

Detection of *mecA/mecC* Genes in Coagulase-Positive *Staphylococcus aureus* Isolated from Raw Beef Meat at the Retail

Gergana Krumova-Valcheva*, Gergana Mateva, Eva Gyurova, Mihail Milanov, Hristo Daskalov

National Food Safety Center, National Diagnostic and Research Veterinary Medical Institute, Sofia, Bulgaria

Abstract

This study aimed to detect coagulase-positive *Staphylococcus aureus* in raw beef, retailed in Bulgaria, and their antimicrobial resistance profile and detection of methicillin-resistant genes. 157 samples of retail packaged raw beef meat from various origins were investigated. The presence of coagulase-positive staphylococci was carried out according to ISO 6888-3. The antimicrobial susceptibility testing was carried out by disk diffusion method and isolates with resistance to at least two antimicrobial classes were verified by the microdilution assay to detect the minimal inhibitory concentration (MIC). Multiplex polymerase chain reaction (PCR) was carried out to confirm MRSA. *S. aureus* was detected in 29.3% (46/157). MRSA were found in 3.8% (6/157). Antimicrobial resistance was found in 32.6% of *S. aureus* isolates with resistance to penicillin being the most common (56.5%). 33.3% (9/27) of the isolates showed resistance to ceftiofur >4mg/L. The *mecA/mecC* genes were identified in 6 out of a total 9 *S. aureus* isolates. The *spa* gene was detected in all tested *S. aureus* isolates, confirming their biochemical results. In conclusion, the raw beef meat, retailed in Bulgaria, appear to be possible source of MRSA and risk to human health. Controlling antibiotics in agriculture and food production is a first step to manage this risk.

Keywords: coagulase-positive *S. aureus*, MRSA, beef, antimicrobial resistance, multiplex PCR

Резюме

Целта на това проучване е да се докаже разпространението на коагулазо-положителни *Staphylococcus aureus* в сурово говеждо месо, продавано в търговската мрежа в България, както и да се установи профилът на антимикробната им резистентност и наличието на гени, определящи метицилинова резистентност. 157 проби от сурово говеждо месо с различен произход бяха изследвани. Наличието на коагулазо-положителни стафилококи се установи в съответствие с ISO 6888-3. Изследването на антимикробната чувствителност беше извършено чрез диск-дифузионния метод, а изолатите с резистентност към поне два антимикробни класа бяха проверени чрез метода за определяне на минималната инхибиторна концентрация (MIC). За потвърждаване на MRSA беше използвана мултиплексна полимеразна верижна реакция (PCR). *S. aureus* беше открит в 29.3% (46/157). MRSA бяха доказани в 3.8% (6/157). Антимикробна резистентност беше установена при 32.6% от изолатите на *S. aureus*, като най-често срещана беше резистентността към пеницилин (56.5%). При 33.3% (9/27) от изолатите се доказа резистентност към цефокситин >4mg/L. Гените за определяне на резистентност към метицилина (*mecA/mecC*) бяха идентифицирани в 6 от общо 9 изолата *S. aureus*. Генът *spa* беше открит във всички тествани изолати *S. aureus*, което потвърди напълно биохимичните им характеристики. В заключение, суровото говеждо месо, продавано в търговската мрежа в България, представлява възможен източник на MRSA и риск за общественото здраве. Контролът на антибиотиците в селското стопанство и производството на храни е първата стъпка за управление на този риск.

Introduction

Staphylococcus aureus subsp. *aureus* is the main coagulase-positive staphylococci. They are widespread, Gram-positive facultative anaerobic

microorganisms. They are normally present in the nasal cavity and the skin of warm-blooded animals and humans. They can cause infection of varying

*Corresponding author: dr.krumova_valcheva@abv.bg
Acta Microbiol. Bulg. 2026; 42(01). <https://doi.org/10.59393/amb26420106>

severity under certain conditions. In addition, they can produce a variety of thermostable enterotoxins when introduced with food and can cause food poisoning and toxic shock syndrome (Turner *et al.*, 2019).

Antimicrobial resistance is more common bi-hazard because of the widespread use of antimicrobials for decades. Food also plays a role in the transmission of resistance to antimicrobial agents. In addition, foods may contain various antibiotic residues that can transmit resistance to pathogenic microorganisms through the ingestion of non-resistant pathogens in food (Pesavento *et al.*, 2007). An integrated surveillance system for AMR along the food chain, based on the One Health concept, should be established to prevent the spread of antimicrobial-resistant bacteria (EFSA, 2019). In this regard there is limited information on the prevalence and antimicrobial resistance of methicillin-resistant *S. aureus* in different foods in Bulgaria (Gogov, 2011).

Cattle farming is considered an agricultural sector with high MRSA occurrence. The research carried out by the European Food Safety Authority (EFSA, 2024) reported the prevalence of MRSA in calves at the time of slaughter (less than 1 year old) in the Netherlands and Belgium. A study carried out by Farahmand *et al.* (2020) examined 42 beef samples from different retails in Tabriz. The results indicated that *S. aureus* was presents in 25 of the samples. Most of the isolates were associated with high levels of antimicrobial resistance and 15 of them were methicillin resistant. A study in Pakistan showed the presence of *S. aureus* in 75% of the tested beef samples and 63% were resistant to cefoxitin (Sadiq *et al.*, 2020).

