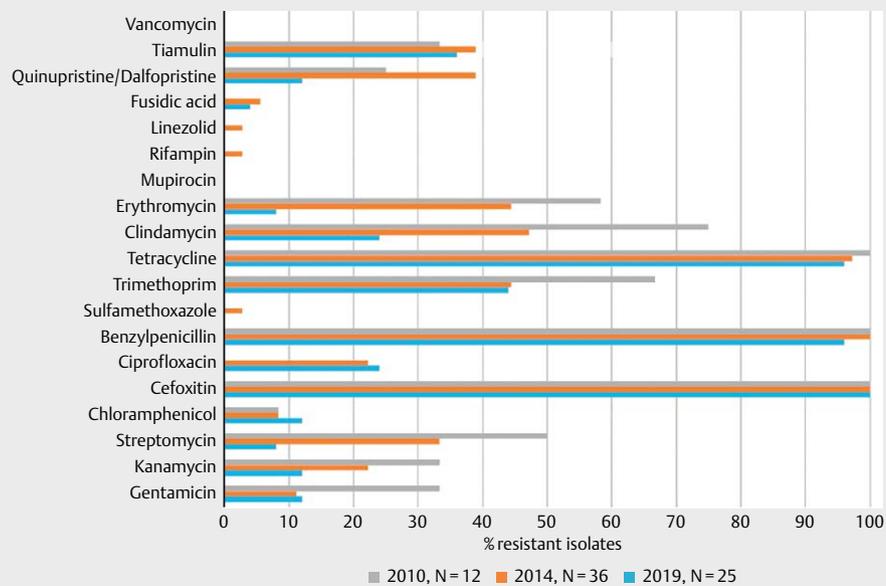
Only including samples from 2014 and 2019, prevalence of MRSA was higher in samples from conventional compared to organic farms (OR 5.3) and increased with herd size (OR 1.002,

► **Table 3** Association of positive samples with region, year, production type and herd size. Only data from 2014 and 2019 included.

► **Tab. 3** Assoziation von positiven Proben mit Region, Jahr, Produktionstyp und Herdengröße. Nur Daten aus 2014 und 2019.

	Coefficient of regression	Standard error	Wald	df ¹	p-value	Odds ratio	95% confidence interval for odds ratio	
							Lower	Upper
Region (Ref: South-West)				2	0.004			
North-West	1.156	0.366	9.991	1	0.002	3.179	1.552	6.511
East	0.441	0.517	0.729	1	0.393	1.555	0.565	4.283
Year	-0.121	0.058	4.281	1	0.039	0.886	0.790	0.994
Production type (Ref: organic)				2	0.005			
Unknown	-18.583	40192.970	0.000	1	1.000	0.000	0.000	
Conventional	1.671	0.512	10.674	1	0.001	5.319	1.952	14.494
Herd size	0.002	0.000	18.375	1	0.000	1.002	1.001	1.003
Constant	238.857	117.761	4.114	1	0.043	5.424* 10 ¹³		

¹df: degrees of freedom.



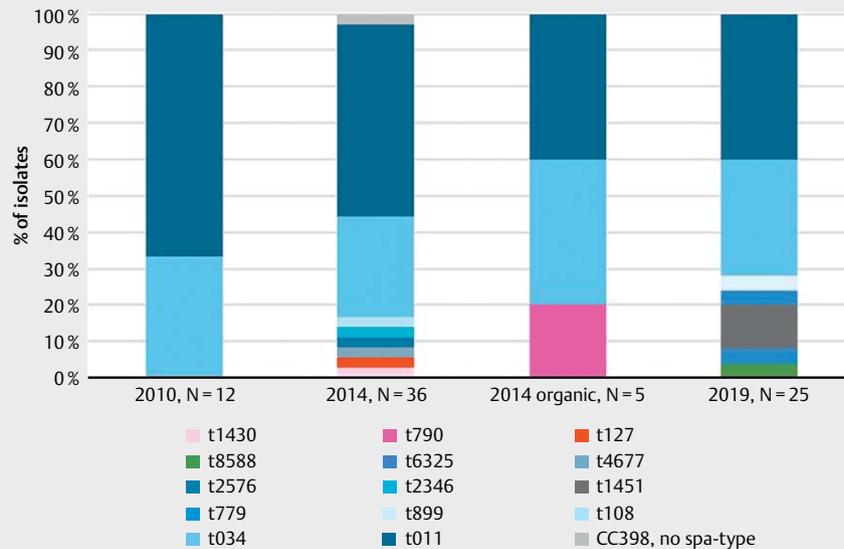
► **Fig. 1** Resistance of isolates from conventional herds and herds with unknown production type to 19 antimicrobials. 2014-data and epidemiological cut offs from [8]. Source: German Federal Institute for Risk Assessment.

