**tehnologija mesa**
*Osnivač i izdavač: Institut za higijenu i tehnologiju mesa, Beograd*
*UDK: 664.9:579.67:579.86*
*BIBLID: 0494-9846*

# Methicillin susceptible and resistant *Staphylococcus aureus* from farm to fork impact on food safety*

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*A b s t r a c t: Methicillin susceptible Staphylococcus aureus (MSSA) are well known as one of the leading cause of food poisoning worldwide for decades. Their food safety importance is due to their ability to form staphylococcal enterotoxins (SE) referred to as superantigens and enterotoxin-like superantigens (SEl) in foodstuffs. The five classical staphylococcal enterotoxin types (A to E) are the most important with regard to food poisoning. Recently, also Methicillin resistant Staphylococcus aureus (MRSA) have been detected in food animals and food with a remarkable prevalence. The majority of these MRSA strains belong to the clonal complex (CC) 398 and were not described in humans prior the detection in food animals. A major concern of public health authorities is the potential spread of these strains into the human population. Meat from poultry displayed the highest prevalence of MRSA whereas the prevalence of MRSA on poultry farms does not seem to be higher than the prevalence in pigs. Moreover, MRSA strains that are not associated with the CC398 (Non-CC398) contribute to the contamination of food items, thus posing an alternate threat to public health. Up to present, the likelihood of MRSA strains isolated from farm to fork to harbour staphylococcal enterotoxine genes seems to be low. However, more research is needed to quantify the risk of colonization of consumers with MRSA via the food chain. Hence, continuous surveillance is needed in order to study whether the presence of enterotoxins or other virulence factors will increase.*

***Key words:*** *MSSA, MRSA, enterotoxins, antibiotic resistance, food chain, food safety.*

## Introduction

From a food safety perspective, coagulase positive staphylococci and in particular *Staphylococcus (S.) aureus* is by far one of the most important foodborne pathogen due to its ability to produce a wide variety of toxins including heat-stable staphylococcal enterotoxins (SEs). Up to present, more than 21 SEs were described, including SEs with a demonstrated emetic activity (SEA-SEE, SEG-SEI, SER-SET) and the so called staphylococcal-like (SE*l*) SEs, which are not emetic in a primate model (SE*l*L, SE*l*Q) or have yet not been tested (SE*l*J, SE*l*K, SE*l*M-SE*l*P, SE*l*U, SE*l*V) (*Argudin et al.*, 2010).

SEs are a major cause of food poisoning worldwide, e.g. around 5.3 % (n = 293) of all reported foodborne outbreaks in 2009, were caused by staphylococcal enterotoxins (EFSA, 2011). As a prerequisite, enterotoxigenic strains of *S. aureus* (naturally or artificially) contaminating varying food matrices have to produce SEs during their exponential growth. After ingestion of food containing preformed SEs symptoms do rapidly occur, including nausea, violent vomiting, abdominal cramps and diarrhea (*Seo and Bohach*, 2007). Usually, the illness is self-limiting and warrants hospitalization in occasional cases, only. Food-stuffs typically involved in foodborne outbreaks due to SE intoxication are (raw) milk and dairy products, meat and products, salads and bakery products, particularly cream-filled pastries and cakes. In most cases food handlers carrying enterotoxin-producing *S. aureus* in their noses or hands are regarded as the main source of food contamination due to improper handling and subsequent storage at elevated temperatures (*Argudin et al.*, 2010). However, *S. aureus* is also present

**Plenary paper on International 56th Meat Industry Conference held from June 12-15th 2011. on Tara mountain;*
**Plenarno predavanje na Međunarodnom 56. savetovanju industrije mesa, održanom od 12-15. juna 2011. godine na Tari.*

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in food animals, and dairy cattle sheep and goats, particularly if affected by subclinical mastitis, are likely contaminants of milk (*Stewart,* 2005).

