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Investigation of the antibiotic resistance of staphylococcus species isolated from foods

Keywords: food, Staphylococcus, antibiotic resistance, MALDI-TOF- MS, bacterial identification, food safety, human pathogen, nosocomial infection

1. SUMMARY

The presence of methicillin-resistant *Staphylococcus aureus* (MRSA) strains in the food chain has been confirmed by several studies in the European Union, but there are only limited data available in Hungary. The objective of the present study was to investigate the antibiotic resistance of Staphylococcus strains isolated from foods, using classical microbiological, molecular biological methods and the MALDI-TOF-MS technique, as well as the multi-locus sequence typing (MLST) of antibiotic resistant strains. During the study, 47 coagulase-positive (CPS) and 30 coagulase-negative (CNS) Staphylococcus isolates were collected. In the course of the MALDI-TOF-MS investigations, all CPS isolates (n=47) were found to be S. aureus species, while 8 different species were identified in the case of the CNS strains. Methicillin resistance was confirmed in two *S. aureus* strains, one of which had a sequence type not yet known, while the other MRSA strain was type ST398, which is the most common type of MRSA strain isolated from farm animals in the EU/EEA.

(The abbreviation "MRSA" is often used in common parlance, but occasionally in the literature to denote "multidrug-resistant Staphylococcus aureus". In the authors' manuscript - the methicillin-resistant pathogen is correctly designated as such. Ed.)

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2. Introduction and literature review

The number of nosocomial infections caused by antibiotic-resistant microorganisms has been increasing in all countries, thus posing a greater and greater challenge to the health care system **[1, 2].** The situation is further exacerbated by the fact that antibiotic-resistant *Staphylococcus* species have already appeared not only in communities and health care, but also in intensive animal husbandry and thus in the food chain [**3**].

In *Staphylococcus* species, genes associated with antibiotic resistance and virulence are found in the mobile genetic elements (MGE), such as chromosome cassettes, pathogenicity islands, plasmids or transposons **[4].** The *mecA* gene is responsible for methicillin resistance: the gene encodes a modified penicillin-binding protein that reduces the binding affinity of most beta-lactam antibiotics, such as penicillin and methicillin. The *mecA* gene is located on the *Staphylococcus* chromosome cassette (SSC*mec*), which is a group of MGE found only in *Staphylococcus* species [**5**]. The transfer mechanism of the *mecA* gene between *Staphylococcus* species is unknown, however, evidence supports horizontal gene transfer between coagulase-positive and coagulase-negative *Staphylococcus* species **[6]**.

The presence of methicillin-resistant *Staphylococcus aureus* (MRSA) strains in the food chain has already been reported in several studies. Some of their authors examined strains isolated from food samples of animal origin, others investigated strains isolated from raw meat samples (pig, fish, poultry). In the Netherlands in 2009, 2,217 different food samples were analyzed, 12% of which contained MRSA strains **[7]**, while in a Danish study 4.6% of 153 pork samples and 7.5% of imported pork samples were infected with MRSA strains **[8]**. MRSA strains have also been identified in Germany in raw milk, pork, turkey and broiler chicken **[9]**. In Hungary, 27 MRSA isolates were identified in the 595 individual milk samples of a dairy farm **[10]**, while in another study only 4 strains out of the 626 *S. aureus* isolates of 42 farms proved to be methicillin-resistant **[11]**. However, beyond these examples, the presence of MRSA in foods from other categories and ready-to-eat foods has not been investigated so far. Molecular typing results of the strains have shown that many types of MRSA are present in the food chain in different countries **[12]**, but the most common type is CC398, accounting for 85% of the MRSA strains isolated from farm animals in the EU and the EEA **[13, 14, 7,15]**.

