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Antibiofilm activity of eugenol against methicillin resistant *Staphylococcus aureus* (MRSA) isolated in retail milk, milk products and hand swabs

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ABSTRACT

A total of 315 samples comprising of raw milk, traditional sweets, frozen milk products and hand swabs were collected from retail shops and vendors from the Mathura district of Uttar Pradesh, India. The prevalence of *Staphylococcus aureus* and methicillin resistant *S. aureus* (MRSA) was found to be 78.0%, 21.3%, 15.0%, 32.7 % and 24.0%, 6.6%, 5.0% and 16.3% in retail raw milk, traditional sweets, frozen milk products and hand swabs, respectively. The overall prevalences of *S. aureus* and MRSA were 31.1% and 10.7% from all the studied sample sources. The confirmed MRSA isolates were analysed for biofilm formation capability by three different methods, namely: Tissue culture plate (TCP), Tube method (TM) and Congo red agar (CRA) assay, and strong biofilm forming MRSA was detected by these methods in 61.7%, 38.2% and 20.5% samples, respectively. The tissue culture plate method was found to be the most sensitive assay of the three. The strong biofilm forming MRSA strains (n=21) were analysed to find the minimum inhibitory concentration (MIC) values against eugenol by the microdilution method. The MIC values of eugenol ranged from 3.125 to 0.190 (v/v) for the MRSA strains. The antibiofilm effect of eugenol on the established biofilms of the MRSA strains was evaluated after challenging with MIC and 2×MIC concentrations of eugenol of 0.19 and 0.39 (v/v). After treatment with eugenol, at the 0.19 (v/v) concentration, the Optical Density (OD) values decreased and ranged from 0.612 to 0.912, while at the 0.39 (v/v) concentration, the OD values decreased and ranged from 0.301 to 0.663. Under Scanning electron microscopy (SEM) analysis, the MRSA biofilms treated with MIC and 2×MIC of eugenol showed loss of cell-to-cell connections, disruption of the organized structures of the biofilms, loss of normal morphology, the surface became rough and the bacterial cells collapsed, possibly due to exudation of the contents. The outcomes of MIC and SEM revealed that eugenol had a remarkable antibiofilm effect on the biofilm of the MRSA strains. This study concluded that eugenol oils exert remarkable antibiofilm effects on MRSA biofilms and can be applied to the surfaces of equipment, food containers, utensils and other materials to inhibit the growth of MRSA biofilms.

Key words: MRSA; eugenol; antibiofilm activity; raw milk

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Introduction

The *Staphylococcus aureus* bacterium is normally found as a commensal, present on the skin and in the upper respiratory tract of animals and humans (VERMA et al., 2023). Milk and milk products can harbour multiple varieties of food-borne pathogens, such as *S. aureus*, *Shigella* like toxin producing *Escherichia coli*, *Salmonella* and methicillin resistant *Staphylococcus aureus* (MRSA) (KAHRAMAN et al., 2024). In last two decades, the presence of MRSA has often been reported in milk and milk-based products worldwide (KHAIRULLAH et al., 2024). *S. aureus* is an important food-borne pathogen that has the ability to form a biofilm over the surfaces of equipment and dairy utensils, and secrete different types of enterotoxins that cause food intoxications in humans (PATEL and RAWAT, 2023). Biofilm is a community of microorganisms encased in a matrix of extracellular polysaccharide (EPS) (MISHRA et al., 2020). The attachment of biofilm- forming food borne pathogens such as *S. aureus* and MRSA to food contact surfaces in food processing plants and local food manufacturing units raises the risk of contamination by these organisms in milk and milk products (WORMANN et al., 2023).