In the EU, Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs lays down certain food safety criteria with regard to SEs to be applicable to products placed on the market during their shelf-life. Accordingly, staphylococcal enterotoxins may have to be undetectable in 25 g of certain cheeses (i.e. cheeses made from raw milk, resp. milk that has undergone a lower heat treatment than pasteurization, resp. ripened or unripened cheeses made from milk or whey that has undergone pasteurization or a stronger heat treatment), milk and whey powder. Hence, process hygiene criteria as layed down in Regulation (EC) No 2073/2005 necessitates the analyses of certain foodstuffs on their amount of coagulase positive staphylococci at the time during the manufacturing process when the number of staphylococci are expected to be highest. Foodstuffs of which this criterion applies for are certain cheeses (as mentioned above), milk and whey powder as well as shelled and shucked products of cooked crustacaens and molluscan shellfish. In case of unsatisfactory results, actions to be taken by the food business operator include improvements in production hygiene and selection of raw materials. Further, the batch has to be tested for the presence of SEs in the case that values > $10^5$ Colony forming units (CFU) per gram are detected.

Methicillin resistant *Staphylococcus aureus* (MRSA) has been described as an important nosocomial pathogen in hospital settings for a long time. Specific strains of MRSA have also been shown to spread in the community. More recently, MRSA were also detected in livestock and companion animals. It has been shown that MRSA from livestock colonize professionals (farmers, veterinarians, etc.) dealing with live farm animals (*Voss et al.,* 2005). The overwhelming majority of the isolates from livestock in Europe have been assigned to clonal complex CC398. As this clonal complex was not described as MRSA in humans prior to the detection in animals it has been named 'livestock-associated (la-) MRSA'.

La-MRSA has also been detected in food, i.e. meat and milk (*de Boer et al.*, 2009; *Käsbohrer et al.,* 2010; *Spohr et al*., 2010) causing concern over a potential spread of MRSA to consumers via the food chain. Contamination of meat with MRSA is likely to occur at various stages of the processing chain by carry over from colonized body sites of the animal to the carcass, by cross contamination, through the environment of processing facilities or by people involved in the handling of carcasses or meat. Data from The Netherlands and from our laboratory show that MRSA of the same clonal lines can be detected in farm animals and food. Among meat samples, the prevalence is highest for meat from turkey and broilers and substantially higher than in pork *(de Boer et al.,* 2009; *Käsbohrer et al.,* 2010*)* while currently there is no evidence that the prevalence of MRSA in poultry is higher than the very high prevalence observed in pigs. However, data on MRSA in poultry is still scarce.

Moreover, data from the Netherlands and preliminary data from Germany indicate that MRSA strains that have not been described in livestock and are not associated with the CC398 (non-CC398) also contribute to the contamination of food items, thus posing an alternate threat to public health (*de Boer et al*., 2009; *Fetsch et al.,* 2009). The origin of this contamination is not clear. While it is likely that colonized people involved in the processing of food contaminated the product it cannot be excluded that the presence of these non-CC398 strains in primary production has been overlooked so far. MRSA ST398 was not noticed in farm animals in Germany before 2007, because *S. aureus* was not a relevant pathogen in the host species and therefore not investigated. According to German data, MRSA CC398 has been present in pigs since at least 2004 (*Meemken et al*., 2009).

Up to present, MRSA isolates of the CC398 usually lack important virulence determinants typical for other community and hospital isolates. Also staphylococcal enterotoxin genes carrying laMRSA isolates have been reported in a small number of cases, only (*Kadlec et al*., 2009; *Laurent et al.*, 2009).

Previous food safety research focussed on Methicillin susceptible *Staphylococcus aureus* and its ability to form staphylococcal enterotoxins in foodstuffs. However, the detection of Methicillin resistant *S. aureus* in livestock and food in recent years raised additional food safety concern. Therefore, this paper aims to gives an overview on studies carried out in Germany on the prevalence, molecular epidemiology and staphylococcal enterotoxin gene content of MRSA isolated from farm to fork and to discuss their possible impact on food safety.

## Materials and Methods

The strain collection of the National Reference Laboratory for coagulase positive staphylococci incl. *Staphylococcus aureus* (NRL-Staph) at the BfR contains MRSA strains isolated in the course of various national and international monitoring programs or research projects. Around 2200 MRSA strains isolated from production holdings (breeding (n =

106) and fattening (n = 145) pigs, turkeys (n = 34), dairy cows (n = 24)), at slaughter (pigs (n = 636), turkeys (n = 202), veal calves (n = 115)) and at retail (raw meat, raw meat preparations and minced meat of pigs (n = 198), veal calves (n = 64), broilers (n = 233) and turkeys (n = 283)) are available.