► **Abb. 1** Resistenz der Isolate konventioneller Herden und von Herden mit unbekanntem Produktionstyp. Daten aus 2014 und epidemiologische cut off Werte aus [8]. Quelle: Bundesinstitut für Risikobewertung.

► **Table 3**). Considering these parameters, prevalence of MRSA was lower in 2019 than in 2014 (OR 0.886).

Antimicrobial resistance of the isolates tended to be higher in the 12 isolates from 2010 than in 2014 and 2019 (► **Fig. 1**). In 2010, 82/228 phenotypic test results (36.0%) were positive. In 2014 this

proportion dropped to 32.7% in the 36 isolates from conventional farms only. In 2019 a further decrease to 25.7% was observed in the 25 available isolates from conventional farms. The differences between 2010 and 2019, and 2014 and 2019 were significant (Chi square = 7.9 and 6.7, respectively).



► **Fig. 2** *spa*-types of isolates of MRSA from bulk tank milk of dairy herds 2010, 2014 and 2019. 2014 data from [8]. Source: German Federal Institute for Risk Assessment.

► **Abb. 2** *spa*-Typen der MRSA Isolate aus Tankmilch aus den Jahren 2010, 2014 und 2019. 2014 Daten aus [8]. Quelle: Bundesinstitut für Risikobewertung.

Typing results

In 2010, all isolates were assigned to the MRSA CC398, with isolates harboring *spa*-types t011 and t034. In 2014, one of the isolates from an organic farm (t790, CC22) and two isolates from conventional farms (t127, CC1 and t1430, CC9) were assigned to other clonal complexes [8]. In one isolate from a conventional farm in 2014, no *spa*-type could be determined. However, by MLST that isolate was assigned to sequence type 398. All other isolates (32 from conventional and 4 from organic farms) were assigned to CC398. In 2019, all isolates were from conventional farms and belonged to the clonal complex 398 with most isolates (18/25, 72%) represented by *spa*-types t011 (10 isolates) and t034 (8 isolates). Seven isolates had other *spa*-types that are associated with the CC398 (► **Fig. 2**).

Discussion

Overall the results in 2019 confirmed the results observed in 2014 [8] and 2010 [6]. In all three years representative sets of samples were collected. An increase in prevalence of MRSA could be expected as *S. aureus* is a constant colonizer of the mammary gland in affected animals and eradication of MRSA from herds is challenging [16]. Meanwhile further herds might become positive through trade of infected or colonized animals or through transmission of MRSA from other animal species to dairy herds as described for pigs in the literature [17]. Moreover, most antimicrobials used in dairy herds in Germany are beta-lactam antimicrobials that might additionally select for MRSA [18]. Therefore, it is encouraging to see no further increase of the prevalence in 2019 as compared to 2014 in both, conventional and organic herds although the reason for this

remains to be investigated. Awareness of the presence of the pathogenic species and the risk of acquiring the bacteria through trade may have increased. However, relevant data on this question are not available.

Resistance of MRSA from bulk tank milk to other antimicrobials than penicillin and cefoxitin did change over the years with resistance to fluoroquinolones numerically increasing and resistance to aminoglycosides, macrolides, lincosamides and trimethoprim decreasing (► **Fig. 1**). Overall, the proportion of positive resistance tests decreased. With respect to the individual substances, it has to be considered that the number of isolates was low in all years and therefore confidence intervals for the estimated prevalence of resistance in the isolates are fairly wide. Significant changes therefore were not observed. Resistance to other substances was constant being typically high to tetracycline and absent to mupirocin and vancomycin. Resistance to other medically important antimicrobials such as linezolid, rifampin and fusidic acid was rarely observed.