Nowadays, antibiofilm agents are used to eradicate the biofilms produced by food-borne pathogens, and may be natural or synthetic (GÓMEZ-SEQUEDA et al., 2020). A list of natural compounds derived from plants with antibiofilm properties is available in the literature that are capable of disrupting pre-formed biofilms and are more efficient than their chemically synthesized counterparts, with fewer side effects (JAFRI and AHMAD, 2021). These are environmental-friendly and effective alternatives to disinfectants, and are necessary to reduce the microbial load and the emergence of antimicrobial-resistant bacterial strains in food-related environments (MILLEZI et al., 2019). To maintain food safety and preservation, plant essential oils (EOs) with antimicrobial properties are a promising alternative (CALO et al., 2015). One of the essential oils, clove and its phyto-compound eugenol (4-allyl-2-methoxyphenol) have antimicrobial, anticarminative, and antispasmodic properties, and are also used as preservative and coating agent to increase the quality of foods (SHARIFMEHR et al., 2019). At present, biofilm formation caused by foodborne pathogens is a serious issue that leads to spoilage of food, as well health problems in consumers. Therefore, this study was designed to observe the antibiofilm effects of eugenol on MRSA isolated from milk, milk-based products and human hands.

Materials and methods

Sampling. A total of 315 samples comprising retail raw milk (n=50), traditional sweets (Burfi (n=40), Peda (n=40), Milk cake (n=40) and Dudhi halva (n=30)), frozen milk products (ice cream and kulfi; n=60) and hand swabs (n=55) were collected from retail shops and vendors in nearby regions of the Mathura district of Uttar Pradesh, India. The samples were brought in chilled condition and processed within 24 hrs, for isolation and identification of *S. aureus* according to the standard microbiological techniques (KOU et al., 2021) with slight modifications.

Isolation and identification of S. aureus. The samples of milk (1ml), milk products (25g) and hand swabs were enriched in 9 ml, 250 ml and 5 ml buffered peptone water (BPW) at 37°C for 24 hrs (HiMedia, India), respectively. The loopful culture growth from the BPW was streaked on Baird Parker agar and incubated at 37°C for 24 hrs. *Staphylococcus spp*. produced specific jet black coloured colonies over this agar A single jet black coloured colony per positive sample was picked and streaked on mannitol salt agar (MSA) and incubated at 37°C for 24 hrs. On MSA staphylococci produced golden yellow colonies surrounded by a yellow zone. A single colony was picked from the MSA, streaked on nutrient agar slant, incubated at 37°C for 24 hrs, and stored at 4°C until further processing.

Molecular detection of S. aureus. After biochemical confirmation, the *S. aureus* were further subjected to monoplex PCR for screening of the housekeeping *nuc* gene according to the procedure given by BRAKSTAD et al. (1992) and the details of the primer are given in Table 1.

Detection of methicillin resistant S. aureus (MRSA). For screening the MRSA, the Antibiotic

sensitivity test (ABST) and selective plating on MeReSa Agar were used according to the procedure given by BAUER et al. (1966) and BHATTACHARYA et al. (2016), respectively. A methicillin disc (5 µg) was used, and isolates with a zone of inhibition ≤21 mm were categorised as MRSA. Further ABST positive strains were streaked over a MeReSa Agar Base (HiMedia) and strains that produced pink-coloured colonies on MeReSa agar were identified as MRSA. Phenotypically confirmed MRSA was subjected to monoplex PCR for detection of the *mec A* gene according to the method of TIWARI and SEN (2006) and the primer details are given in Table 1.

Biofilm production by phenotypic assays. MRSA isolates were observed for biofilm forming capacity *in vitro* by three different assays, *viz.* Congo red agar (CRA) assay, the Tube method (TM) and Tissue culture plate (TCP) assay. In the CRA assay, black coloured colonies with a dry crystalline consistency on CR agar indicated biofilm producers, whereas colonies showing a red colour were considered non-biofilm producers (PANDA et al., 2016). In TM, a visible film lining the wall and the bottom of the tube were considered to demonstrate a positive reaction and a strong biofilm former (CHRISTENSEN et al., 1985). In the TCP assay, the OD values were considered as an index of bacteria adhering to the surface and forming biofilms. Strains were classified into three categories: weak biofilm producers, when ODc < OD ≤ (2×ODc), moderate biofilm producers, when (2×ODc) < OD ≤ (4×ODc), and strong biofilm producers, when (4×ODc) < OD (RODRIGUEZ-LAZARO et al., 2018).