All genotypically confirmed MRSA isolates were typed using SCC*mec*- and *spa* typing protocols as described elsewhere (*Shopsin et al.*, 1999; *Zhang et al.,* 2005). In addition, of one isolate per identified *spa* type the Multi locus sequence type (MLST) was determined (*Enright et al.*, 2000). *Spa*- and Multi--locus sequence type (MLST) were determined using the software Ridom Staphtype and the *S. aureus* MLST database.

A subset of MRSA isolates (n = 100), all of CC398, was further investigated for the presence of staphylococcal enterotoxine genes (sea, seb, sec, sed, see, seg, she, sei, sej, sek, sel, sem, sen, seo, seq, ser, seu) by PCR as described by *Argudin et al.* (2011).

Susceptibility testing and evaluation of resistance were performed with all isolates in accordance with Clinical and Laboratory Standards Institute guidelines by broth dilution or disc diffusion method, respectively.

## Results

### Typing and antimicrobial resistance testing results of MRSA isolated from farm to fork

**Pigs:** Among the isolates from holdings of breeding and fattening pigs, *spa* types t011, t034 and t108 and SCC*mec* type V predominate and the proportion of Non-CC398 MRSA (ST9, ST39, ST97) was less then five percent. CC398 MRSA do also predominate in pigs at slaughter, however the proportion of *spa* types t011, t034 and t108 varied greatly per abattoir (*Tenhagen et al.*, 2009). In raw meat at retail, the proportion of Non-CC398 MRSA among the isolates was highest (up to 13 %) as was the percentage of SCC*mec* types other than V, with 11 % of the isolates harbouring SCC*mec* type IVa. Resistance patterns of isolates from pigs along the food chain differed slightly, overall showing high frequencies of resistance against beta-lactames and tetracycline (both up to 100 %), lincosamides and macrolides (clindamycin and erythromycin, both up to 80 %) and aminoglycosides (gentamicin and kanamycin, ~ 20 %).

**Cattle:** At farm level, samples from dairy cows resp. bulk milk harboured CC398 MRSA with *spa* types t034 and t011 and SCC*mec* types V resp. III, only. Moreover, all MRSA isolates from veal calves at slaughter belonged to CC398. The proportion of Non-CC398 MRSA among the isolates from raw veal meat at retail was low (< 2 %). As among isolates with porcine origin, resistance to beta-lactames and tetracycline (~100 %), lincosamides (up to 72 %) and macrolides (up to 61%) were highest. Remarkably, isolates from veal calves at slaughter showed a significantly higher proportion of resistance against streptogramin antibiotics (quinupristin/dalfopristin 27%) and folate synthesis inhibitors (sulfamethoxazole/trimethoprim (42 %) then pig isolates.

**Poultry:** As among CC398 MRSA from porcine and cattle origin, t011 and t034 with SCC*mec* type V were also the most often *spa* types among poultry isolates. MRSA from poultry at farm level are scarce up to present. Only isolates from turkey flocks were available for analysis, displaying a high proportion of Non-CC398 MRSA (21 %, mainly *spa* type t002 (ST5)). Interestingly, the high percentage of SCC*mec* types IVa was exclusively detected in MRSA of *spa* type t011. At slaughter, the proportion of Non-CC398 MRSA among turkey isolates was lower than at farm level (< 8 %) whereas around 15 % of the isolates from raw turkey meat at retail level were associated with Non-CC398 *spa* types (mainly t002).

Raw meat from broilers showed a high proportion of Non-CC398 MRSA (~ 30 %), with *spa* type t1430 (ST9) predominating. Interestingly, this *spa* type was not detected in raw meat of other origin and was most often associated with SCC*mec* type IVa. Overall, MRSA isolates from raw meat from poultry (broiler and turkey) harboured significantly more often type IVa SCC*mec* than those from other origin. Resistance to beta-lactames, tetracycline, lincosamides and macrolides among poultry isolates were equally frequent as among pig and cattle isolates. However, the percentage of MRSA strains from poultry in particular from raw meat and raw meat preparations being resistant to ciprofloxacin (~ 30% of the isolates) was higher than in isolates from other origins.

### Staphylococcal enterotoxin gene content of MRSA isolated from farm to fork

None of the subset of 100 CC398 MRSA isolates derived from various sources from farm to fork in Germany carried any of the staphylococcal enterotoxin genes tested (*Argudin et al*., 2011).