The presence of additional methicillin-resistant *Staphylococcus* (MRS) species in foods has been investigated by fewer studies. In Nigeria, 13 *Staphylococcus* species (*S. xylosus*, *S. epidermidis*, *S. simulans*) showed methicillin resistance out of 255 isolates from traditional foods **[16]**. In a study in Poland, out of 58 strains isolated from ready-to-eat foods, 33 *Staphylococcus* strains (*S. epidermidis, S. simulans, S. xylosus, S. hycus, S. lentus, S. saprophyticus*) showed resistance to at least one type of antibiotic **[17]**.

In the European Union, testing for antibiotic resistance in *Staphylococcus* strains isolated from foods and farm animals is currently voluntary, so in 2016 only Germany, Switzerland, Denmark and Spain reported information related to this topic. The incidence of MRSA varied from country to country, but in the case of a comparison it should be taken into account that the studies were performed on strains isolated from different animal species, meats and meat products **[18]**. A small proportion of human infections can be traced back to MRSA strains of the CC398 type, and they are also mainly limited to occupational exposures, such as veterinary medicine and intensive animal husbandry. Nevertheless, the virulence factors detectable in CC398-type MRSA strains allow for high pathogenicity, so continuous revision is essential both in animals and in foods **[19]**. The need for surveillance is also justified by the possible presence of antibiotic resistance of other *Staphylococcus* species, which allows for the spread of resistance and poses a risk to consumer health.

A prerequisite for a successful surveillance system is the availability of a uniform, economically acceptable, rapid and reliable method for the species-level identification of the microorganisms and the determination of antibiotic resistance, a promising cornerstone of which could be matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) based on peptide identification. In the early 2000s, several studies reported specific fragment ions that allow rapid identification of antibiotic-resistant *Staphylococcus* strains. The most often studied biomarker was the fragment ion with an m/z value of 2,414, the appearance of which in the mass spectrum correlates with the expression of *psm-mec*, characteristic of MRSA strains [**20**]. The applicability of the 2,414 m/z fragment ion for detection and discrimination has been demonstrated in several studies **[21, 22]**.

In addition to the studies of the biomarkers of MRSA strains, methicillin resistance-specific fragment ion peaks of other *Staphylococcus* species have been analyzed in other studies. In an earlier article, two specific fragment ion values were determined: the ion fragment peak with an m/z value of 7,239, which is a biomarker of methicillin-resistant *S. epidermidis*, and the fragment ion peak with an m/z value of 9,674, which is the biomarker of methicillin-resistant *S. haemolyticus* **[23]**.

The objective of our own experiments was to investigate the methicillin resistance of *Staphylococcus* strains isolated from foods using classical microbiological, molecular biological methods and the MALDI-TOF-MS technique, as well as the multi-locus sequence typing (MLST) of antibiotic-resistant strains for the epidemiological study of the strains.

3. Materials and methods

3.1. The isolates collected and culture conditions

77 *Staphylococcus* isolates, isolated according to the requirements of standard MSZ EN ISO 6888-1:2008, were analyzed in the Microbiological Laboratory of WESSLING Hungary Kft. in the period between August 2019 and September 2020. The isolates were grown on a Baird-Parker (Biokar, France) selective culture medium at 37 °C for 24±1 hour and colonies characteristic of *Staphylococci* were inoculated onto Columbia blood agar (Neogen, UK) (37 °C, 24±1 hours). In the course of culturing, 47 strains showed a positive coagulase reaction, while 30 isolates proved to be coagulase-negative, which was also confirmed by a latex agglutination rapid test (PASTOREX™ STAPH-PLUS). Isolates were collected from raw meat, meat products and ready-to-eat foods: poultry (n=14), beef (n=5), pork (n=42), game (n=1), fish (n=1), dairy product (n=3), ready-to-eat foods (n=3), vegetables (n=2) and dry pasta (n=6).

3.2. Identification of the isolates using the MALDI-TOF-MS technique

The 77 isolates collected were identified using a Bruker Microflex LT MALDI-TOF mass spectrometer and the MALDI BioTyper 3.1 (Bruker Daltonics) software. A formic acid suspension protocol was used in which a single colony was collected from the Columbia blood agar using a sterile loop, and then it was suspended in 40 µl formic acid. To the suspension was added 40 µl of acetonitrile, 1 µl of which was applied to one of the positions of the plate. After the solvent evaporated, 1 µl of α-HCCA (10 mg/ml α-Cyano-4-hydroxycinnamic acid) matrix solution was applied and the solvent was allowed to evaporate once more. 6 parallel measurements were performed for each sample.