Screening of the biofilm forming gene (bap) in MRSA isolates. Biofilm formers MRSA isolates were screened for biofilm forming *bap* (biofilm associated protein) gene. Monoplex PCR was conducted according to CUCARELLA et al. (2004) and the primer details are depicted in Table 1.

Determination of minimum inhibitory concentration (MIC) of eugenol. The broth microdilution method was used to determine the MIC of eugenol according to the guidelines given by CLSI (2019).) Isolates of MRSA were inoculated in Muller Hinton broth (MHB) overnight at 37°C then further, fresh MHB was used to make a 10 fold dilution which was incubated at 37°C until the exponential phase was attained. Eugenol was diluted serially two-fold in 0.1% (v/v) Dimethyl sulfoxide (DMSO) to prepare concentrations of eugenol ranging from 0.01 to0.04%. In the microdilution method, 96 well plates were used and they were all loaded with 200 µl mixture containing 100 µl MHB with the culture and 100 µl of diluted oil in different concentrations. The solubility of the eugenol was increased by DMSO. A bacterial suspension with 5×10^5 colony forming units (CFU/ml) was added to each well, which was confirmed by the total viable count. Positive control wells were created without eugenol and with only ATCC *S.aureus* 25923 culture, while negative control wells were without bacteria and with only media. The plates were incubated at 37°C for 24 hrs. OD was measured as 600nm and MIC was calculat-

Table 1. Primer details for housekeeping (*nuc*), antibiotic resistance (*mec*A) and biofilm forming (*bap*) genes

S. No.	Targeted gene (F/R)	Primer sequences	Amplicon size (bp)	References
1.	*nuc* F	5'GCGATTGATGGTGGATACGGTT3'	267	BRAKSTAD et al. (1992)
	nuc R	3'AGCCAAGCCTTGACGAACTAAAGC5'		
2.	*mec*A F	5'GTAGAAATGACTGAACGTCCGATGA3'	310	TIWARI and SEN (2006)
	*mec*A R	5'CCAATTCCACATTGTTTCGGTCTAA3'		
3.	*bap* F	5'CCCTATATCGAAGGTGTAGAATTGCAC3'	971	CUCARELLA et al. (2004)
	bap R	5'GCTGTTGAAGTTAATACTGTACCTGC3'		

ed as the lowest concentration of eugenol without visible growth.

Antibiofilm effect of eugenol on established MRSA biofilms. Crystal Violet microtiter plate assay was performed using the procedure given by CHRISTENSEN et al. (1985) with slight modifications. MRSA isolates were inoculated in Tryptic soya broth (TSB) medium at 37°C for 12 hrs and diluted to 1:200 with fresh sterile TSB medium containing 0.5% glucose. They were dispensed into 96 well plates to grow biofilms over 24 hrs. Further, the biofilms washed with PBS were challenged with the MIC of eugenol for 6 hrs at 37°C. Thereafter, the medium was discarded and the wells were washed with sterile PBS at least 3 times, and stained with 50 µl crystal violet (0.1%) for 15 minutes at 37°C. The CV stains attached to the biofilms in the wells were dissolved with 200 µl of ethanol and the OD value was observed at 570 nm using a spectrophotometer (Eppendorf). The experiment was repeated in triplicate to obtain the mean value under each treatment.

Statistical analysis. The chi-square test was used to compare the efficacy of different biofilm forming methods, and the OD values for different concentrations of eugenol were calculated as the means of individual experiments performed in triplicate and compared with those of the control group (0%) by one way analysis of variance (ANOVA) using SPSS (ver. 16).

