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Research article

OCCURRENCE, ENTEROTOXIGENIC PROFILE, AND ANTIMICROBIAL RESISTANCE OF *STAPHYLOCOCCUS AUREUS* ISOLATES FROM BOVINE MASTITIS MILK SAMPLES

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This study included dairy farms registered as milk producers in the entity of Republika Srpska, Bosnia and Herzegovina. A total of 180 milk samples originating from cows were collected from dairy farms across several locations during 2024 and 2025. Out of the 180 tested raw milk samples obtained from dairy cows suspected to suffer from clinical or subclinical mastitis the presence of *Staphylococcus aureus* was confirmed in 44 samples, representing 24.4% (SD: 3.2%; 95% CI: 18.2% – 30.7%) of all analyzed samples. The presence of one or more *se* genes was detected in nine isolates (20.5%; 95% CI: 11.0% – 33.7%). Among the total of 44 *S. aureus* isolates, only one isolate exhibited phenotypic resistance to cefoxitin using ETEST® Cefoxitin, which was molecularly confirmed by detection of the *mecA* gene. All analyzed *S. aureus* isolates showed resistance to at least one of the tested antimicrobial agents, while 25% (95% CI: 12.2% – 37.8%) of isolates showed resistance to two antimicrobial agents. A total of 15.9% (95% CI: 6.6% – 30.1%) of isolates displayed antimicrobial multidrug resistance (MDR), defined as resistance to three or more classes of antimicrobial drugs.

Keywords: *S. aureus*, *se* genes, MRSA antimicrobial resistance

INTRODUCTION

Staphylococci are ubiquitous in the environment and can be found in air, dust, sewage, water, as well as on humans and animals. They are opportunistic pathogens or commensals that inhabit the skin and mucous membranes of animals and humans and can spread within the host's environment, surviving for extended periods in these areas [1].

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Mastitis is a disease that causes major economic losses in the dairy industry worldwide due to reduced milk yield and quality, financial costs associated with milk rejection and treatment expenses, as well as the culling of animals from intensive production [2]. *Staphylococcus aureus* has a pronounced ability to cause staphylococcal mastitis, which is one of the most prevalent forms of contagious mastitis in dairy animals. The disease may take peracute, acute, subacute, or chronic forms. The level of milk contamination may be lower than 100 CFU/mL of *S. aureus* in milk, although experimental findings have shown values between 10^3 and 10^5 CFU/mL [3]. The mammary gland is the most important reservoir of the pathogen, which is mostly transmitted from-animal-to-animal via milking equipment, utensils, milking machines, and the hands of milkers [4].

According to data collected from seven counties in the northwestern part of the Republic of Croatia between 2014 and 2018, *S. aureus* was confirmed in 14.24% of 1,264 analyzed milk samples from various inflammatory conditions [5]. During 2024 a total of 179 milk samples from clinical and subclinical mastitis cases were analyzed from 48 farms across Bosnia and Herzegovina. A total of 88 (49.2%) mastitis milk samples were confirmed, among which *S. aureus* infection was detected in 10 samples (11.4%) [6].

In addition to the significant economic losses associated with mastitis, infections of the mammary gland represent an important public health issue. *Staphylococcus aureus* is excreted from the infected udder, and many isolates of this pathogen from milk possess *se* genes [7]. Grispoldi et al. [8] analyzed *S. aureus* isolates from twelve dairy farms in central Italy and confirmed 17 *S. aureus* isolates out of 60 tested samples. The observed prevalence of *se* genes in *S. aureus* isolates was 47.1%, 35.3%, 29.4%, and 5.9% for *see, sea, sed* and both *seb* and *sec,* respectively. Six out of the seven enterotoxin-producing isolates were capable of expressing the corresponding enterotoxins. In the study from Russia, fifty-three (88%) isolates demonstrated enterotoxigenic properties, with the following gene distribution: *sea* in 32 isolates (53.3%), *seb* in 2 (3.3%), *sec* in 30 (50%), *sed* in 3 (4%), *see* in 28 (46.6%), *seg* in 42 (70%), *sei* in 6 (10%), s*elp* in 2 (3.3%) [9].

