

# Characterisation of *Staphylococcus aureus* isolated from meat processing plants – a preliminary study

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## Abstract

**Introduction:** Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) belonging to the clonal complex 398 (CC398) emerged recently in livestock as a new type of MRSA, which may cause zoonotic infections. This study presents data on the characterisation of *S. aureus* isolated from the meat processing plants. **Material and Methods:** *S. aureus* was isolated from 90 samples collected in the raw meat warehouse, from devices and surfaces of meat processing plants, and from finished meat products. The isolates were subjected to molecular analysis in order to investigate the presence of enterotoxin genes, the *mecA* gene, and to verify whether they belong to the clonal complex 398. The genetic relatedness of the isolates was determined using pulsed-field electrophoresis. Likewise, antimicrobial susceptibility was tested. **Results:** From 21 *S. aureus* strains isolated, five belonged to the CC398, two of which were recognised as MRSA and three as methicillin-sensitive *Staphylococcus aureus* (MSSA). The most prevalent enterotoxin genes were *seg* and *sei*. Two MRSA CC398 isolates, three MSSA CC398, and one MSSA were classified as multidrug-resistant. **Conclusion:** The first isolation of MSSA CC398 from beef in Poland indicates contamination of beef by strains belonging to this clonal complex. The occurrence of multidrug-resistant enterotoxigenic *S. aureus* isolates in the finished meat products constitutes a potential risk for the consumers.

**Keywords:** meat, *Staphylococcus aureus*, clonal complex 398, antibiotic resistance.

## Introduction

*Staphylococcus aureus* is considered as one of the most important pathogenic bacteria due to its ability to cause food-borne diseases and various types of infections. The methicillin-resistant *S. aureus* (MRSA) is of particular importance. Traditionally, two groups are distinguished among its strains: hospital-acquired MRSA (HA-MRSA) and community-acquired MRSA (CA-MRSA) (8, 13). Recently, a new type of isolates emerged into the *S. aureus* population, belonging to the clonal complex 398 (CC398) and defined as livestock-associated MRSA (LA-MRSA) (5, 29). Initially *S. aureus* CC398 was found to predominate among MRSA in pigs in Europe, but it may also colonise calves, cows, sheep, and poultry (11, 31). Transmission of LA-MRSA occurs especially in humans with

occupational exposure to livestock, particularly pig farming (13). It is also likely that LA-MRSA may be acquired through contaminated meat products (8, 31). These strains may cause similar infections in humans as *S. aureus* or MRSA (27), although most of the virulence factors typical for *S. aureus* are not detected among isolates of the clonal complex 398 (12). However, the recent reports indicate the presence of enterotoxin genes in these strains (4). Given the changing epidemiology, updated information on *S. aureus* strains is needed for the proper control measures of this pathogen (5, 29).

The aim of this study was preliminary characterisation of *S. aureus* occurring in the meat processing plants. The *S. aureus* isolates were subjected to molecular analysis in order to investigate the presence of enterotoxin genes, the *mecA* gene, and

to verify whether they belong to the CC398. Additionally, antimicrobial susceptibility of the isolates was tested.

## Material and Methods

**Isolation and identification of *S. aureus*.** A total number of 90 samples were obtained from three different meat processing plants from the area of Warsaw; 30 samples from each plant. From each plant, 10 samples were collected in the raw meat warehouse, 10 samples from the devices and surfaces, and 10 from the finished meat products. Staphylococci were isolated using a modified method with enrichment culture in Mueller-Hinton broth, as the objective of the study was not to determine the microbiological quality of samples, but to isolate *S. aureus*. The samples were taken by vigorous swabbing of the surface with sterile swab moistened with saline. Each swab was put in 10 mL of Mueller-Hinton broth (bioMérieux, France), containing 6.5% NaCl, and incubated at 37°C for 24 h. The 10 µL of this culture was inoculated on Columbia agar with 5% sheep blood (bioMérieux, France). Colonies formed after incubation were evaluated on the basis of colony morphology and haemolytic activity. All isolates producing colonies characteristic for *S. aureus* were tested with a tube test for coagulase and catalase production according to standard procedures. SlidexStaph test (bioMérieux, France) was used for initial identification of *S. aureus* isolates.