The aims of our study were to detect coagulase-positive *S. aureus* and MRSA in raw beef sold at retail in Bulgaria, their antimicrobial resistance as well as the presence of the *mecA/mecC* gene.

Materials and Methods

Sample collection

Retail packaged raw beef samples (n=157) were randomly selected from Bulgarian retails between August 2019 and December 2021. The samples origin was as follows: Bulgaria (n=124), Belgium (n=1), Ireland (n=9), The Netherlands (n=16), Romania (n=1), USA (n=2) and of unknown (n=5). Each sample is in an individual commercial package weighing at least 200 g. The samples were transported under refrigerated conditions (4 °C) up to 24 h after collection and tested immediately upon

receipt.

Isolation of coagulase-positive *S. aureus*

Isolation of coagulase-positive *S. aureus* was carried out according to ISO 6888-3:2003. Five potential colonies from Baird-Parker agar were randomly selected and then cultures on Braih-Heart agar (BHI) (Merck, France) at 37 °C for 24 hours. The presumptive isolates *Staphylococcus* spp. were tested by coagulase and catalase tests. All coagulase- and catalase-positive isolates were identified to the species by Apistaph (Biomerieux, France) according to the manufactures instructions.

Antimicrobial susceptibility testing

Disk diffusion method according to the EUCAST (EUCAST, 2021; Humphties *et al.*, 2018) was used to tested susceptibility to ampicillin (AMP, 2µg.disk⁻¹), penicillin (P, 1 UI), cefoxitin (FOX, 30mg.disk⁻¹), ciprofloxacin (CIP, 5 µg.disk⁻¹), oxacillin (OX, 1 µg.disk⁻¹), clindamycin (CD, 2 µg.disk⁻¹), erythromycin (E, 15 µg.disk⁻¹) and chloramphenicol (C, 30 µg.disk⁻¹) of the 45 isolates coagulase-positive *S. aureus*. We reported the results in mm and interpretation was based on the protocol describes in CLSI (2020). The calculation of multiple antibiotic resistances (MAR) for each *S. aureus* isolate was done with follow equation (Singh *et al.*, 2010):

$$MAR\ index = \frac{Number\ of\ resistance\ antibiotics}{Total\ number\ of\ tested\ antibiotics}$$

MAR index higher than 0.2 means that the tested bacterium originates from high-risk contamination source where antibiotic are frequently applied.

Isolates showing resistance to at least two antimicrobial classes by disk diffusion method (n=27) were verified using the microdilution assay according to the CLSI method to detect minimal inhibitory concentration (MIC) (CLSI, 2020). We used Sensititre Staphylococci plate – EUST (Thermo Trek Diagnostics, OH, USA) and prepare the test according to ISO 20776-1:2019. The antimicrobial agents used in the MIC test and the interpretation ranges are listed in Table 1.

Molecular analysis

The conformation of MRSA was carried out using the multiplex polymerase chain reaction (PCR). We used them to identify *S. aureus* by amplification of *spa* gene and to confirm methicillin resistance by amplification of the *mecA/mecC* genes (Stegger *et al.*, 2012) (Table 2).

Table 1. List of antimicrobial agents, their subclasses, scope of testing and interpretation of sensitivity results

Antimicrobial subclasses	Antimicrobial agents	Scope of testing (mg/L)	Interpretation		Source
			S ≤	R >	
Macrolides	Clindamycin (CLI)	0.12-4	0.25	0.5	EUCAST, 2021
	Erythromycin (E)	0.25-8	1	2	EUCAST, 2021
Tetracyclines	Tetracycline (TET)	0.5-16	1	2	EUCAST, 2021
Anzamyacin	Rifampin (RIF)	0.016-0.5	0.06	0.5	EUCAST, 2021
Aminoglycosides	Streptomycin (STR)	4-32	16	16	EUCAST, 2021
	Kanamycin (KAN)	4-64	8	8	EUCAST, 2021
Penicillins	Penicillin (P)	0.12-2	0.125	0.125	EUCAST, 2021
Phenicol	Chloramphenicol (CHL)	4-64	8	8	EUCAST, 2021
Pleuromutilins	Tiamuline (TIA)	0.5-4	2	2	EUCAST, 2021
Streptogramins	Quinupristin/Dalfopristin (SYN)	0.5-4	1	2	EUCAST, 2021
Glykopeptide	Vancomycin (VAN)	1-16	2	2	EUCAST, 2021
Aminoglycoside	Gentamycin (GEN)	1-16	1	1	EUCAST, 2021
Fluoroquinolones	Ciprofloxacin CIP	0.25-8	0.001	1	EUCAST, 2021
Cephalosporins	Cefoxitin (FOX)	0.5-16	4	4	EUCAST, 2021
Oxazolidinones	Linezolid (LZD)	1-8	4	4	EUCAST, 2021
Pseudoammonium acid	Mupirocin (MUP)	0.5-256	1	1	EUCAST, 2021
	Fusidat (FUS)	0.25 – 4	1	1	EUCAST, 2021
Miscellaneous agents	Trimethoprim (TMP)	2-31	4	4	EUCAST, 2021
	Sulfamethoxazole (SMX)	64-512	128	128	EUCAST, 2021