Scanning electron microscopy (SEM). SEM was performed to observe the effect of eugenol on the integrity, structure and other morphological changes of the MRSA biofilms. First, a single colony was inoculated into 5 ml of TSB and incubated at 37°C for 12 hrs. The bacterial culture was matched with 0.5 McFarland and further diluted to 100 times, with a range of bacterial cells of from 10^5 to 10^6 cells per ml. A cover slip of 1 cm diameter was placed at the bottom of 24 well plates and each well was filled with 1.8 ml of TSB supplemented with sucrose (1% w/v) and 200 µl of bacterial inoculum, and incubated for 24-48 hrs at 37°C. Thereafter, the wells were rinsed 3 times with 2 ml of PBS to eliminate non-adherent bacteria. Then the established biofilms were challenged with fresh media containing eugenol (0.39 v/v and 0.19 v/v) and further incubated for 6 hrs. The plate was gently washed three times with sterile PBS to remove the planktonic cells. Pre-fixing of samples was performed by immersion in 2% glutaraldehyde in 0.1 M phosphate buffer. The samples were treated with gradient ethanol (30%, 50%, 70%, 90% and 100%) (CUI et al., 2020). The antibiofilm effect of eugenol was observed under a Scanning electron microscope (JEOL-JSM 6510 LV), at the University Sophisticated Instruments Facility (USIF), AMU, Aligarh, Uttar Pradesh, India.

Results

Isolation of S. aureus. A total of 98 presumptive *S. aureus* were isolated from 315 samples (a single colony was taken from each positive sample) and phenotypically confirmed by production of jet black coloured colonies on Baird Parker agar and golden yellow colonies on MSA, and by a series of biochemical tests. The prevalence of *S. aureus* was found to be 78.0% (39/50), 21.3% (32/150), 15.0% (9/60) and 32.7% (18/55) in retail raw milk, traditional sweets, frozen milk products and hand swabs, respectively with an overall prevalence of 31.10% in all studied sources (Table 2). All the phenotypically confirmed isolates were *nuc* gene bearers, thus the house keeping gene was present in all the *S. aureus* isolates with a prevalence of 100.0% (Table 2).

Phenotypic and genotypic detection of MRSA. Further, phenotypically and genotypically confirmed *S.aureus* isolates (n=98) were subjected to antimicrobial sensitivity testing against methicillin (5 µg) using ABST, and the results showed that 34.6% (34/98) isolates were resistant to methicillin, showing a zone of inhibition less than or equal to 21 mm diameter, while the intermediate and sensitive scores for methicillin were 36.7% (36/98) and 28.57% (28/98), respectively. Methicillin resistant ABST positive isolates also produced pink coloured colonies over MeReSa agar and were further phenotypically confirmed as MRSA. Thus, in some sources, the prevalence of MRSA was found to be 24.0% (12/50), 6.6% (10/150), 5.0% (3/60) and 16.3% (18/55) in retail raw milk, traditional sweets, frozen milk prod-

ucts and hand swabs, with an overall prevalence of 10.7% (34/315). The percent positivity of the *mecA* gene was 41.6%, 30.0%, 33.3% and 33.3% in retail raw milk, traditional sweets, frozen milk products and hand swabs, with an overall positivity of 35.29% (Table 2).

Biofilm production by phenotypic assays. In the CRA assay, 20.5% (7/34) MRSA produced black coloured colonies on CRA and were found positive, while 79.4% (27/34) of the isolates produced red coloured colonies and were negative for CRA. In TM, 38.2% (13/34) isolates produced slime on the bottom and walls of the tubes, and were categorized as strong biofilm formers. The remaining isolates produced slime either on the bottom or the wall, and were considered moderate 2.94% (1/34) or non-biofilm formers 29.4% (10/34). In the TCP assay, 61.7% (21/34), 23.5% (8/34) and 14.7% (5/34) were strong, moderate and weak biofilm producers. In the three phenotypic assays, 61.7% MRSA isolates were revealed to be biofilm formers by the TCP method, 38.2% by TM and 20.5%, by CRA assay. A chi-square test showed significantly ($P<0.001$) more positive results with TCP than TM and CRA assay, thus TCP was the most sensitive of all three assays (Table 3).