**Preparation of bacterial DNA.** A single colony of pure culture of each isolate was transferred to 25 µL of lysostaphin (100 mg/mL, A&A Biotechnology, Poland) and incubated at 37°C for 10 min. Next 25 µL of proteinase K (20 mg/mL, A&A Biotechnology, Poland) and 75 µL of Tris-HCl buffer were added to the bacterial suspension, which was incubated at 37°C for 10 min. The mixture was centrifuged for 5 min at 15,000 rpm, and the resulting supernatant containing the isolated DNA was used as a template in PCR reactions.

**Detection of *S. aureus*-specific fragment of *nuc* gene, *mecA* gene, clonal complex 398, and enterotoxin genes.** *S. aureus* was identified based on the amplification of the *nuc* gene described by Sasaki *et al.* (26). All obtained isolates were screened by duplex PCR using primers specific for *mecA* gene and clonal complex 398 (28). Strain MRSA CC398 S0358, kindly provided by the University of Copenhagen, Faculty of Health and Medical Sciences, Department of Veterinary Disease and Biology, served as a positive control (14). The detection of enterotoxin genes *sea*, *seb*, *sec*, *sed*, and *see* was performed by the method described by Becker *et al.* (2). Additionally, multiplex PCR was used for amplification of *sei*, *sej*, *sel*, and *sem* genes (7). PCR reactions were conducted using Dream Taq™ Master Mix (2x) (Thermo Scientific, USA) according to the manufacturer's instructions. All

amplified products (10 µL) were resolved on 1.5% agarose gel stained with ethidium bromide and documented with Versa Doc Model 1000 Imaging System with Quantity One 4.4.0 software (BioRad Laboratories, USA).

**Pulsed-field gel electrophoresis (PFGE).** PFGE was performed according to the harmonised protocol for *S. aureus* (23) with minor modifications (18). The restriction fragments obtained by digestion of staphylococcal DNA with *Sma*I (20 U/µL, Thermo Scientific, USA) were separated using a CHEF-DR II System (BioRad Laboratories, USA). Gel images were analysed by Gel Compare II version 4.6 (Applied Maths, Belgium) and cluster analysis was performed by UPGMA using dice similarity coefficient with optimisation set at 0.5 % and position tolerance at 1.5%. The isolates were clustered using an 80% homology cut-off, above which the isolates were considered to be closely related and assigned to the same PFGE cluster (30).

**Antimicrobial susceptibility testing.** All *S. aureus* isolates were evaluated for their antimicrobial susceptibility by disk diffusion method according to the EUCAST guidelines (10). The following antimicrobials were tested: amoxicillin (AMX, 25 µg), cefoxitin (FOX, 30 µg), gentamicin (GEN, 10 µg), erythromycin (ERY, 15 µg), clindamycin (CLI, 2 µg), ciprofloxacin (CIP, 5 µg), tetracycline (TET, 30 µg), and trimethoprim/sulfamethoxazole (SXT, 1.25/23.75 µg).

## Results

In total, 21 (23.2%) out of 90 samples were positive for *S. aureus*. The majority of the isolates were recovered from pork in the raw meat warehouse. The details concerning the place of isolation and type of meat are shown in Table 1.

Five out of 21 obtained isolates were assigned to the CC398 (Table 2). Three of them originated from finished meat products and two from raw poultry meat before processing. Moreover, two of the five *S. aureus* CC398 isolates were recognised as MRSA, one from finished pork product, and one from raw turkey meat. The most prevalent enterotoxin genes were *seg* and *sei* identified in five MSSA (methicillin-sensitive *S. aureus*) isolates, one MRSA CC398, and one MSSA CC398 that was also positive for *sea* gene. Additionally, one MSSA isolated from the knife used during meat processing was positive for enterotoxin B gene. All isolates were negative for *sec*, *sed*, *see*, *sel*, and *sem* enterotoxin genes.