To identify the isolates, the MALDI Biotyper 3.1 software was used, which compares the mass spectra obtained with the reference mass spectra in its database and calculates a compliance factor (score). In case of a log score value of 2.300 – 3.000, identity is highly probable. At a log score value this high, the species is considered to be identified. If the log score value is between 2.000 and 2.299, the identity is less certain, so in this case only the genus of the microorganism can be considered identified. When the log score value is between 1.700 and 1.999, even the identification of the genus cannot be considered sufficiently certain. If a log score value between 0.000 and 1.699 is returned by the evaluation software, identification should be considered unsuccessful. Identification of the coagulase-positive *Staphylococcus* strains included in the study was carried out earlier **[24]**.

3.3. Antibiotic susceptibility testing

3.3.1. Investigation of the methicillin resistance specific peaks by the MALDI-TOF-MS method

The mass spectra obtained were exported to the flexAnalysis 3.4 software (Bruker Daltonics) and manual analysis and comparison of the mass spectra was performed. Smoothing of the mass spectra was carried out with the Savitzky–Golay filter, while baseline correction was performed using the TopHat algorithm. During the analysis the presence of methicillin resistant-specific fragment ion values was examined (***Table 1***).

Table 1. Methicillin resistance (MR) specific peaks analyzed in this study

Species	MR specific fragment ion	Literature reference
S. aureus	m/z 2.414	**[20]**
S. aureus	m/z 2.414	**[22]**
S. aureus	m/z 2.414	**[21]**
S. epidermidis	m/z 7.239	**[23]**
S. haemolyticus	m/z 9.674	**[23]**

3.3.2. Disk diffusion method

When examining the antibiotic resistance of the strains, the guidelines of the CLSI (Clinical and Laboratory Standards Institute, 2019) were followed **[25]**. A bacterial suspension equivalent to 0.5 McFarland unit was applied to the surface of a Mueller-Hinton agar (Oxoid, UK), and then Cefoxitin 30 µg disks were placed on the surface of the medium. The strains were incubated at 37 °C for 18 hours. In the case of MRSA strains, the reference range of the clearance zone was 6-19 mm volt, while for *mecA* negative species it was 20-32 mm.

3.3.3. Selective differentiation agar

In addition to the above, CHROMagar MRSAII selective differentiation medium (BD, UK) was used for the detection of methicillin-resistant *Staphylococcus aureus* species in the antibiotic resistance assays. Isolates were incubated at 37 °C for 24 – 48 hours under aerobic conditions. Strains that formed mauve colonies, morphologically similar to those of *Staphylococci*, were considered MSRA bacteria. The disk diffusion method (Cefoxitin 30 µg) and the MRSA CHROMagar assay were repeated twice for each strain isolated from a food (n=77). During the study, the reference MRSA strain ATCC 33591 was used as a positive control and the MRSA strain ATCC 29213 was used as a negative control.

3.3.4. mecA gene complex

Detection of the *mecA* gene was performed according to the protocol of the Danish National Food Institute (NFI), published in 2012 **[26]**. During the study, MRSA strain ATCC 43300 was used as a positive control and MRSA strain ATCC 29213 was used as a negative control. Genomic DNA was isolated from the bacteria and the *mecA* gene sequence was amplified using PCR. The primers used are listed in ***Table 2***.

Table 2. Primers used for the amplification of the mecA gene

Primer name	Primer sequence
mecA_fwd	5'- GGGATCATAGCGTCATTATTC-3'
mecA_rev	5'- AACGATTGTGACACGATAGCC-3'

3.4. MLST study of *methicillin-resistant* Staphylococcus *strains*

According to the study of Thomas et al. **[27]**, genomic DNA was isolated from the bacteria, and then the gene sequences specific for the 7 *Staphylococcus aureus* species were amplified using PCR (***Table 3***). The nucleotide sequences of the purified PCR products were determined and the sequence data were evaluated in the *BioNumerics 7.6* software.