Screening of the bap gene in MRSA isolates. In this study, none of the MRSA isolates found in raw

Table 2. Prevalence of *S. aureus*, MRSA, *nuc* and *mec*A genes in retail raw milk, milk products and hand swabs

Sample source	**Total samples**	**No. of *Staphylococcus* spp isolates**	**No. of *S. aureus isolates***	**Prevalence of *S. aureus* (%)**	**No. of MRSA isolates**	**Prevalence of MRSA (%)**	**Prevalence of *nuc gene*%**	**Prevalence of *mec* A *gene* %**
Retail raw milk	50	47	39	78.0	12	24.0	100	41.6
Traditional sweets	150	124	32	21.3	10	6.6	100	30.0
Frozen milk products	60	12	9	15.0	3	5.0	100	33.3
Hand swabs	55	34	18	32.7	9	16.3	100	33.3
Total (n)	315	217	98	31.1	34	10.7	100	35.29

Table 3. Comparison of different MRSA biofilm formation methods

***S. aureus* isolates**	**MRSA isolates**	**Biofilm formation**	**Congo Red agar assay (CRA) (%) (T1)**	**Tube method (TM) (%) (T2)**	**Tissue Culture plate assay (TCP) (%) (T3)**
n=98	n=34	Strong	20.5 (7/34)	38.2 (13/34)	61.7 (21/34)
		Moderate	-	2.94 (1/34)	23.5 (8/34)
		Weak/ none	79.41 (27/34)	29.4 (10/34)	14.7 (5/34)
		Total Biofilm former (Strong + Moderate)	20.5 (7/34)	41.17 (14/34)	85.2 (29/34)

n= Number of samples; $\chi^2_{T1T2}=3.376$ (P=0.066); $\chi^2_{T1T3}=12.174^{**}$ (P=0.000); $\chi^2_{T2T3}=14.233^{**}$ (P=0.000)

milk, traditional sweets, frozen milk products and hand swabs had biofilms with the *bap* gene.

Determination of the minimum inhibitory concentration (MIC) of eugenol. Twenty-one strong biofilm former MRSA strains were tested to find the MIC values of eugenol, and the range of MIC values was 3.125 - 0.190 (v/v).

Antibiofilm effect of eugenol on established biofilms of MRSA strains. The antibiofilm attribute of eugenol was evaluated after challenging with MIC and 2×MIC of 0.19 and 0.39 eugenol (v/v) on established MRSA biofilms. The mean OD values of the control and two different concentrations of eugenol 0.19 and 0.39 (v/v) were observed at 570 nm. The OD values of the control for all the strains ranged from 0.813 to 1.419, while after treatment with eugenol, at a concentration of 0.19 (v/v), the OD values decreased and the values ranged between 0.612 and 0.912 and at 0.39 (v/v) concentration, the OD values decreased further and ranged between 0.301 and 0.663 (Table 4). On statistical analysis of the increase in concentration of eugenol oil from 0.19 to 0.39, a significant ($P<0.01$) decrease in the OD values of the MRSA strains was found (Table 5).

Scanning electron microscopy (SEM). The MIC and 2×MIC of eugenol were challenged on

Table 4. The effect of different eugenol concentrations against MRSA biofilms (OD_{570})