Twelve distinct PFGE profiles were observed among 16 *S. aureus* isolates (Fig. 1). The remaining five isolates belonging to the CC398 were nontypeable by PFGE using *Sma*I. All PFGE profiles showed relatively high level of similarity. Some strains originated from the same plants and the same type of meat showed 100% identity in Gel Compare analysis of

PFGE profiles. Additionally, one isolate (6/04) from beef and one from pork (5/04) in the same plant exhibited identical PFGE profiles.

The antimicrobial resistance patterns of examined isolates are presented in Table 2. In general, 28.6% of *S. aureus* isolates were susceptible to all antimicrobials

tested, and 33.3% showed only amoxicillin resistance. Two out of three MSSA CC398, two MRSA CC398, and one MSSA isolates exhibited multidrug resistance phenotype with resistance to three or more classes of antimicrobial agents.

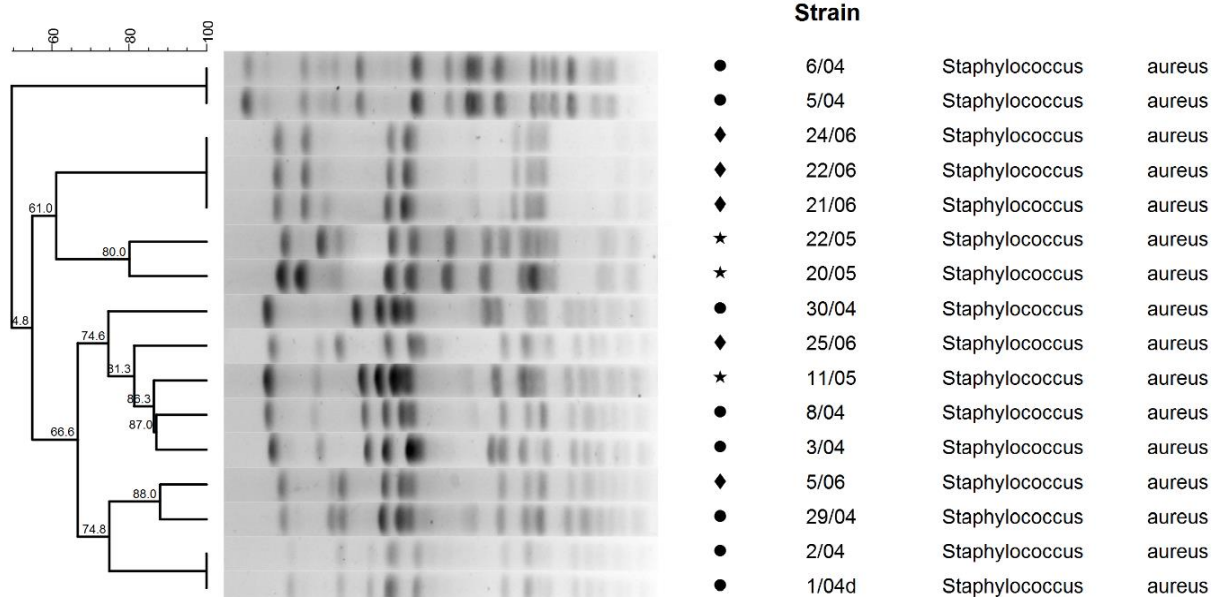
**Table 1.** The number of *S. aureus* isolates and the place of isolation in the meat processing plants

Place of isolation	Type of meat – number of <i>S. aureus</i> isolates		
	Pork	Beef	Poultry
Raw meat warehouse	8	2	3 (2 turkeys, 1 chicken)
Finished meat products	2	3	2 (1 turkey, 1 guinea fowl)
Knife surface used in the meat processing plant	1	0	0