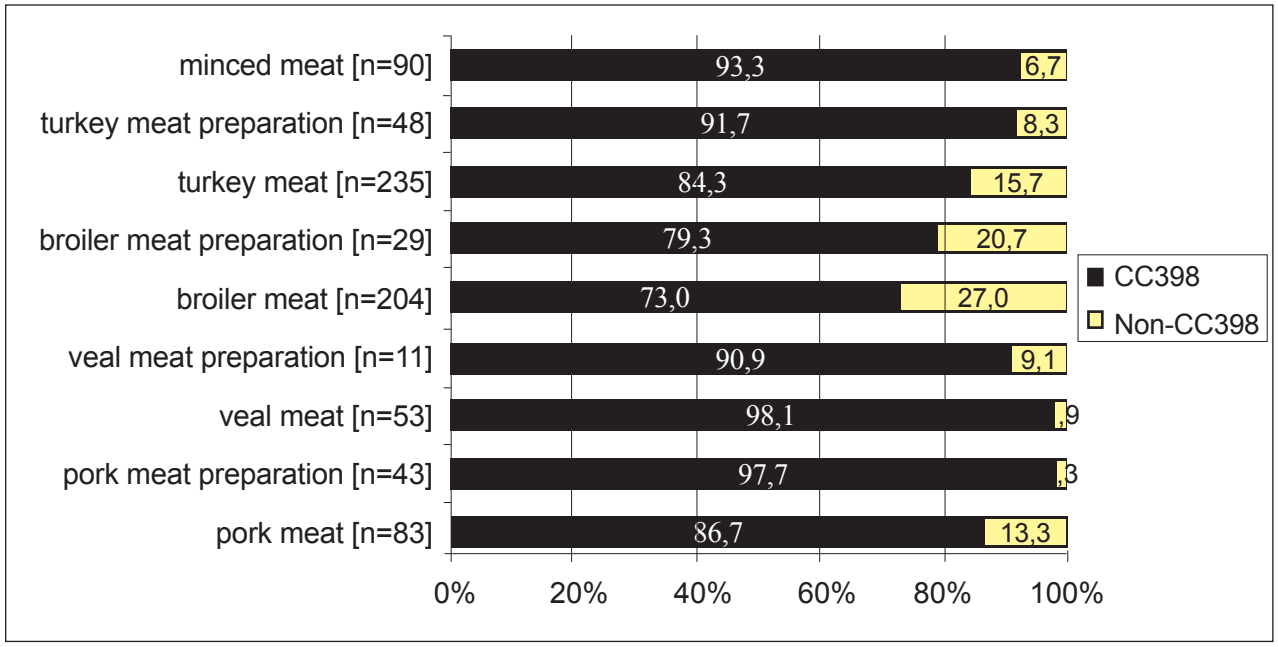


**Figure 1.** Percentage of Non-CC398 MRSA found in isolates from food at retail
**Slika 1.** Procenat Ne- CC398 MRSA utvrdjenih u izolatima hrane u maloprodaji

Legenda/Legend:
Minced meat/Mleveno meso
Turkey meat preparation/Proizvodi od ćurećeg mesa
Turkey meat/Ćureće meso
Broiler meat preparation/Proizvodi od pilećeg mesa
Broiler meat/Pileće meso
Veal meat preparation/Proizvodi od teletine
Veal meat/Teleće meso
Pork meat preparatio/Proizvodi od svinjskog mesa
Pork meat/Svinjsko meso

## Discussion

For a long period of time MRSA were seen as a problem of human medicine and hospital hygiene alone. However, the MRSA complex has a zoonotic component, which is closely linked to strains of the clonal complex (CC) 398 and to food animal populations (*Meemken et al.,* 2009). People dealing with livestock are at higher risk of colonization than those without direct contact to the animals (*Cuny et al.,* 2009; *Mulders et al.*, 2010).

Currently, the majority of laMRSA isolates from farm to fork belong to *spa* types which can be assigned to clonal complex CC398. As shown in Fig.1, MRSA from food at retail showed the highest diversity among the isolates. In particular, MRSA from poultry showed the highest proportion of Non-CC398 MRSA. Additionally, prevalences from farm to fork varied greatly between and within the different species. Whereas meat from poultry displayed a substantially higher prevalence of MRSA than red meat, the prevalence of MRSA on poultry farms does not seem to be higher than the prevalence in pigs. Specific clonal lineages seem to predominate in specific origins, e.g. MRSA of *spa* type t1430 (belonging to ST9) harbouring SCC*mec* type IVa and with a high ciprofloxacin resistance rate isolated from raw broiler meat respectively, MRSA of *spa* type t002 (ST5) isolated from raw turkey meat. These findings call for strain and clonal lineage specific risk characterization to assess the consumers risk associated with MRSA in food.