Table 2. Nucleotide sequence of primers and amplicon size

Target gene	Name of the primers	Nucleotide sequence (5' - 3')	Amplicon size (bp)
<i>mecA</i>	mecA P4	TCCAGATTACAACCTTCACCAGG	162
	mecA P7	CCACTTCATATCTTGTAACG	
<i>Spa</i>	spa-1113F	TAAAGACGATCCTTCGGTGAGC	180-600
	spa-1514R	CAGCAGTAGTGCCGTTTGCTT	
<i>PVL</i>	pvl-F	GCTGGACAAAACCTTCTTGGAATAT	85
	pvl-R	GATAGGACACCAATAAATTCTGGATTG	
<i>mecC</i>	mecA _{LGA251} MultiFP	GAAAAAAAGGCTTAGAACGCCTC	138

PCR amplification was carried out in a 25 µl reaction mixture containing 2 µl of isolated DNA and 23 µl of mastermix (6.5 µl PCR H₂O, 12.5 µl 2×Green PCR MasterMix, 2 µl Primer Mix 1 (containing 10 pmol each of spa-1113F, mecAP4, pvl-F and mecALGA251 MultiFP), 2 µl Primer Mix 2 (containing 10 pmol each of spa – 1514R, mecAP7, pvl-R, mecALGA251 MultiRP). The PCR was conducted in an C 100 Touch Thermal Cycler (BioRad, USA) by initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 s, extension at 58°C for 1 min and at 72°C for 1 min and separation at 72°C for 10 min. *S. aureus mecA* positive strain was used as a positive control. The negative control consisted of PCR-grade

water without any template. DNA fragments were visualized after horizontal electrophoresis in a 2% agarose gel (SeaKem Agarose) in 1×TAE buffer at 130 V for 30 min.

Gel coloration was performed in ethidium bromide solution for 20 min. The results were visualized with UV light (>2500 µW/cm³). GeneRuler 100bp DNA Ladder (Fermentas) was used as a 100 bp marker to determine molecular weight. All isolates positive for *mecA/mecC* gene were identified as MRSA.

Statistical data

The statistical significance was evaluated with Chi-square (χ^2) tests using Epitools (Sergeant, 2018).

Results

Prevalence of coagulase-positive *S. aureus* in raw beef

S. aureus was detected in 29.3% (46/157) of the tested raw beef samples. Coagulase-negative staphylococci were detected in 46.5% of the samples (73/157), and the absence of staphylococci was found in 24.2% (38/157). The spread of *S. aureus* and coagulase-negative staphylococci in raw beef samples, depending on their origin, is presented in Fig. 1.

Analysis of the results showed the highest prevalence of *S. aureus* in raw beef meat in samples with unknown origin (75.0%, 3/4), followed by those originating from the Netherlands (37.5%, 6/16), Ireland (33.3%, 3/9) and from Bulgaria (27.4%, 34/124). *S. aureus* not detected in any of the tested samples from Belgium, Romania and the USA, but were not statistically significant. MRSA was detected in 3.8% (6/157). All of them originated from Bulgaria. MRSA is included in the number of coagulase-positive *S. aureus*.

Antimicrobial resistance of coagulase-positive *S. aureus*

In the disk diffusion method, 32.6% (15/46) of the *S. aureus* isolates demonstrate susceptibility to all antimicrobials. Analysis of the resistance profile of the 46 isolates showed that resistance to penicillin was the most common (56.5%), followed by resistance to ampicillin (41.3%), oxacillin and clindamycin (10.9%). The MAR index ranged from

0.125 to 0.5 with a mean of 0.263 and a standard deviation of 0.109 (Table 3).

Twenty-seven isolates coagulase-positive *S. aureus* tested for MIC, showed different percentages of resistance to the different antibiotics (Fig. 2). The highest resistance was observed to sulfamethoxazole (88.9%), followed by tetracycline (59.3%), penicillin (51.9%) and ceftiofur (33.3%). 34.8% (9/27) of the isolates showed resistance to ceftiofur >4mg/L, which were identified as MRSA. Total susceptibility was recorded for mupirocin, linezolid, vancomycin and rifampin. Multidrug resistance was found in 77.8% (21/27) isolates tested.

All MRSA isolates were 100% resistant to chloramphenicol (MIC ≥ 512 mg/L), but sensitive to clindamycin, rifampin, kanamycin, erythromycin, ciprofloxacin, linezolid and mupirocin. All isolates are multi-drug resistant to at least three different classes of antibiotics. Among the tested MRSA isolates, one was found to be resistant to 8 out of the 19 tested antibiotics, displaying MIC against ceftiofur ≥ 16 mg/L.

Detection of *mecA/mecC* genes of *S. aureus* isolates

Nine isolates that showed ceftiofur resistance were tested for the presence of the *mecA/mecC* genes. MRSA genes were identified in 6 out of a total of 9 isolates (Fig. 3).