**Table 2.** Characteristics of *S. aureus* isolates from meat processing plants

Meat processing plant	Source	Isolate	MSSA/MRSA	CC398	Presence of enterotoxin genes	Resistance pattern
A	pork - R <sup>a</sup>	1/04	MSSA	-	-	-
A	pork - R	2/04	MSSA	-	<i>seg, sei</i>	GEN
A	pork - R	3/04	MSSA	-	<i>seg, sei</i>	-
A	pork - R	5/04	MSSA	-	<i>seg, sei</i>	AMX
A	beef - R	6/04	MSSA	-	<i>seg, sei</i>	AMX
A	beef - R	8/04	MSSA	-	<i>seg, sei</i>	AMX, GEN, TET
A	pork - FP <sup>b</sup>	23/04	MRSA	+	<i>seg, sei</i>	AMX, FOX, SXT, GEN, ERY, CLI, TET
A	beef - FP	29/04	MSSA	-	-	AMX, GEN
A	beef - FP	30/04	MSSA	-	-	-
B	chicken - R	11/05	MSSA	-	-	AMX
B	turkey - R	14/05	MSSA	+	<i>sea, seg, sei</i>	AMX, GEN, ERY, CLI, TET, CIP
B	turkey - R	15/05	MRSA	+	-	AMX, FOX, SXT, GEN, ERY, CLI, TET
B	guinea fowl - FP	20/05	MSSA	-	-	-
B	turkey FP	22/05	MSSA	-	-	-
C	knife - E <sup>c</sup>	5/06	MSSA	-	<i>seb</i>	GM
C	pork - FP	11/06	MSSA	+	-	AMX, GEN, ERY, TET
C	beef - FP	16/06	MSSA	+	-	AMX
C	pork - R	21/06	MSSA	-	-	AMX
C	pork - R	22/06	MSSA	-	-	AMX
C	pork - R	24/06	MSSA	-	-	AMX
C	pork - R	25/06	MSSA	-	-	-

<sup>a</sup> R – raw meat; <sup>b</sup> FP – finished product; <sup>c</sup> E – environmental sample; AMX – amoxicillin; FOX – cefoxitin; GEN – gentamicin; TET – tetracycline; SXT – trimethoprim/sulfamethoxazole; ERY – erythromycin; CLI – clindamycin; CIP – ciprofloxacin



**Fig. 1.** Dendrogram showing the level of similarity of *SmaI* restriction PFGE patterns of the genomic DNA of *S. aureus*. Different shapes indicate various meat processing plants (● – meat processing plant A; ★ – meat processing plant B; ◆ – meat processing plant C)

## Discussion

The occurrence of *S. aureus*, in particular MRSA and enterotoxigenic strains in foods of animal origin, represents a relevant issue with regard to food safety (8, 15). The presence of LA-MRSA in raw meat was described in different countries. In the Netherlands MRSA (mostly the CC398) was detected in 10.7% of pork samples, 15.2% of beef, and 35.3% of turkey meat (9). *S. aureus* is common in pork samples; however, its prevalence varies between countries. For instance, in Germany *S. aureus* was detected in 4.2% of pork samples (3), in Taiwan in 9% (21), whereas in the USA in 45.6% of pork samples (25). Contamination of raw meat may result from colonisation of farm animals with *S. aureus*, particularly the CC398 (8). However, contamination of meat through human handling during processing is also possible (31). The results presented in this paper confirmed the presence of *S. aureus*, including MSSA and MRSA belonging to the CC398 in raw pork and poultry meat and food of animal origin in Poland, which was described previously by others (4, 19, 20, 24). According to our knowledge, this is the first report on the isolation of MSSA belonging to the CC398 from beef. It is significant that despite a small number of beef samples tested, one isolated strain was recognised as *S. aureus* CC398. Determination of the prevalence of strains from the CC398 among cattle and beef requires further studies.