Table 3. Genes used in the MLST method and data of the primers used in their amplification

Gene	Primer	Primer DNA sequence (5'-3')
Carbamate kinase (arcC)	arc up_1	TTG ATT CAC CAG CGC GTA TTG TC
	arc dn_2	AGG TAT CTG CTT CAA TCA GCG
Shikimic acid dehydrogenase (aroE)	aro up_3	ATC GGA AAT CCT ATT TCA CAT TC
	aro dn_4	GGT GTT GTA TTA ATA ACG ATA TC
Glycerol kinase (glpF)	glp up_5	CTA GGA ACT GCA ATC TTA ATC C
	glp dn_6	TGG TAA AAT CGC ATG TCC AAT TC
Guanylate kinase (gmk)	gmk up_7	ATC GTT TTA TCG GGA CCA TC
	gmk dn_8	TCA TTA ACT ACA ACG TAA TCG TA
Phosphate acetyltransferase (pta)	pta up_9	GTT AAA ATC GTA TTA CCT GAA GG
	pta dn_10	GAC CCT TTT GTT GAA AAG CTT AA
Triose phosphate isomerase (tpi)	tpi up_11	TCG TTC ATT CTG AAC GTC GTG AA
	tpi dn_12	TTT GCA CCT TCT AAC AAT TGT AC
Acetyl coenzyme A acetyltransferase (yqiL)	yqi up_13	CAG CAT ACA GGA CAC CTA TTG GC
	yqi dn_14	CGT TGA GGA ATC GAT ACT GGA AC

4. Results

4.1. Identification results of the isolated strains

In the MALDI-TOF-MS study, all coagulase-positive *Staphylococcus* (CPS) strains (n=47) were found to be *S. aureus* species (***Tables 4 and 6***). In the case of coagulase-negative *Staphylococcus* (CNS) strains, 8 different species (*S. xylosus, S. saprophyticus, S. pasteuri, S. epidermidis, S. warneri, S. chromogenes, S. piscifermentans, S. haemolyticus*) were identified (***Tables 4 and 7***). 30% of the CNS isolates (n=30) were found to be *S. warneri* species, while 23% were found to be *S. pasteuri* species. 70% of the *S. aureus* strains and all of the CNS strains were isolated from meat and meat products ***(Figures 1 and 2)***. In the case of *S. aureus* strains, 64% of the meats and meat products came from pigs, while this was true for 70% of the CNS strains. Within meat and meat products, the distribution of isolates coming from poultry and beef was nearly the same.