Excessive and unnecessary use of antibiotics in mastitis treatment represents a significant public health concern. One of the priorities in veterinary medicine and public health is the restrictive use of antibiotics, along with the implementation of alternative therapeutic approaches, aimed at reducing antibiotic usage and the occurrence of antimicrobial resistance (AMR) in dairy products [10]. The World Health Organization (WHO) [11], along with numerous expert groups and researchers, agrees that the spread of AMR represents a major problem that requires a global and coordinated action plan for its resolution. Resistance of staphylococci to penicillin is mediated by the *blaZ* gene, which encodes β-lactamase [12]. Unlike penicillinase-mediated resistance, which is relatively narrow in spectrum, methicillin resistance confers resistance to all penicillins, cephalosporins, β-lactamase inhibitors, and carbapenems [13]. Based on antimicrobial susceptibility, *S. aureus* resistance to methicillin is defined as the minimum inhibitory concentration (MIC) of cefoxitin greater than or equal to 8 μg/mL [14]. In Croatia, ten methicillin-resistant *Staphylococcus*

aureus (MRSA) strains (4.2%) were confirmed in subclinical mastitis samples collected between 2014 and 2019 [15].

The aim of this study was to investigate the occurrence of *S. aureus* in milk samples from cows with clinical or subclinical mastitis originating from registered dairy farms in the entity of Republika Srpska, Bosnia and Herzegovina, as well as to examine the enterotoxigenic potential of *S. aureus* isolates using PCR detection of specific genes encoding staphylococcal enterotoxins (SE) synthesis.

Furthermore, antimicrobial susceptibility of the *S. aureus* isolates was determined using the disk diffusion method. Screening for methicillin resistance was performed using ETEST® Cefoxitin (MIC determination). If phenotypic resistance to cefoxitin was detected, PCR detection of the *mecA* and *mecC* genes was performed to confirm MRSA isolates.

MATERIALS AND METHODS

During 2024 and 2025, a total of 180 milk samples were collected from dairy cows suspected of having clinical or subclinical mastitis. All farms included in the study were registered milk producers located in the entity of Republika Srpska, Bosnia and Herzegovina. The farms were randomly selected, and the milk sampling was carried out with the agreement of the farm owners and competent veterinary stations monitoring the health status of dairy herds. All sampled dairy cows were previously diagnosed with subclinical or clinical mastitis by the responsible farm veterinarians based on clinical signs of udder inflammation, including changes in milk appearance and a positive result of the California Mastitis Test (CMT). Milk samples were collected immediately after mastitis diagnosis and prior to any antimicrobial treatment.

Milk samples from cows with mastitis were taken and analyzed in accordance with the National Mastitis Council guidelines [16]. From each culture-positive milk sample, one characteristic *S. aureus* isolate was selected for further microbiological analysis. Columbia Blood Agar supplemented with 5% sheep blood (HiMedia, India) was used as the primary culture medium for isolation of the pathogen. In accordance with Fetsch [17] selective differential Mannitol Salt Agar (HiMedia, India) and Baird-Parker Agar (Oxoid, UK) were additionally used to support the selective isolation and presumptive identification of *S. aureus*. Standard microbiological tests were used to confirm the suspected colonies, including Gram staining, coagulase production testing (BD BBL™ Rabbit Coagulase Plasma, Thermo Fisher Scientific, USA), and catalase activity detection. All confirmed isolates were stored in Tryptic Soy Broth (HiMedia, India) with 15% glycerol at -70°C.

For detection of methicillin resistance, the protocol of the DTU National Food Institute for MRSA isolation from food and farm environments was applied [18]. After pre-enrichment in Mueller-Hinton Broth (Oxoid, UK), isolates were inoculated onto chromogenic selective Brilliance MRSA 2 Agar (Oxoid, UK). After incubation,

suspected colonies were inoculated on Columbia Blood Agar with 5% sheep blood for further biochemical identification and confirmation.