S. No.	MRSA strain	Source	Eugenol concentration 0.39%		Eugenol concentration 0.19%		(Control) Eugenol concentration 0%	
			Mean	±SD	Mean	±SD	Mean	±SD
1.	MM3	Milk	0.407	±0.00	0.761	±0.11	0.936	±0.17
2.	MM7	Milk	0.301	±0.05	0.812	±0.17	0.912	±0.19
3.	MM11	Milk	0.302	±0.17	0.912	±0.10	1.419	±0.16
4.	MB1	Burfi	0.521	±0.16	0.814	±0.15	0.997	±0.24
5.	MP1	Peda	0.451	±0.16	0.821	±0.19	0.985	±0.20
6.	MP3	Peda	0.515	±0.23	0.912	±0.20	1.345	±0.16
7.	MP4	Peda	0.567	±0.22	0.713	±0.18	0.843	±0.09
8.	MMC1	Milk cake	0.321	±0.05	0.812	±0.14	0.919	±0.22
9.	MMC3	Milk cake	0.402	±0.17	0.612	±0.15	0.834	±0.11
10.	MK1	Dudhi halwa	0.592	±0.20	0.615	±0.18	0.813	±0.13
11.	MK2	Dudhi halwa	0.562	±0.11	0.613	±0.21	0.815	±0.16
12.	MIC1	Ice cream	0.591	±0.19	0.715	±0.19	0.916	±0.21
13.	MKU1	Kulfi	0.612	±0.23	0.761	±0.14	0.943	±0.15
14.	MKU2	Kulfi	0.663	±0.21	0.812	±0.21	0.987	±0.21
15.	MHSW1	Hand swab of shop worker	0.361	±0.26	0.813	±0.17	0.954	±0.22
16.	MHSW3	Hand swab of shop worker	0.351	±0.10	0.856	±0.21	0.976	±0.14
17.	MHSW4	Hand swab of shop worker	0.413	±0.22	0.893	±0.12	0.991	±0.12
18.	MHSV1	Hand swab of vendor	0.405	±0.20	0.714	±0.20	0.881	±0.17
19.	MHSV2	Hand swab of vendor	0.456	±0.14	0.613	±0.20	0.821	±0.19
20.	MHSV4	Hand swab of vendor	0.582	±0.11	0.631	±0.07	0.813	±0.20
21.	MHSV5	Hand swab of vendor	0.496	±0.16	0.699	±0.14	0.893	±0.14
22.	ATCC 25923		0.512	±0.21	0.713	±0.21	0.923	±0.20

Table 5. Comparison of the mean OD_{570} values of MRSA biofilms treated with different concentrations of eugenol

S. No.	Concentration (v/v)	OD value (Mean ± SE)
1.	Control (0%)	0.9507 ± 0.0320^{c}
2.	0.19	0.7553 ± 0.0210^{b}
3.	0.39	0.4719 ± 0.0108^{a}

the established MRSA biofilms and structural changes were observed by SEM at 5000X magnification. The SEM analyses of the control of MRSA revealed that the biofilm was very well organized in a 3D structure, with intact cell-to-cell connections (Fig 1A). The morphology of the untreated MRSA cells was smooth and regular, with an intact cell membrane. After challenging the established MRSA biofilms with a 0.19 (v/v) concentration of eugenol, it was observed that the bacterial cells were not well organized and started scattering, and there was a loss of the normal cell morphology (Fig 1B). Conversely, with a 0.39 (v/v) concentration of eugenol on the MRSA biofilm, completely disorganised biofilm structures, leakage of intracellular contents, and dead cells were observed (Fig. 1C).