As previously reported, herd size affected the probability of a bulk tank milk sample of being positive with larger herds being more likely to be positive than smaller herds [8, 19]. Interestingly, prevalence of MRSA differed between regions with the South-West having the lowest prevalence. However, the ranking of the North-West and the East changed over time. While in 2010 and 2014 prevalence was highest in the North-West, it tended to be higher in the East in 2019. A potential reason for this shift is the substantially bigger herd size in the East (► **Table 1**). Bigger herds tend to buy more animals, thus increasing the risk of MRSA being introduced into the herds.

Organic herds were less likely positive for MRSA than conventional herds. This had been observed in 2014 [8], but was confirmed in 2019. Structural aspects as smaller herd size and presumably less frequent antimicrobial treatments among organic herds as compared to conventional herds might explain this observation in part. Additionally, trade of animals between conventional and organic herds is legally limited, which probably contributes to lower prevalence [20]. Trade of animals has repeatedly been identified as a likely driver for MRSA spread in different animal species [21, 22].

Diversity of MRSA in the bulk tank milk samples tends to increase over time. Although most isolates belonged to *spa*-types associated with the livestock associated clonal complex CC398, the proportion of other *spa*-types than t011 and t034 increased. Whether this is due to evolving strains within the herds or introduction of new strains is not clear. Since only one isolate from each positive sample was *spa*-typed, more than the observed diversity is possible as herds may harbor more than one *spa*-type [23, 24]. Therefore, the absence of non-CC398 MRSA in 2019 as opposed to 2014 has to be interpreted carefully. The changes in the patterns clearly indicate that MRSA in dairy herds should be regularly monitored.

Resistance to antimicrobials in the isolates decreased over time. Overall, antimicrobial use in dairy cows is comparatively low [25] and limited by the need to observe withdrawal periods for milk after treatment. This increases the indirect costs of treatment substantially and therefore can be considered an incentive for farmers and veterinarians not to use antimicrobials. Over time, antimicrobial use in dairy cows in Germany has been fairly constant [25].

The presence of MRSA in dairy herds needs to be considered an occupational health risk as reported in recent studies from Germany, Italy and Poland [5, 26–28]. The risk of MRSA being transmitted to humans via consumption of raw milk needs to be considered. So far, the risk of transmission via food is considered as low [29]. However, this observation is based on meat as a source of pathogenic bacteria. Bacterial counts of MRSA in bulk tank milk are overall low to our knowledge ($< 10^3$ cfu/ml, unpublished own data), limiting the risk of colonization. Minimum bacterial concentrations for colonization of humans via food have never been determined. Similarly low levels of MRSA have also been observed in broiler meat [30]. However, in contrast to broiler meat, cows' milk is also consumed raw despite public warnings because of other zoonotic agents. Studies reported a high prevalence of MRSA among milk fed calves in MRSA positive herds, assuming that it might be transmitted to the neonates with unpasteurized milk [27, 31]. Milk and colostrum of individual positive cows might contain more staphylococci than bulk tank milk that is always diluted with the milk of the majority of negative cows. Still, other modes of transmission are possible during parturition and via contact to other calves. Further studies on the transmission pathways between cows and calves on dairy farms are needed.

CONCLUSION FOR PRACTICE

In conclusion, MRSA continue to challenge biosecurity systems of dairy herds. Farmers and veterinarians need to be alert to avoid introduction of the bacteria into the herd. So far, there is no evidence that antimicrobial use in dairy cows is a driver of the spread of MRSA in the herd. However, this has been shown in fattening pigs [21] and has been suspected in veal calves [2]. The risk of acquiring MRSA through occupational exposure remains high for workers in positive herds. This also requires alertness as there is a risk of clinical disease for colonized persons having to undergo surgery or other medical invasive procedures. Therefore, this group of persons should be educated to alert medical personnel when entering the health system.

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Conflict of interest

The authors declare that they have no conflict of interest.

Dedication

We dedicate this article to Prof. Rolf Mansfeld to the occasion of his well-earned retirement. The authors as well as German herd management owe him most important impulses and insights. Without these, herd management in Germany would not be where it stands today.

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