Methicillin resistance was phenotypically screened using ETEST® Cefoxitin strips (bioMérieux, France) with a MIC range of 0.016 – 256 mg/L, while definitive confirmation of MRSA isolates was performed by PCR detection of the *mecA* and/or *mecC* genes.

A total of 44 *S. aureus* isolates were confirmed and further analyzed. Molecular confirmation of species identity and detection of enterotoxigenic potential (*se* genes), as well as methicillin resistance genes (*mecA* and *mecC*), were performed using accredited methods based on Real-Time PCR and conventional PCR techniques [18,19]. The ability of the isolates to synthesize SE was assessed using the VIDAS® SET2 immunoassay (bioMérieux, France), based on the ELFA method (Enzyme-Linked Fluorescent Assay), in accordance with ISO 19020:2017 [20].

The determination of susceptibility of the 44 *S. aureus* isolates to selected antimicrobial agents was performed using the Kirby-Bauer disk diffusion method [14], on Mueller-Hinton agar (HiMedia, India). For testing antimicrobial susceptibility of the 44 isolates, commercially available disks (BioX Diagnostics, Belgium) were used at the following concentrations [14]: penicillin G (10 IU), cefoxitin (30 μg), gentamicin (10 μg), tetracycline (30 μg), ciprofloxacin (5 μg), nitrofurantoin (300 μg), trimethoprim-sulfamethoxazole (1.25/23.75 μg), chloramphenicol (30 μg), rifampin (5 μg), and clindamycin (2 μg).

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS), version 20.0 (IBM Corp., USA) [21]. For all statistical tests, the confidence level was set at 5% ($\alpha = 0.05$), and the significance of the tested differences was expressed as the corresponding p-values; differences were considered statistically significant when $p < 0.05$. Fisher's exact test was used to assess the significance of associations between occurrence of *S. aureus* and different sampling locations, and Monte Carlo simulation was applied in cases where expected cell frequencies were below the recommended threshold.

RESULTS

Out of the total of 180 raw milk samples collected from dairy cows with clinical or subclinical mastitis, the presence of *S. aureus* was confirmed in 44 samples, representing 24.4% of all analyzed samples (SD: 3.2%; 95% CI: 18.2% – 30.7%). From each positive milk sample one *S. aureus* isolate was taken for further analysis. The results of cultural isolation and identification of *S. aureus* were further verified by the Real-Time PCR detection of the *ftsZ* gene, specific to this bacterial species. The geographical distribution of the *S. aureus* isolates is presented in Table 1.

Table 1. Geographical distribution of *S. aureus* isolates from mastitis milk samples.

Location	No. of milk samples	No. of *S. aureus* isolates (%; 95% CI)
Banja Luka	24	4 (17%; 6.7% – 35.9%)
Bijeljina	37	16 (43%; 27.1% – 60.5%)
Gradiška	30	7 (23%; 11.8% – 40.9%)
Derventa	15	2 (13%; 1.7% – 40.5%)
Prijedor	10	1 (10%; 1.8% – 40.4%)
Prnjavor	15	3 (18%; 4.3% – 48.1%)
Šamac	11	2 (27%; 10.0% – 57.3%)
Nevesinje	20	4 (20%; 8.1% – 41.6%)
Gacko	18	5 (28%; 12.5% – 51.1%)
Total	**180**	**44** (24.4%; 18.2% – 30.7%)

Based on the Monte Carlo simulation applied within Fisher's exact test, the analysis of *S. aureus* isolation frequencies from mastitis milk samples collected from different locations (Table 1) showed no statistically significant differences among the examined locations (Pearson χ^2 (8) = 10.73; p = 0.217; Monte Carlo p = 0.304). The largest deviation from the expected frequency was observed in Bijeljina, where the number of positive samples was higher than expected. These findings indicate that geographic location was not a significant determinant of *S. aureus* distribution within the studied population.