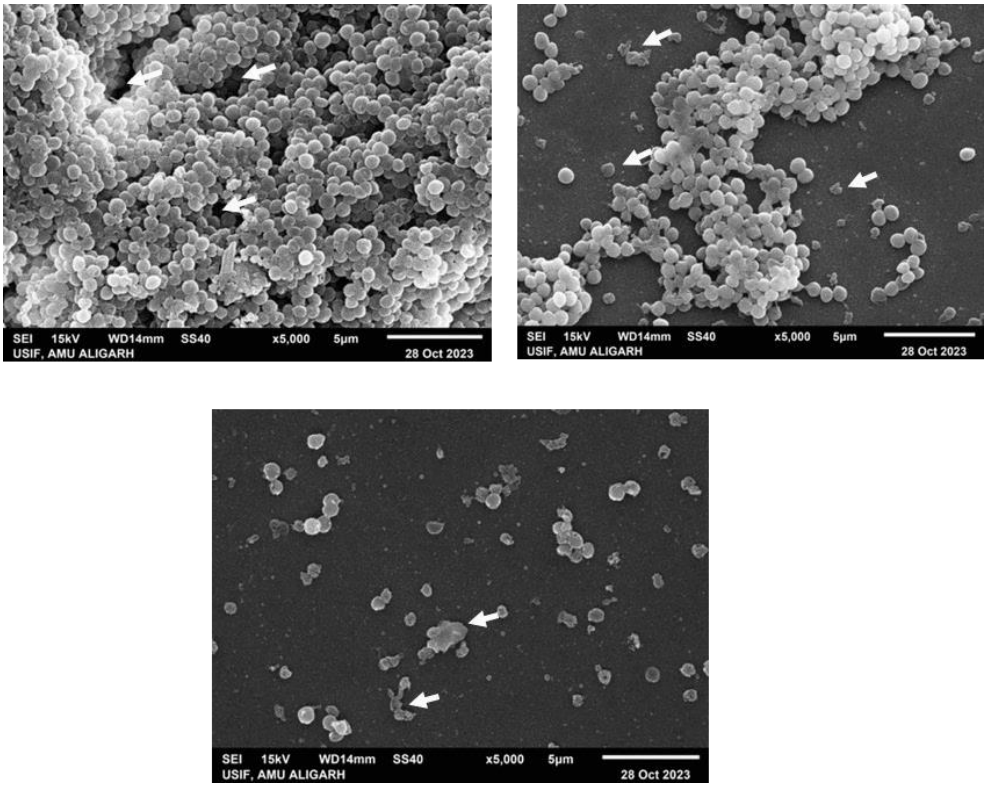

Fig 1. Scanning electron microscopy images (5000X) showing the antibiofilm effect of eugenol on MRSA biofilm

(A) Control sample of MRSA biofilm growing without eugenol, white arrow showing its well-organised 3D structure; (B) Biofilm grown with eugenol 0.19 (v/v) conc., white arrows showing loss of morphology and cell to cell connection; (C) Biofilm grown with eugenol 0.39 (v/v) conc., white arrows showing complete disruption of the organized structure of the biofilm and dead cells

Discussion

Milk and milk products can harbour a number of spoilage and pathogenic microorganisms, including multidrug resistant food-borne pathogens. Of these, MRSA causes a wide spectrum of infections in both animals and humans (MASTOOR et al., 2022; JHA et al., 2024). In the last two decades, the presence of MRSA in foods and food-producing animals, including milk and milk products and their biofilm forming capabilities has often been reported worldwide, raising public health concerns (PICOZZI et al., 2017). In order to monitor, prevent and control biofilm forming food borne MRSA contamination, it is necessary to understand its sources, phenotypic and genotypic characteristics, and transmission dynamics.

A similar prevalence value of *S. aureus* (70.0%) was revealed from raw milk in Riyadh by ALGHIZZI and SHAMI (2021) while TIBEBU et al. (2021) found 25.53% in cow's milk followed by 19.23% in hand swabs. LAKHANPAL et al. (2019) also reported prevalences of 26.6% and 10.0% of *S. aureus* in traditional sweets and frozen milk products from Himachal Pradesh, India. The discrepancies in the prevalence values of *S. aureus* may be due to differences in the procedure of sample collection, processing and laboratory practices.

In the present study, screening of the house keeping *nuc* gene done in *S. aureus* isolates and all were *nuc* gene bearers showing the 100% prevalence of this gene. In other studies researchers revealed 37.32% and 50.62% *nuc* genes from various milk sources in Turkey and Bangladesh, respectively (KEYVAN et al., 2020; SHAHID et al., 2021).

In other studies, the prevalence values of MRSA in raw retail milk were reported in the following countries: Nigeria (7.14%), China (51.6%), Ethiopia (33.3%) and India (23.8%), respectively, in the studies by ALIYU et al. (2020); KOU et al. (2021); GIRMAY et al. (2020) and SWETHA et al. (2017), respectively. In traditional sweets, in comparison with 6.6% in our study, a 3.0% prevalence of MRSA was reported from India in the study by THAKER et al. (2013) and a similar prevalence value of 8.4% was reported from Brazil by KRONING et al. (2016). In our study, hand swab samples showed 5.0% MRSA prevalence, while higher values, 19.0%, 36.36% and 10.0%, were reported in the studies by TIBEBU et al. (2021); SINGH et al. (2013); SAYED et al. (2010), respectively, which is significantly higher than in the present study. The differences in the values may be due to differences in the geographical conditions and the processing procedures of samples.