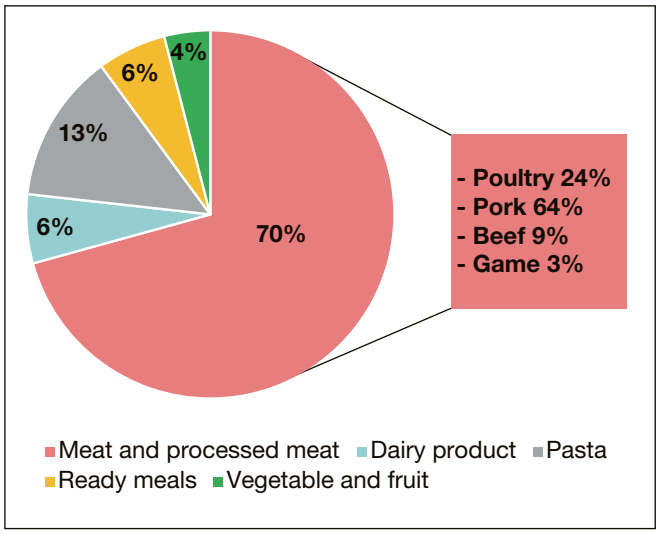

Figure 1. Food types tested

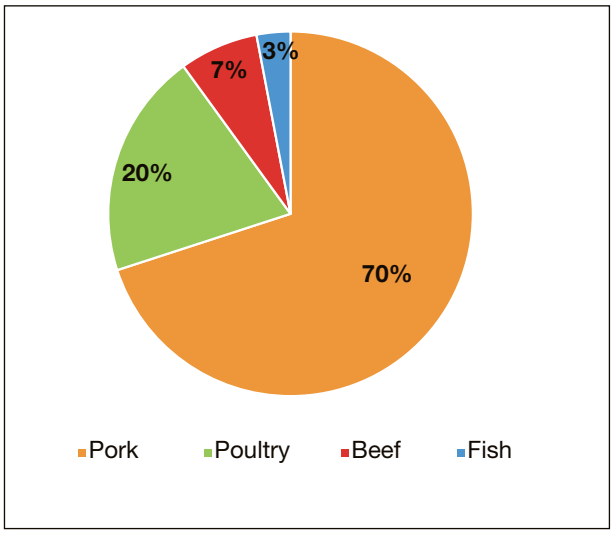

Figure 2. Raw materials of meats and meat products

The mean identification log score values of the isolates and their standard deviations are summarized in ***Table 4***. The mean identification log score value of *S. aureus* isolates exceeded 2.400. The lowest log score value was 2.304, and even in this case, identification can be considered safe. When examining CNS isolates, each species was identified with a log score value above 2.300 and the standard deviation did not exceed 0.1 in any of the cases.

Table 4. Identification log score values of the identified coagulase-positive and -negative Staphylococcus species

Species	Mean identification log score value	Lowest log score value	Highest log score value	Standard deviation of log score values
S. aureus (n=47)	2.415	2.304	2.571	0.072
S. xylosus (n=1)	2.412	-	-	-
S. saprophyticus (n=3)	2.406	2.395	2.415	0.010
S. pasteuri (n=7)	2.387	2.325	2.462	0.050
S. epidermidis (n=4)	2.348	2.304	2.396	0.038
S. warneri (n=9)	2.365	2.324	2.406	0.028
S. chromogenes (n=1)	2.372	-	-	-
S. haemolyticus (n=4)	2.353	2.307	2.413	0.054
S. piscifermentans (n=1)	2.318	-	-	-

4.2. Methicillin resistance results determined by the MALDI-TOF-MS technique

In the analysis of the mass spectra obtained from the isolates, 3 antibiotic resistance-specific peaks were examined. The m/z 2,414 peak is a protein product of the *mecA* gene **[28]**, so the presence or absence of this peak was examined for all strains. The detectability of the m/z 7,239 peak was examined only in *S. epidermidis* species, while the presence/absence of the m/z 9,674 peak was examined only in *S. haemolyticus* species due to the species specificity of the peaks.

The m/z 2,414 peak was detected in two *S. aureus* strains out of 77 isolates, one of which came from goose liver (SA-17), while the other came from pork butt (SA-47). For the additional 75 isolates, this peak did not appear even at a low intensity (***Table 5***). In the analysis, mass spectra of the two *S. aureus* strains that proved to be methicillin-resistant were marked in red, while the mass spectra of the other *S. aureus* strains that did not have a methicillin resistance-specific peak were marked in black ***(Figures 3 and 4).***