The presence of one or more *se* genes was detected in 9 of the 44 *S. aureus* isolates (Table 2), representing 20.5% (95% CI: 11.0% – 33.7%). When considered in the context of the total number of analyzed milk samples (N = 180), *se* genes were confirmed in 5.0% of samples (95% CI: 2.7% – 10.0%).

Table 2. Distribution of *se* genes (n=9) in *S. aureus* isolates (n=44) from mastitis milk

Number of isolates positive for different *se* combinations (%)											
No. of isolates	**All *se***	***sea***	***seb***	***sec***	***sed***	***see***	***seg***	***sei***	***sea/ seg***	***seb/ sec***	***sec/ see***
44	9 (20.5%)	-	-	3 (6.8%)	-	-	-	-	2 (4.5%)	3 (6.8%)	1 (2.3%)

Among the examined *se* genes, *sec* was the most frequently detected (7 isolates, 78%), followed by *seb* (3 isolates, 33%), *sea* (2 isolates, 22%), *seg* (2 isolates, 22%), and *see* (1 isolate, 11%). Three isolates (33.3%; 95% CI: 7.4% – 65.2%) carried exclusively the *sec* gene, while six isolates (66.7%; 95% CI: 34.7% – 90.1%) were found to harbor two *se* genes.

All nine *se*-positive isolates were further analyzed using the VIDAS® SET2 immunoassay to evaluate their ability to produce seven staphylococcal enterotoxins (SEA, SEB, SEC1, SEC2, SEC3, SED, and SEE). The geographical distribution of *se* gene presence and SEA–SEE production is shown in Table 3.

Table 3. Geographical distribution of se gene (n=9) presence in *S. aureus* isolates from mastitis milk and their ability to produce SEA–SEE

Detected *se* profile	No. of isolates	Location	Detected SE
seb/sec	1	Bijeljina	SEB/SEC
sec	2		SEC
sec/see	1		SEC/SEE
sea/seg	2		SEA
sec	1	Nevesinje	SEC
seb/sec	1	Banja Luka	SEB/SEC
seb/sec	1	Gacko	SEB/SEC

Results of the antimicrobial susceptibility testing of the 44 *S. aureus* isolates are presented in Table 4. All isolates exhibited resistance to at least one tested antimicrobial agent, while 25% (95% CI: 12.2%–37.8%) of isolates showed resistance to two antimicrobial agents. A total of 15.9% (95% CI: 6.6%–30.1%) of isolates displayed MDR.

Table 4. Antimicrobial susceptibility of *S. aureus* isolates from mastitis milk (n = 44).

Antimicrobial substance	No. (%) of isolates		
	S (%)	I (%)	R (%)
Penicillin G	0	0	44 (100%)
Cefoxitin	41 (93.18%)	0	3 (6.82%)
Gentamicin	36 (81,82%)	7 (15,91%)	1 (2.27%)
Tetracycline	28 (63.64%)	4 (9.09%)	12 (27.27%)
Ciprofloxacin	41 (93.18%)	2 (4%)	1 (2.27%)
Nitrofurantoin	37 (84.09%)	1 (2.27%)	6 (13.64%)
Trimethoprim – Sulfamethoxazole	42 (95.45%)	2 (4.55%)	0
Chloramphenicol	42 (95.45%)	2 (4.55%)	0
Rifampin	39 (88.64%)	0	5 (11.36%)
Clindamycin	38 (86.36%)	3 (6.82%)	3 (6.82%)

S = susceptible, **I** = intermediate (moderately susceptible), **R** = resistant

Complete resistance was observed to penicillin G (100%; 95% CI: 93.4–100%). In contrast, high susceptibility rates were observed for chloramphenicol (95.5%; 95% CI: 85.7–98.9%), trimethoprim-sulfamethoxazole (95.5%; 95% CI: 85.7–98.9%), and ciprofloxacin (93.2%; 95% CI: 82.4–97.8%).