None of the subset of 100 MRSA isolates of the CC398 ST398 harboured any of the staphylococcal enterotoxin genes tested. This is in line with previous studies, in which a low occurence of either seb or sed and seg was reported for MRSA CC398 isolates from Germany and France (*Kadlec et al.,* 2009; *Laurent et al.,* 2009). It has been speculated that the environment contributes to the low presence of virulence determinants in this clone (*Argudin et al.*, 2011). However, the presence of enterotoxin genes among Methicillin susceptible *S. aureus* (of undetermined CCs) can be high (*Nitzsche et al.,* 2007). Hence, MRSA of Non-CC398 and isolated from farm to fork seem to harbour staphylococcal entero-

toxin genes at a much higher percentage than CC398 MRSA (Fetsch et al., personal communication). Thus, the horizontal gene transfer between strains of different clonal lineages harbouring staphylococcal enterotoxin genes is likely to occur and calls for further investigations.

While the presence of MRSA in food is well established there is a substantial lack of knowledge on the quantity of MRSA in meat and commodities and on dose-response relationship. Therefore, a valid quantitative assessment of the consumers risk is currently not feasible. Up to now, there is no evidence that MRSA ST398 is spread via the food chain. However, animal products remain a potential source of MRSA. More research is needed to quantify the risk of colonization of consumers with MRSA via the food chain and to monitor a possible evolve of isolates with a potential higher pathogenic potential, i.e. staphylococcal enterotoxin gene content.

**Acknowledgement:**The authors gratefully acknowledge the local authorities and regional laboratories for providing isolates in the course of various national monitoring programs. A. Richter and M. Spohr kindly provided strains from turkey flocks and dairy cows, respectively. The support of U. Kämpe, J. Kowall, A. Beyermann and W. Barownik is also gratefully acknowledged.

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# Meticilin-rezistentni sojevi *Staphylococcus aureus*, od farme do trpeze – uticaj na bezbednost hrane

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*R e z i m e: Sojevi Staphylococcus aureus (MSSA - Methicillin susceptible) osetljivi na meticilin su dobro poznati kao vodeći uzroci trovanja hranom širom sveta. Njihov značaj sa stanovišta bezbednosti hrane proizilazi iz njihove sposobnosti da formiraju u prehrambenim proizvodima stafilokokne enterotoksine (SE) koji se još nazivaju superantigenima i superantigene koji su slični enterotoksinima (SEl). Pet klasičnih tipova stafilokoknih enterotoksina (od A do E) su najvažniji sa stanovišta trovanja hranom. Nedavno su otkriveni i meticilin-rezistenti sojevi Staphylococcus aureus (MRSA) u korisnim domaćim životinjama i hrani sa značajnom prevalencom. Većina ovih MRSA sojeva pripada klonalnom kompleksu (CC) 398 i nisu registrovani kod ljudi pre nego što su otkriveni kod domaćih životinja. Najveći razlog za zabrinutost nadležnih organa za javno zdravlje je potencijalno širenje ovih sojeva na ljudsku populaciju. Meso šivine je pokazalo najvišu prevalencu MRSA, dok na živinarskim farmama prevalencija MRSA nije veća nego kod svinja. Štaviše, MRSA sojevi koji nisu povezani sa CC398 (Ne-CC398) doprinose kontaminaciji prehrambenih proizvoda, i na taj način predstavljaju dodatnu opasnost po javno zdravlje. Do sada, verovatnoća da će MRSA sojevi izolovani od farme do trpeze imati gene stafilokoknih enterotokcina se čini da je mala. Međutim, potrebna su istraživanja kako bi se kvantifikovao rizik od kolonizacije potrošača sa MRSA preko lanca ishrane. Prema tome, stalni nadzor je potreban kako bi se ispitalo prisustvo enterotoksina ili drugih virulentnih faktora.*

***Ključne reči:*** *MSSA, MRSA, enterotoksini, rezistentnost na antibiotike, lanac ishrane, bezbednost hrane.*

Paper recieved: 16.05.2011.