More than 20 types of staphylococcal enterotoxins (SEs) showing phylogenetic relationship and sequence similarities have been described so far (15). SEs have been traditionally divided into classical (SEA to SEE) and new enterotoxins (SEG, SEH, SEI, SEJ, SEK, SEL, SEM, SEN, SEO, SEP, SER, and SEU) (29). A study conducted in Japan showed that 21.7% of *S. aureus* strains obtained from raw meat or food products of animal origin were positive for one or more classical enterotoxin genes (A-E) (17). Recently, isolation of *S. aureus* harbouring new enterotoxin genes from the finished meat products, and also from raw milk and dairy products has been reported with increased frequency (1, 25). Pu *et al.* (25) reported that 85% of *S. aureus* strains isolated from pork and beef were positive for at least one classical or new enterotoxin genes, and 66% harboured two or more of these genes. With regard to new STs, SEG and SEI are the most frequently detected and their presence was demonstrated in 66% of *S. aureus* strains isolated from meat samples in USA (1, 25). Moreover, the presence of classical and new staphylococcal enterotoxin genes was demonstrated also among coagulase-negative staphylococci obtained from beef and lamb meat (16) and from milk (24).

The results from Poland presented by Bystroń *et al.* (4) showed that 55% of *S. aureus* isolated from pigs and pork harboured genes encoding classical enterotoxins and 45% carried genes encoding new enterotoxins. Similarly to our results, the *seg* and *sei*

genes were the most prevalent. However, in our study only two isolates were positive for classical enterotoxin genes. In general, enterotoxin genes are not widespread among the MRSA CC398 lineage (12). Some reports described the CC398 strains carrying enterotoxin genes (*seb*, *sed*, *see*, *seg*, *sei*, *seg*, *sek*, *sem*, *ser*) genes (4). European Union Regulation 1441/2007 specifies the detection of SE in food of animal origin, but according to this regulation only the presence of classical enterotoxins is investigated (6). Therefore, the food is currently not examined for the presence of new enterotoxins. Thus, it could be assumed that the true incidence of new enterotoxin genes remains unknown.

PFGE typing is one of the best molecular methods of epidemiological analyses of *S. aureus*. One of the aims of this research was to compare *S. aureus* strains isolated from different meat processing plants. *S. aureus* belonging to the CC398 are nontypeable using *SmaI* (31) and this was observed in five strains detected in our study. In general, a high similarity was observed among the obtained PFGE profiles. Two MSSA isolates (6/04 and 5/04) recovered from beef and pork in the same plant exhibited 100% identity in PFGE profiles. Moreover, both of them had the same resistance patterns and were positive for the *seg* and *sei* resistant genes, implying that the raw meat could have been contaminated during processing.

In general, LA-MRSA isolates, representing multidrug-resistant phenotype, usually are resistant to all  $\beta$ -lactams, tetracyclines, macrolides, lincosamides, and streptogramins (12, 31). Accordingly, two MRSA CC398, three MSSA CC398, and one MSSA isolates were multidrug-resistant in our study. These results are in accordance with the data obtained in the USA, where multidrug resistance was commonly observed among MRSA as well as *S. aureus* from pork (1, 25). However, the majority of MSSA isolates described in our study were susceptible to all antimicrobials tested or showed only the amoxicillin resistance. The presence of resistant bacteria in food of animal origin is highly undesirable, since resistant genes may be transferred from MRSA to bacteria of the human microbiota.

The study confirms that *S. aureus* strains may adapt to different hosts and are constantly evolving. Łuczak-Kadłubowska *et al.* (22) showed the presence of different new clones among the population of MRSA in Poland. Therefore, continuous monitoring of the genetic diversity of *S. aureus*, particularly MRSA, is needed. This study presents preliminary data on the characterisation of *S. aureus* obtained from the meat processing plants. Nevertheless, the isolation of MRSA CC398, multidrug-resistant, and enterotoxigenic strains is alarming and further investigations should be undertaken. Extensive studies are necessary to identify the potential sources of contamination of the finished meat products and to establish a potential risk.

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