Out of the total of 44 *S. aureus* isolates, only one isolate exhibited phenotypic resistance to cefoxitin, with a measured MIC of 8 mg/L as determined by ETEST® Cefoxitin strips. Molecular confirmation of methicillin resistance was performed using a duplex Real-Time PCR technique, which detected the *mecA* gene responsible for methicillin resistance. When considering all the 180 analyzed milk samples originating from cows with clinical or subclinical mastitis, MRSA prevalence was 0.6%. The MRSA isolate exhibited resistance to penicillin G, cefoxitin, gentamicin, tetracycline, rifampin, and clindamycin. Intermediate susceptibility was observed to nitrofurantoin and trimethoprim-sulfamethoxazole. The MRSA isolate showed high susceptibility to ciprofloxacin and chloramphenicol.

DISCUSSION

In this study, the overall prevalence of *S. aureus* in milk samples from cows with subclinical or clinical mastitis was 24.4%, while the *mecA* gene was detected in one of the 44 isolates (2.9%), corresponding to 0.6% of the total number of analyzed samples (n=180). These results indicate that *S. aureus* remains an important pathogen in bovine mastitis within the studied population, whereas methicillin-resistant isolates are still relatively rare.

Data from European countries show substantial variability in the prevalence of *S. aureus* in mastitis milk. In Slovakia and the Czech Republic, *S. aureus* accounted for 16.5% of intramammary infections, with 2.9% of isolates carrying the *mecA* gene [22].

A Serbian study [23] reported the prevalence of 5.4% in subclinical mastitis cases. In Germany, analyses of over one million milk samples revealed *S. aureus* in 12.4% of cases [24]. Similarly, in Norway [25], among 36,431 milk samples submitted for bacterial culture from 2019 to 2020, *S. aureus* was the most frequently detected udder pathogen (24.5%), followed by *Streptococcus dysgalactiae* (13.3%) and *Streptococcus uberis* (9.0%). Phenotypic and molecular analyses of clinical mastitis samples from three regions in Poland [26] showed that *Streptococcus uberis* was the most common pathogen (38%), followed by *S. aureus* (22%), A large Irish study [27], involving 7,833 milk samples collected from 2020 to 2023, reported *S. aureus* prevalence ranging from 21.4% to 25.6%, which closely correspond to our findings. Our results on the prevalence of *S. aureus* are also very similar to those obtained in Norway [25] and Poland [26]. Overall, the above-mentioned data suggest that the prevalence of *S. aureus* in mastitis milk varies considerably between countries and regions, likely due to differences in herd management, sampling strategies, detection methods, and regional epidemiology of mastitis.

Analysis of *se* genes in the *S. aureus* isolates revealed that 9 out of 44 isolates (20.5%; CI 11.0–33.7%) carried one or more *se* genes. When expressed as a proportion of all analyzed milk samples (N = 180), *se* genes were detected in 5.0% (CI 2.7–10.0%). Among these, *sec* was the most frequently detected gene (78%), followed by *seb* (33%), *sea* (22%), *seg* (22%), and *see* (11%).