Some isolates were found to be resistant by the MIC method without this being confirmed by the presence of the responsible gene. This is most

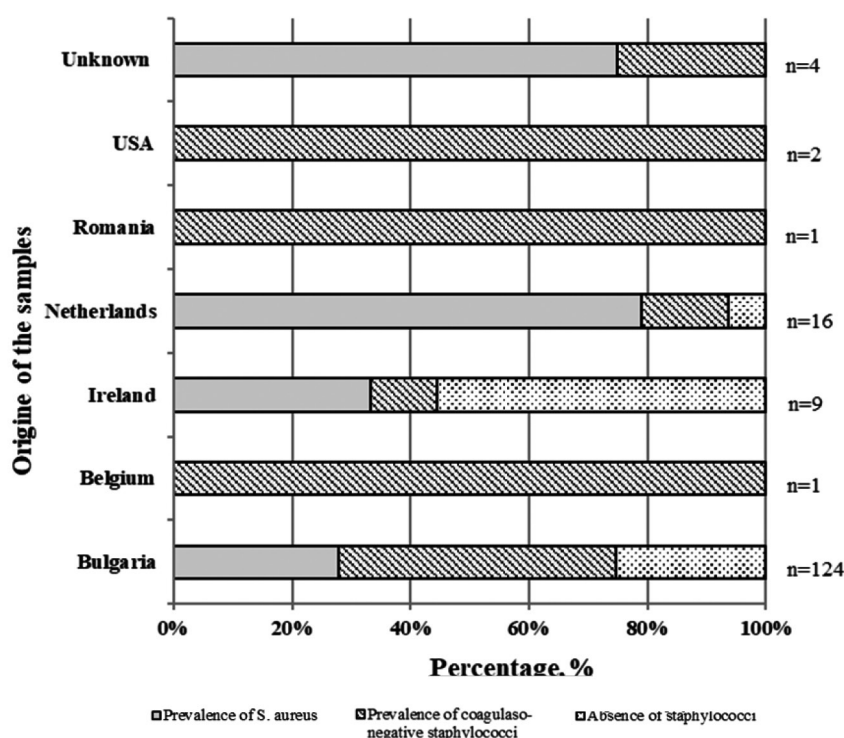


Fig. 1. Prevalence of *S. aureus* and coagulase-negative staphylococci in raw beef samples, depending on their origin

Table 3 Antimicrobial resistance profiles of the coagulase-positive *S. aureus* isolates, tested by disc-diffusion method

Isolates	Resistance profile	MAR index
<i>S. aureus</i> 145-1	AMP,P,FOX,OX,	0.5
<i>S. aureus</i> 1188	P, FOX, OX,CD	0.5
<i>S. aureus</i> 195-2	AMP, P, CD	0.375
<i>S. aureus</i> 284-2	AMP, P,OX	0.375
<i>S. aureus</i> 547- 2	AMP, P, E	0.375
<i>S. aureus</i> 1010- 2	AMP, P, CD	0.375
<i>S. aureus</i> 767	P, CD,E	0.375
<i>S. aureus</i> 792- 2	P, CD,E	0.375
<i>S. aureus</i> 32	AMP, P,	0.25
<i>S. aureus</i> 89-1	AMP, P	0.25
<i>S. aureus</i> 290-1	AMP, P	0.25
<i>S. aureus</i> 230-1	AMP, P	0.25
<i>S. aureus</i> 198	AMP, P	0.25
<i>S. aureus</i> 337	AMP, P	0.25
<i>S. aureus</i> 423-2	AMP, P,	0.25
<i>S. aureus</i> 432-1	AMP, P,	0.25
<i>S. aureus</i> 449	AMP, P,	0.25
<i>S. aureus</i> 624- 2	AMP, P,	0.25
<i>S. aureus</i> 956- 2	AMP, P	0.25
<i>S. aureus</i> 966- 1	P, OX	0.25
<i>S. aureus</i> 784	P, OX,	0.25
<i>S. aureus</i> 17	E	0.125
<i>S. aureus</i> 258-2	AMP	0.125
<i>S. aureus</i> 292	P	0.125
<i>S. aureus</i> 344	P	0.125
<i>S. aureus</i> 417-1	P	0.125
<i>S. aureus</i> 609- 2	AMP	0.125
<i>S. aureus</i> 624- 1	AMP	0.125

Legend: AMP: ampicillin; P: penicillin; FOX: ceftioxitin; OX: oxacillin; CD: clindamycin; E: erythromycin;

likely because the primer pairs used were only for major resistance genes. It is important to note that in approximately 25% of MRSA strains, the entire genome may consist of the presence of mobile genetic elements that determine methicillin resistance [1]. The *spa* gene was detected in all *S. aureus* isolates (n=9), confirming their biochemical results.

Discussion

For the first time in Bulgaria, a study that provides complex information on the spread of coagulase-positive *S. aureus*, their antimicrobial resistance, detected of MRSA and detected of the resistance genes in the isolates from raw beef from different origins has been conducted. The occurrence of *S. aureus* in beef meat retailed in Bulgaria was 30.6%. Analysis of available scientific data showed a higher prevalence of *S. aureus* in beef samples. Similar to our results in Romania was reported in cow milk samples (Morar *et al.*, 2021). Kim *et al.* (2020) also detected *S. aureus* in 13.1% of beef samples in Korea. In contrast to our data, they reported 12.9% positive for *S. aureus* in imported beef samples and only 13.4% positive in domestic beef meat samples.