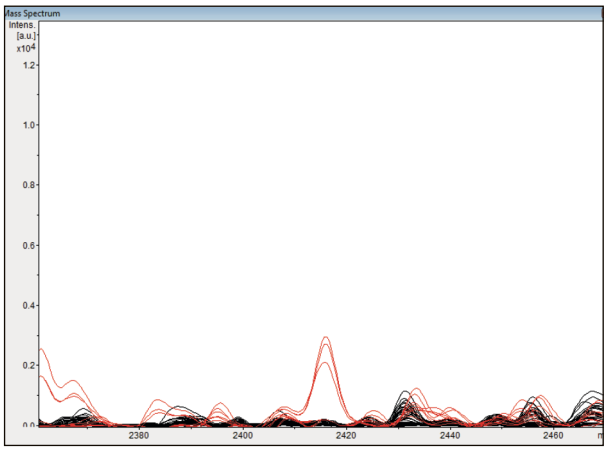

Figure 3. Mass spectrum of the m/z 2,414 transition

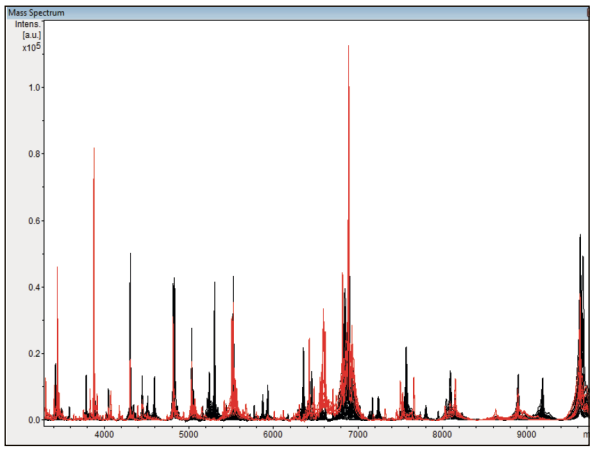

Figure 4. Mass spectra of the 47 Staphylococcus strains

Table 5. Frequency of specific ion fragment values in Staphylococcus strains isolated from foods

Species	Frequency of specific ion fragment values		
	m/z 2,414	**m/z 7,239**	**m/z 9,674**
S. aureus (n=47)	4,3%	-	-
S. xylosus (n=1)	-	-	-
S. saprophyticus (n=3)	-	-	-
S. pasteuri (n=7)	-	-	-
S. epidermidis (n=4)	-	-	-
S. warneri (n=9)	-	-	-
S. chromogenes (n=1)	-	-	-
S. haemolyticus (n=4)	-	-	-
S. piscifermentans (n=1)	-	-	-

The m/z 7,239 peak could not be detected in any of the *S. epidermidis* strains (n=4), while the m/z 9,674 peak did not appear in any of the 4 *S. haemolyticus* species.

4.3. Results of the disk diffusion method and the MRSA CHROMagar selective differentiation agar

In the study of the 77 strains, the diameter of the clearance zone ranged from 23 to 29 mm in the case of 75 strains. The strain isolated from goose liver (SA-17) had a clearance zone with a diameter of 9 mm, while the strain isolated from pork butt (SA-47) had a clearance zone with a diameter of 17 mm, and the same strains also formed mauve-colored colonies on the MRSA CHROMagar selective differentiation medium (***Tables 6 and 7***).

Table 6 Antibiotic resistance test results of the S. aureus strains identified in foods