Several studies have reported that the *sec* gene is the dominant enterotoxigenic marker in *S. aureus* isolates associated with mastitis in both bovines and goats, suggesting a potential role of SEC in mastitis pathogenesis [28]. However, it should be emphasized that the detection of *se* genes and *in vitro* SE production indicate enterotoxigenic potential, but do not necessarily reflect *in vivo* toxin expression or direct pathogenic relevance. Although epidemiological studies have shown a high prevalence of the *sec* gene among bovine mastitis isolates and suggest a possible involvement of SEC in disease development, it remains unclear whether SEC acts as a direct virulence factor in bovine mastitis [28]. A Serbian study [29] detected SE production in 5 out of 75 (6.67%) *S. aureus* isolates originating from cows with clinical and subclinical mastitis, all of which produced SEC, further corroborating our findings. In a Turkish study [30], 27 out of 106 *S. aureus* isolates (25.5%) were identified as enterotoxigenic, which is close to our results regarding the overall prevalence of enterotoxigenic isolates (20.5%). On the other hand, none of the Turkish isolates produced SEC, while 25 (23.6%) and 2 (1.9%) of the isolates tested positive for SEA and SEB production, respectively. These findings differ from our findings for production of SEC (15.9%), SEA (4.5%) and SEB (6.8%), probably due to differences in mastitis prevalence and treatments, as well as in the applied sampling and testing methodologies. Data from central Russia [9] further illustrate the high complexity of enterotoxin gene profiles in *S. aureus* isolates from subclinical mastitis. Multiplex PCR analysis revealed the following occurrence rates: *sea* (53.3%), *sec* (50%), *see* (46.6%), *seg* (70%), *sei* (10%), while *seb* and *sed* were relatively rare (3.3% and 4%, respectively). These *se* profiles show a broad genetic repertoire and different enterotoxigenic potential within a single pathogen population, which is similar to the diversity of *se* genes detected in our study but also varying in the type and combination of the detected *se* genes.

The prevalence of *mecA*-positive isolates in this study was low and consistent with European data, but continued monitoring is important to prevent the spread of methicillin-resistant strains. A meta-analysis on MRSA isolated from bovine mastitis cases [31] observed regional differences in prevalence of the pathogen. The highest prevalence was reported in Asia (6.47%; 95% CI: 4.33–8.97), whereas Europe showed the lowest prevalence (1.18%; 95% CI: 0.18–2.83). In England [32], the *mecA* gene was detected at a prevalence rate of 0.27%, whereas in Slovakia and the Czech Republic, *mecA* was confirmed in 2.9% of isolates [33]. Presence of the *mecA* gene in small numbers of isolates from mastitis milk suggests that methicillin resistance is still relatively rare in the studied European populations of dairy cows, but its presence represents a potential risk for the spread of MRSA in the herds and possible transmission to humans. This is consistent with the above-mentioned reports from

other countries, where the prevalence of MRSA isolates is low, but not negligible. The absence of the *mecC* in our MRSA isolate is consistent with reports of others, who indicate that *mecC*-positive strains are less prevalent and genetically distinct from *mecA*-positive isolates [34, 35].

Each of the *S. aureus* isolates analyzed in our study exhibited resistance to at least one of the tested antimicrobial agents, primarily penicillin G (100%). Additionally, 15.9% of isolates exhibited MDR, defined as resistance to three or more antimicrobial drugs from different classes. A global meta-analysis of antimicrobial resistance in the context of bovine intramammary infections worldwide between 1969 and 2020 [36] confirmed that penicillin resistance is the most prevalent among *S. aureus* isolates. However, the MDR prevalence observed in our study (15.9%) is higher than the 4.3% reported across ten European countries [36], suggesting potential regional differences in antimicrobial drug usage practices and AMR development. The same authors analyzed results of studying contagiousness and AMR in a total of 211 *S. aureus* isolates from bovine mastitis across ten European countries, where nine strains (4.3%) displayed MDR, detected only in four countries: Belgium (n = 4; 1.8%), Austria (n = 1; 0.5%), Italy (n = 3; 1.4%), and Germany (n = 1; 0.5%). These findings are lower than that observed in our study (15.9%), highlighting regional differences in AMR patterns and emphasizing the need for continuous monitoring and prudent antimicrobial use in dairy herds in Bosnia and Herzegovina.

Overall, our results indicate a moderate prevalence of *S. aureus*, a low frequency of *mecA* positive isolates, and a modest proportion of *se* gene-positive isolates, which is consistent with trends reported across Europe. These findings align with the widely observed pattern of a low, yet persistent, occurrence of MRSA characterized by β-lactam resistance. However, the detection of MDR isolates, together with the confirmed presence of the *mecA* gene, highlights the need for systematic antimicrobial resistance monitoring, as well as continued education of veterinarians and farmers regarding prudent antibiotic use and prevention of the spread of resistant strains.