Other previous studies have reported significantly higher levels compared to our findings. In Turkey, Sanlibaba (2022) reported a significantly higher prevalence of *S. aureus* in beef meat (63.5%). Similar data was reported in Georgia – presence of *S. aureus* in 63.0% of beef samples (Jackson *et al.*, 2013). More than 75% prevalence of *S. aureus* in various beef products was reported in Pakistan (Sadiq *et al.*, 2020), China (Wu *et al.*, 2019) and Turkey (Guran *et al.*, 2021). This significant difference may be due to the different beef products tested in these studies compared to those in our study. Furthermore, the sample size in these

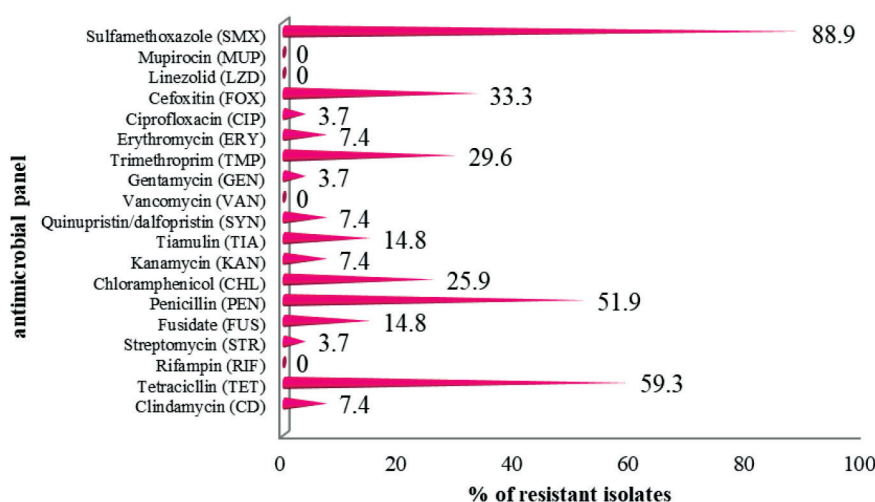


Fig. 2. Overall percentage resistance of coagulase-positive *S. aureus*, confirmed by the MIC method

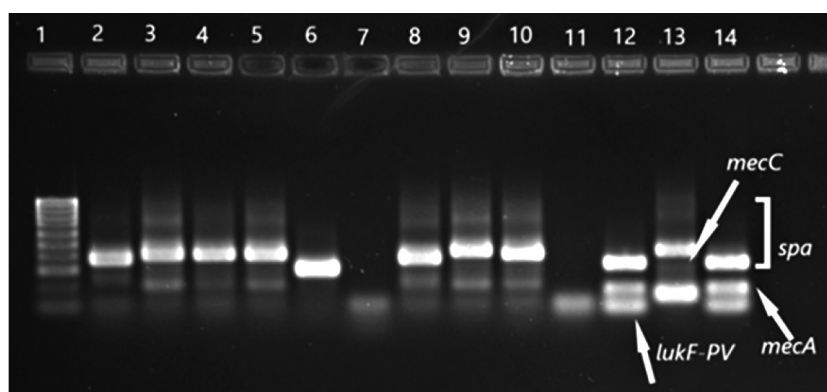


Fig. 3. Detection of the *mecA/mecC* genes by polymerase chain reaction in beef isolates showing resistance to cefoxitin by the MIC method

Legend: 1 - DNA loader, 100 bp; 11-negative control; 12 and 14 - *PVL*-positive control possessing *spa* (180-600 bp), *lukF-PV* (85 bp) and *mecA* (162 bp) genes; 13 - MRSA-positive control possessing *spa* (180-600 bp) and *mecC* (138 bp) genes; 2 ÷ 6; 8-10 - MRSA-positive isolates from chilled beef; 7 - MRSA-negative isolates.

studies was relatively smaller, which may also explain the difference in percentages. The different ranges of beef samples, which suggest secondary contamination during cutting, meat processing and packaging, would also account for the higher percentage of positive samples.

The MRSA is a serious public health problem. Mortality rates due to MRSA infections have remained high in recent years. In some Asian countries such as Taiwan, China, Japan and South Korea the spread of MRSA in hospitals reaches up to 70-80% (Chuang and Huang, 2015). The situation among the clinical isolates in Bulgaria is also alarming – 33.6% (Bozhkova, 1999). In the present work, the overall prevalence of MRSA was 5.7% (n=9) with predominantly in samples from Bulgaria (8 out of 9 isolates). The most studies on the detection of MRSA in retail beef found that the prevalence ranged from <1 to 14.3% (Kim *et al.*, 2020; Jackson *et al.*, 2013; Wu *et al.*, 2019; Guran *et al.*, 2021; Dorjochoo *et al.*, 2023). The Czech Republic reported a prevalence rate of 40% in mixed ground meat that also contained beef (Tegegne *et al.*, 2021), while Pakistan reported a higher prevalence rate of 63.0% (Sadiq *et al.*, 2020).

In contrast, the study conducted in Korea did not detect MRSA in any of the beef samples (Kim *et al.*, 2020). Similarly, Tegegne *et al.* (2021) found MRSA in minced meat, but not in chilled beef in the Czech Republic. Antimicrobial resistance of *S. aureus* can be transmitted through contaminated meat to consumers (Hanson *et al.*, 2011) or suggesting contamination of meat from human sources (Dhup *et al.*, 2015). In our study we found highest resistance to penicillin and ampicillin by disk diffusion method and to sulphamethoxazole and tetracycline by MIC method. This was not unexpected as these

antibiotics have a broad spectrum of action and are very commonly used to treat variety infections in animals and humans. Similar highly expressed resistance to penicillin by disk diffusion method has been published by other authors (Sanlibaba, 2022, Dorjochoo *et al.*, 2023). In Turkey and China, 100% penicillin resistance has been reported (Wu *et al.*, 2019; Guran *et al.*, 2021).