Food category	ID	Food	log score value	Identification result	Clearance zone (mm)	MRSA Chromagar	*mecA* gene
Dairy product	SA-1	Trappista cheese	2.458	*S. aureus*	24	-	-
	SA-10	Trappista cheese	2.440	*S. aureus*	26	-	-
	SA-18	Milk dessert	2.513	*S. aureus*	25	-	-
Dry pasta	SA-2	Dry pasta	2.476	*S. aureus*	25	-	-
	SA-49	Dry pasta	2.308	*S. aureus*	28	-	-
	SA-7	Dry pasta	2.304	*S. aureus*	23	-	-
	SA-21	Dry pasta	2.486	*S. aureus*	27	-	-
	SA-22	Dry pasta	2.448	*S. aureus*	24	-	-
	SA-11	Dry pasta	2.429	*S. aureus*	25	-	-
Meat	SA-12	Chicken legs	2.465	*S. aureus*	23	-	-
	SA-13	Chicken legs	2.345	*S. aureus*	27	-	-
	SA-16	Marinated chicken	2.332	*S. aureus*	24	-	-
	SA-44	Whole duck	2.401	*S. aureus*	28	-	-
	SA-17	Goose liver	2.447	*S. aureus*	9	+	+
	SA-20	Duck plate	2.432	*S. aureus*	24	-	-
	SA-28	Duck leg	2.353	*S. aureus*	23	-	-
	SA-29	Duck crackling	2.571	*S. aureus*	26	-	-
	SA-4	Bacon	2.319	*S. aureus*	23	-	-
	SA-31	Bacon	2.546	*S. aureus*	26	-	-
	SA-5	Sausage	2.477	*S. aureus*	26	-	-
	SA-6	Pork tenderloin	2.366	*S. aureus*	23	-	-
	SA-32	Pork tenderloin	2.466	*S. aureus*	26	-	-
	SA-25	Sausage	2.429	*S. aureus*	25	-	-
	SA-33	Pork tenderloin	2.329	*S. aureus*	23	-	-
	SA-24	Pork shoulder	2.343	*S. aureus*	28	-	-
	SA-34	Pork tenderloin	2.350	*S. aureus*	29	-	-
	SA-9	Pork shoulder	2.425	*S. aureus*	23	-	-
	SA-35	Pork shoulder	2.500	*S. aureus*	24	-	-
	SA-36	Pork shoulder	2.387	*S. aureus*	23	-	-
	SA-37	Pork shoulder	2.429	*S. aureus*	24	-	-
	SA-15	Crackling	2.342	*S. aureus*	25	-	-
	SA-38	Sausage to bake	2.474	*S. aureus*	26	-	-
	SA-02	Pork shoulder	2.383	*S. aureus*	26	-	-
	SA-01	Sausage	2.395	*S. aureus*	23	-	-
	SA-39	Pork knuckle	2.562	*S. aureus*	24	-	-
	SA-40	Pork knuckle	2.516	*S. aureus*	26	-	-
	SA-41	Head	2.403	*S. aureus*	23	-	-
	SA-47	Pork butt	2.354	*S. aureus*	17	+	+
	SA-3	Beef	2.333	*S. aureus*	29	-	-
	SA-8	Beef	2.484	*S. aureus*	25	-	-
	SA-48	Guts	2.362	*S. aureus*	28	-	-
	SA-42	Rabbit meat	2.371	*S. aureus*	24	-	-

Ready-to-eat foods	SA-43	Hamburger meat	2.382	*S. aureus*	23	-	-
	SA-50	Grilled chicken	2.305	*S. aureus*	28	-	-
	SA-45	Chocolate	2.339	*S. aureus*	23	-	-
Vegetables	SA-46	Fresh salad mix	2.525	*S. aureus*	28	-	-
	SA-27	Carrot paté	2.421	*S. aureus*	25	-	-

Table 7. Antibiotic resistance test results of the coagulase-negative Staphylococcus strains identified in foods

Food category	ID	Food	log score value	Identification result	Clearance zone (mm) (cefoxitin)	MRSA Chromagar
Poultry	061SH	Egg	2.384	*S. haemolyticus*	24	-
	062SH	Egg	2.307	*S. haemolyticus*	26	-
	178SE	Chicken breast	2.340	*S. epidermidis*	26	-
	392SP	Duck crackling	2.318	*S. piscifermentans*	26	-
	473SE	Egg cream	2.353	*S. epidermidis*	25	-
	529SW	Paté	2.324	*S. warneri*	26	-
Pork	573SX	Crackling paté	2.412	*S. xylosus*	24	-
	377SS	Pork butt	2.395	*S. saprophyticus*	24	-
	378SS	Pork butt	2.408	*S. saprophyticus*	24	-
	051SP	Pork shoulder	2.354	*S. pasteuri*	26	-
	527SE	Ground meat	2.304	*S. epidermidis*	23	-
	052SP	Pork shoulder	2.462	*S. pasteuri*	25	-
	528SE	Ground meat	2.396	*S.epidermidis*	23	-
	530SW	Ground meat	2.364	*S.warneri*	24	-
	426SW	Pork shoulder	2.348	*S.warneri*	24	-
	868SS	Pork butt	2.415	*S. saprophyticus*	28	-
	911SW	Ground meat	2.371	*S.warneri*	24	-
	393SW	Pork shoulder	2.403	*S.warneri*	24	-
	427SW	Pork butt	2.362	*S.warneri*	23	-
	976SP	Pork shoulder	2.325	*S.pasteuri*	24	-
	050SP	Pork shoulder	2.397	*S.pasteuri*	26	-
	051SP	Pork butt	2.392	*S.pasteuri*	25	-
	052SW	Pork butt	2.334	*S.warneri*	26	-
	472SP	Ground meat	2.341	*S.pasteuri*	26	-
	065SW	Pork tenderloin	2.375	*S.warneri*	23	-
	510SH	Crackling paté	2.413	*S.haemolyticus*	26	-
	512SH	Pork butt	2.309	*S.haemolyticus*	24	-
Beef	435SW	Beef patty	2.406	*S. warneri*	24	-
	434SC	Beef neck	2.372	*S. chromogenes*	28	-
Fish	979SP	Carp slices	2.436	*S. pateuri*	24	-