It should be noted that this study included only cows with mastitis from registered farms, which may limit the generalizability of the findings to other production systems or unregistered herds. Broader surveillance studies are therefore guaranteed to provide a more comprehensive epidemiological assessment. Continuous monitoring of AMR and virulence factors in mastitis pathogens remains essential for improving animal health, milk quality, and food safety, as well as for reducing potential public health risks associated with the emergence and spread of resistant *S. aureus* strains.

CONCLUSION

The estimated prevalence of *S. aureus* in milk from cows with clinical and subclinical mastitis (24.4%) is consistent with reports from European and global dairy production systems. These findings confirm that *S. aureus* remains a significant mastitis pathogen

within the studied population. The observed prevalence should be interpreted in the context of farm level risk factors, including milking hygiene, bedding type, and general herd management, all of which have been shown to greatly influence the occurrence of *S. aureus* as a mastitis pathogen.

The detected low frequency of MRSA isolates, together with the presence of enterotoxigenic strains and MDR, emphasize the importance of continuous surveillance of both AMR and virulence determinants. Although MRSA prevalence was low, its detection highlights the necessity for ongoing monitoring and prudent antimicrobial use in dairy herds.

Bacteriological monitoring of milk from dairy cow provides valuable insight into trends in pathogen occurrence and supporting evidence-based mastitis control strategies, identification of risk factors, and the development of evidence-based mastitis prevention strategies.

Given the ongoing structural changes in milk production and herd management in industrialized countries, updated knowledge of pathogen prevalence, including AMR profiles such as MRSA, is essential for both animal health and milk safety, as well as public health.

Authors' contributions

BV carried out the molecular genetic studies. OS, DK and ECK helped with sample collection and analyses. NK performed the statistical analysis. DA and TI designed the study, coordinated its realization, as well as drafting the manuscript. All authors read and approved the final manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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ZASTUPLJENOST, ENTEROTOKSINOGENI PROFIL I ANTIMIKROBNA REZISTENCIJA *STAPHYLOCOCCUS AUREUS* IZOLATA IZ UZORAKA MLEKA KRAVA SA MASTITISOM

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Istraživanjem su obuhvaćene farme mlečnih krava koji su registrovani proizvođači mleka na području entiteta Republika Srpska, Bosna i Hercegovina. Ukupno je prikupljeno 180 uzoraka mleka poreklom od mastitičnih grla. Uzorkovanje je vršeno na farmama mlečnih krava na više lokaliteta tokom 2024. i 2025. godine. Od ukupno 180 ispitanih uzoraka sirovog mleka prikupljenih od mlečnih krava sa sumnjom na klinički i subklinički mastitis prisustvo *Staphylococcus aureusa* potvrđeno je u 44 uzorka, što predstavlja 24,4% (SD: 3,2%; 95% CI: 18,2% – 30,7%) od ukupnog broja analiziranih uzoraka. Prisustvo jednog ili više *se* gena utvrđeno je kod devet analiziranih izolata, što čini 20,5% (95% CI: 11,0% – 33,7%). Od ukupno 44 izolata *S. aureusa* samo je jedan izolat pokazao fenotipsku rezistenciju na cefoksitin primenom Cefoksitin ETEST®-a i molekularnom konfirmacijom meticilinske rezistencije putem detekcije *mecA* gena. Svaki od analiziranih izolata *S. aureusa* pokazao je otpornost na delovanje barem jednog od testiranih antimikrobnih supstanci, dok je 25% (95% CI: 12.2% – 37.8%) izolata pokazalo rezistenciju na dva antimikrobna agensa. Ukupno 15,9% (95% CI: 6,6% – 30,1%) izolata ovog patogena je pokazalo antimikrobnu multirezistenciju (otpornost na 3 i više antimikrobnih lekova različitih klasa).