The MAR ranged from 0.125 to 0.5 and suggests that *S. aureus* identified in this study may have been originated from an environment where antibiotics were commonly used. In our study using MIC method, we identified low levels of resistance (3.7% - 7.4%) to streptomycin, gentamicin, ciprofloxacin, erythromycin and clindamycin. These are highly effective antimicrobials for treatments of infections caused by both Gram-positive and Gram-negative bacteria. Clindamycin is often preferred as a first-line antibiotic to treat animals (Kayili and Sanlibaba, 2020). In a study carried out in Korea (Kim *et al.*, 2020), resistance to ciprofloxacin ranged from 1% to 2.7% in beef meat, which was relatively closer to our results. In contrast, Jackson *et al.* (2013) reported that all *S. aureus* isolated from retail beef were resistant to ciprofloxacin.

Resistance to sulfamethoxazole in our study by the MIC method was 88.9%. In contrast, other authors have reported 8.2% resistance (Kim *et al.*, 2021). Özdemir (2022) tested 17 isolates from commercially sold ground beef and found a total resistance to sulfamethoxazole 100% for MRSA isolates and 41.2% for methicillin-susceptible *S. aureus*. In comparison to other studies, the differences in the results could also be due from differences in the animal population, the inappropriate use of antibiotics, and the inclusion of antibiotics in feed as a growth-promoting agent.

We also found high sensitivity to chloramphenicol (74.1%). Similar results were reported by other authors, who identified chloramphenicol as the antimicrobial with the lowest resistance among *S. aureus* (Kim *et al.*, 2020). Some authors report 100% susceptibility to chloramphenicol (Jackson *et al.*, 2013; Morar *et al.*, 2021). The most likely reason for this is that it has not been used for treatment in recent years. The vancomycin is the most often recommended for treating infections caused by MRSA (Soares *et al.*, 2012), but none of the tested isolates in our study showed resistance to them.

Multidrug resistance detected in isolates from raw beef meat is described as resistance to 3 or more classes of antibiotics. In present study, multidrug resistance was found in 77.8% of the tested *S. aureus* and 100% in the MRSA by MIC method. These results indicate that multidrug resistant *S. aureus* in beef can be a transmission risk to humans and cause infections that may be difficult to treat. Wu *et al.* (2019) reported similar worrying data. Multidrug resistance levels in retail beef vary across different geographical regions, with the occurrence of multidrug resistance *S. aureus* observed in 100% in China (Wu *et al.*, 2019), 31.2% in Romania (Morar *et al.*, 2021), 96.87% of retail beef in Turkey (Salinbaba, 2022) and 40.0% in Mongolia (Dorjgochoo *et al.*, 2023).

The *mecA* gene is specific to staphylococci and encodes resistance to β -lactam antibiotics. It is used as a biomarker to detect MRSA (Jain *et al.*, 2008). We detected *mecA/mecC* in 6 out of 9 isolates showing MIC to cefoxitin >4 mg. Similar results were obtained by Sadiq *et al.* (2020). They were detected the *mecA* gene in 63% of the tested isolates. In contrast, in Georgia and Romania was reported 6.3 and 6.1 % prevalence of MRSA in beef samples, respectively (Jackson *et al.*, 2013, Morar *et al.*, 2021). MRSA was also reported in 42.5% in Bulgaria in 2011 (Gogov, 2011). These differences may be explained by the various sources of the samples as well as the use of different molecular techniques in the countries to detect the *mecA* gene product. Our study shows discrepancy between phenotypic resistance and molecular detection of the *mecA/mecC* genes. The resistance to cefoxitin >4 mg/L was found in 9 out of 27 isolates using MIC method, whereas the *mecA/mecC* gene was confirmed only in 6. Some studies have detected the *mecA/mecC* gene in staphylococcal isolates that were susceptible to cefoxitin (Osman *et al.*, 2016). However, the consistent prevalence of MRSA in beef suggests that potential variations

are not comparable between studies. A possible explanation for this phenomenon is that the resistance pattern of phenotypic expression may differ depending on the temperature or osmolality of the medium used. This would probably make it difficult to study MRSA sensitivity using conventional laboratory procedures.

This study adds to the existing literature with data on the phenotypic and molecular characteristics of *S. aureus* in beef. However, this analysis has potential drawbacks that cannot be ignored. Initially, the identification of methicillin-resistant *S. aureus* strains was based on the detection of the *mecA* gene and testing the MIC, which revealed some inconsistencies. Furthermore, the absence of monitoring programs for methicillin-resistant *S. aureus* in beef does not allow for a large-scale monitoring with larger number of meat and meat products consumed in Bulgaria.