4.4. mecA *gene detection results*

Based on the results of the MALDI-TOF-MS analyses, the disk diffusion method and the MRSA selective differentiation medium, it was found that the *S. aureus* strains isolated from goose liver and pork butt (SA-17, SA-47) carry methicillin resistance, and this was confirmed by the *mecA* gene responsible for PBP2a synthesis, which could also be detected in the two strains (***Table 6***).

4.5. MLST types of the MRSA strains

MLST typing of the two MRSA strains was also performed during the study, using data available on the PubMLST website (https://pubmlst.org/saureus/). Of the two MRSA strains, the BioNumerics 7.6 software

could only assign the sequence type in the case of the strain isolated from pork butt. The strain isolated from goose liver belonged to a sequence type not yet known, while the strain isolated from pork butt belonged to sequence type 398 (***Table 8***).

Table 8. MLST types of the MRSA strains isolated from foods

Food	ID	Species	„Repeat succession" code	MLST type
Pork butt	SA-47	*S. aureus*	08-16-02-25-34-24-25	ST398
Goose liver	SA-17	*S. aureus*	09-02-16-34-17-34-16-34	Unknown

5. Summary and conclusions

During the study, 77 *Staphylococcus* isolates were collected from various food matrices according to the methods described in standard MSZ EN ISO 6888-1:2008. Although the standard allows the separation of coagulase-positive and coagulase-negative *Staphylococcus* species, it does not allow species identification, which is significant because of the different virulence factors and pathogenicities of the species. Identification of the 47 coagulase positive and 30 coagulase-negative *Staphylococcus* strains isolated from foods was performed using the MALDI-TOF-MS technique, with high identification log score values. In addition, by analyzing the mass spectra obtained and based on methicillin resistant-specific ion fragment values determined in previous studies, methicillin resistance was found in two *S. aureus* strains, which was confirmed by the disk diffusion method, selective differentiation agar and the detection of the *mecA* gene. Thanks to the specific ion fragment values, the diagnostic time can be significantly reduced, which is not negligible from economic and therapeutic points of view. However, it should be taken into consideration that due to the high variability of MRSA strains, the sensitivity and specificity of these ion fragment values are not 100%, so confirmatory studies are required.

Multi-locus sequence typing (MLST) of the two MRSA strains isolated from foods was also carried out for the epidemiological study of the strains. The isolate coming from pork belonged to type ST398, which is the most common type of MRSA strain isolated from farm animals in the EU/EEA. However, taking into account the specific host adaptation capabilities of type ST398 strains, which allow them to adhere not only to pigs, but also to other animal species and the human body, contamination and infection may occur in a number of ways during the technological steps in food processing.

Given that 2 of the 47 *S. aureus* strains isolated from foods proved to be methicillin-resistant, this fact confirms the dangers posed by globally increasing antibiotic resistance, thus indicating the severity and urgency of the situation.

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