Conclusion

For the first time, a study on the detection of *mecA/mecC* genes in coagulase-positive *S. aureus* isolated from raw beef meat at the retail was obtained. It can be concluded that the existing antibiotic resistance presented in isolates from raw beef meat poses risk to consumers. In particular, this relates to the increasing use of minimally thermally-treated beef products or raw ones. The occurrence of cefoxitin-resistant *S. aureus* isolates which are *mecA*-negative suggests other mechanisms of transmission of methicillin resistance. It should be ensured that antimicrobials are used correctly in animals and humans in order to control the increasing risk of antimicrobial resistance. Monitoring antimicrobial resistance profiles of MRSA isolates is necessary to understand epidemiological changes. This should guide the implementation of strong policies and strategies for the use of antibiotics in the prevention and treatment of both humans and animals. Limiting their use and appropriately controlling their use in agriculture and food production is a first step in the fight against antimicrobial resistance.

Acknowledgment

We want to thank Petranka Marinova, Lyudmila Kovacheva and Svetlana Krumova for technical support in the sample testing.

Funding

This study was funded by the National Diagnostic and Research Veterinary Medical Institute, Sofia, Bulgaria [scientific code 4.45, 2019-2022 of the Research plan].

References

- Bozhkova, K. (1999). Methicilin resistant staphylococci – distribution in clinical specimen and in vitro susceptibility to some antibacterial drugs. *Scr. Sci. Med.* **31**: 115-120.
- CLSI (2020). Performance standards for Antimicrobial Susceptibility Testing. 30th edition. M100. Wayne, PA: Clinical and Laboratory Standard Institute.
- Chuang, Y., Y. Huang (2015). Livestock-associated methicillin-resistant *Staphylococcus aureus* in Asia: an emerging issue? *Int. J. Antimicrob. Agents* **45**: 334–340. <https://doi.org/10.1016/j.ijantimicag.2014.12.007>.
- Dhup, V., A. Kearns, B. Pichon, H. Foster (2015). First report of identification of livestock-associated MRSA ST9 in retail meat in England. *Epidemiol. Infect.* **143**: 2989-92. <https://doi.org/10.1017/S0950268815000126>.
- Dorjgochoo, A., A. Batbayar, A. Tsend-Ayush, O. Erdenebayar, B. Byambador, J. Sarantuya, Y. Munkhdelger (2023). Detection of virulence genes of *Staphylococcus aureus* isolated from raw beef for retail sale in the markets of Ulaanbaatar city, Mongolia. *BMC Microbiol.* **23**: 372. <https://doi.org/10.1186/s12866-023-03122-2>.
- EUCAST. Breakpoint tables for interpretation of MICs and zone diameters. Version 11.0. <https://www.eucast.org/2021> [accessed 15 March 2022]
- European Food Safety Authority (2019). Scientific report of EFSA and ECDC (EFSA and ECDC). The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food, 2017. *EFSA J.* **17**: 5598. <https://doi.org/10.2903/j.efsa.2019.5598>.
- European Food Safety Authority (EFSA) & European Centre for Disease Prevention and Control (ECDC) (2024). The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2021–2022. *EFSA J.* **22**: e8583. <https://doi.org/10.2903/j.efsa.2024.8583>.
- Farahmand, S., M. Haeili, D. Darban-Sarokhalil (2020). Molecular typing and drug resistance patterns of *Staphylococcus aureus* isolated from raw beef and chicken meat samples. *Iranian J. Med. Microbiol.* **14**: 478-489. <https://doi.org/10.30699/ijmm.14.5.478>.
- Gogov, Y (2011). Investigation on methicillin-resistant strains *Staphylococcus aureus* (MRSA) isolated from animal origin foodstuffs. *Vet. Assembly* **3-4**: 30-33 [in Bulgarian].
- Guran, H., A. Bozan Bayrak, W. Alali, C. Yesiloglu (2021). Prevalence and antimicrobial susceptibility pattern of methicillin-resistant *Staphylococcus aureus* isolates from retail raw meats in Turkey. *Int. Food Res. J.* **29**: 089–1100. <https://doi.org/10.47836/ifrj.29.5.11>.
- Hanson, B., A. Dressler, A. Harper, R. Scheibel, S. Wardyn, L. Roberts, J. Kroeger, T. Smith (2011). Prevalence of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) on retail meat in Iowa. *J. Infect. Public Health* **4**: 169-174. <https://doi.org/10.1016/j.jiph.2011.06.001>.
- Humphries R., J. Ambler, S. Mitchell, M. Castanheira, T. Dingle, J. Hindler, L. Koeth, K. Sei (2018). CLSI methods development and standardization working group best practices for evaluation of antimicrobial susceptibility tests. *J. Clin. Microbiol.* **56**: e01934-17. <https://doi.org/10.1128/JCM.01934-17>.
- ISO 6888-3:2003. Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species). Part 3: Detection and MPN technique for low numbers.
- ISO 20776-1:2019. Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices. Part 1: Broth micro-dilution reference method for testing the in vitro activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases.
- Jackson, C., J. Davis, J. Barrett (2013). Prevalence and characterization of methicillin-resistant *Staphylococcus aureus* isolates from retail meat and humans in Georgia. *J. Clin. Microbiol.* **51**: 1199-207. <https://doi.org/10.1128/JCM.03166-12>.
- Jain, A., A. Agarwal, R. Verma (2008). Cefoxitin disc diffusion test for detection of methicillin-resistant staphylococci. *J. Med. Microbiol.* **57**: 957–961. <https://doi.org/10.1099/jmm.0.47152-0>.
- Kayili, E., P. Sanlibaba (2020). Prevalence, characterization and antibiotic resistance of *Staphylococcus aureus* isolated from traditional cheeses in Turkey. *Int. J. Food Prop.* **23**: 1441–1451. <https://doi.org/10.1080/10942912.2020.1814323>.
- Kim, Y., H. Kim, S. Kim, M. Kim, H. Kwak (2020). Prevalence and characteristics of antimicrobial-resistant *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* from retail meat in Korea. *Food Sci. Anim. Resour.* **40**: 758-771. <https://doi.org/10.5851/kosfa.2020.e50>.
- Morar, A., A. Ban-Cucerzan, V. Herman, E. Tîrziu, K. Sallam, S. Abd-Elghany, K. Imre (2021). Multidrug resistant coagulase-positive *Staphylococcus aureus* and their enterotoxins detection in traditional cheeses marketed in Banat Region, Romania. *Antibiotics* **10**: 1458. <https://doi.org/10.3390/antibiotics10121458>.
- Osman, K., A. Amer, J. Badr, N. Helmy, R. Elhelw, A. Orabi, M. Bakry, A. Saad (2016). Antimicrobial resistance, biofilm formation and *mecA* characterization of methicillin-susceptible *S. aureus* and Non-*S. aureus* of beef meat origin in Egypt. *Front. Microbiol.* **7**: 222. <https://doi.org/10.3389/fmicb.2016.00222>.
- Özdemir, F. (2022). Antimicrobial resistance, multilocus sequence, and spa typing of *Staphylococcus aureus* isolated from retail raw meat products. *Biomed Res. Int.* **2022**: 6035987. <https://doi.org/10.1155/2022/6035987>.
- Pesavento, G., B. Ducci, N. Comodo, A. Lo Nostro (2007). Antimicrobial resistance profile of *Staphylococcus aureus* isolated from raw meat: A research for methicillin resistant *Staphylococcus aureus* (MRSA). *Food Control* **18**: 196-200. <https://doi.org/10.1016/j.foodcont.2005.09.013>.
- Sadiq, A., M. Samad, Saddam, N. Basharat, S. Ali, Roohullah, Z. Saad, A. Khan, Y. Ahmad, A. Khan, J. Khan (2020). Methicillin-resistant *Staphylococcus aureus* (MRSA) in slaughter houses and meat shops in capital territory of Pakistan during 2018–2019. *Front. Microbiol.* **11**: 577707. <https://doi.org/10.3389/fmicb.2020.577707>.
- Sanlibaba, P. (2022). Prevalence, antibiotic resistance, and enterotoxin production of *Staphylococcus aureus* isolated from retail raw beef, sheep, and lamb meat in Turkey. *Int. J. Food Microbiol.* **361**: 109461. <https://doi.org/10.1016/j.ijfoodmicro.2022.109461>.

- org/10.1016/j.ijfoodmicro.2021.109461.
- Sergeant, E. (2018). Epitools epidemiological calculators. <http://epitools.ausvet.com.au> (accessed in December 2023).
- Singh, A., S, Yadav, S, Singh, P, Bharti (2010). Prevalence of *Salmonella* in chicken eggs collected from poultry farms and marketing channels and their antimicrobial resistance. *Food Res. Int.* **43**: 2027-2030. <https://doi.org/10.1016/j.foodres.2010.06.001>.
- Soares, G., L. Figueiredo, M. Faveri, S. Cortelli, P. Duarte, M. Feres (2012). Received mechanisms of action of systemic antibiotics used in periodontal treatment and mechanisms of bacterial resistance to these drugs. *J. Appl. Oral Sci.* **20**: 295–309. <https://doi.org/10.1590/s1678-77572012000300002>.
- Stegger, M., P. Andersen, A. Kearns, B. Pichon, M. Holmes, G. Edwards, F. Laurent, C. Teale, R. Sakov, A. Larsen (2012). Rapid detection differentiation and typing of methicillin-resistant *Staphylococcus aureus* harbouring either *mecA* or the new *mecA* homologue *mecA* (LGA251). *Clin. Microbiol. Infect.* **18**: 395-400. <https://doi.org/10.1111/j.1469-0691.2011.03715.x>.
- Tegegne, H., I. Kolackova, M. Florianová, T. Gelbicová, J. Madec, M. Haenni, R. Kapriskova (2021). Detection and molecular characterisation of methicillin-resistant *Staphylococcus aureus* isolated from raw meat in the retail market. *J. Glob. Antimicrob. Resist.* **26**: 233–238. <https://doi.org/10.1016/j.jgar.2021.06.012>.
- Turner, N., B. Sharma-Kuinkel, S. Maskarinec, E. Eichenberger, P. Shah, M. Carugati, T. Holland, V. Fowler Jr (2019). Methicillin-resistant *Staphylococcus aureus*: An overview of basic and clinical research. *Nat. Rev. Microbiology* **17**: 203–218. <https://doi.org/10.1038/s41579-018-0147-4>. (2019)
- Wu, S., J. Huang, F. Zhang, Q. Wu, J. Zhang, R. Pang, H. Zeng, X. Yang, M. Chen, J. Wang, J. Dai, L. Xue, T. Lei, X. Wei. Prevalence and characterization of food-related methicillin-resistant *Staphylococcus aureus* (MRSA) in China. *Fron. Microbiol.* **10**: 304. <https://doi.org/10.3389/fmicb.2019.00304>.