In this study, the methicillin resistant *mec* gene was screened in MRSA isolates and overall, 35.29% were *mecA* gene bearers. These results were consistent with the study by ALIYU et al. (2020) who reported a prevalence of 33.3% from milk products in Nigeria, while SHAHID et al. (2021) and OMARA et al. (2021) reported a prevalence of 12.2% and 72.6% in Bangladesh and Giza, respectively, where the outcomes were lower and higher than in the present study. MRSA represents those *S. aureus* strains that possess the *mec*A gene garbling penicillin-binding protein 2a, which mediates resistance to methicillin and all other b-lactam antibiotics, so it represents a global health problem (ALGHIZZI and SHAMI, 2021). Due to its importance and prevalence, nosocomial infection caused by MRSA has been listed as one of the three most difficult infectious diseases in the world by the World Health Organization (MOGHADDAM et al., 2021).

Overall our data revealed that MRSA is frequently detected in retail raw milk, traditional milk-based products (burfi, peda, milk cake and dudhi halva), frozen milk products (ice cream and kulfi) and hand swabs (milk shop workers and vendors) in the Mathura region. Failure to follow hygienic practices, lack of personal hygiene, and poor food processing methods lead to contamination of milk and milk products. These pathogenic organisms are also spread through unclean hands that serve as a source of contamination. Differences in prevalence are largely due to differences in the hygienic practices involved in the processing and storing of milk and milk products, and the surrounding environmental conditions. So, the collection, production, transportation and sale of retail raw milk and milk products in local shops and by vendors should be standardized.

Biofilm production is recognized as an important virulence factor of some food-borne pathogens

including MRSA, and is a major concern for the dairy industry. It frequently related to a lack of monitoring standards and unhygienic practices during the processing and handling of milk and milk products (JHA et al., 2024). The results of biofilm production were in contrast with YOUNIS et al. (2021), who reported 26.6.0% non-biofilm formers and 73.3% biofilm formers. KOU et al. (2021) revealed 66.1% strong 32.3.0% moderate and 1.6% weak biofilm formers which is similar to the current study. The findings of our study suggest that the TCP assay is a more reliable method for detection of biofilm forming MRSA compared to the TM and CRA methods.

The discrepancies in the categorization of biofilm phenotypes could result from differences in the interpretation of results. Therefore, it is important to align the method and interpretation of biofilm formation. At the same time, MRSA has varying abilities to form biofilm and it is important to explain the various sources fully (SOLTAN et al., 2018). It has been demonstrated previously that the phenotypic expression of biofilm production, ability is influenced by number of factor including composition of medium also (RAHIMI et al., 2016).

In current study, none of the biofilm-forming MRSA strains harboured the biofilm forming *bap* gene, with a prevalence value of 0.0%. In contrast to these results, other studies showed the *bap* gene occurred in biofilm forming *S. aureus* isolates in 5.0% and 10.0% of samples, (MARQUES et al. (2017) and BALLAH et al. (2022)), respectively. In agreement with the present study, in the study by NOTCOVICH et al. (2018) it was found that the phenotypically confirmed biofilm former *S. aureus* was negative for the *bap* gene Similarly, this study also revealed no association between the formation of biofilms on a phenotypic basis and the presence of *bap* genes in biofilm formation. In other studies, an explanation of the absence of the *bap* gene was found, and it was said that pathogenicity islands are able to be transmitted horizontally between strains and are not present in every strain (TORMO et al., 2005). Moreover, our results confirmed the hypothesis proposed by the researchers that the *bap* gene has not yet spread among MRSA isolates of animal and human origin (MOSSELHY et al., 2021).

The natural antibiofilm agent eugenol was shown to have an inhibiting effect on MRSA biofilms. The range of the MIC of eugenol for MRSA biofilms was between 3.125 and 0.19 (v/v). These results are in agreement with YADAV et al. (2015) who reported the MIC range varied between 0.01% and 0.04% in South Korea, and with EL-FAR et al. (2021) who stated that the MIC values of eugenol for MRSA ranged from 3.125% to 0.01%.

These results were consistent with the results of YADAV et al. (2015), the range of OD was found to be between 0.456 and 1.602 at 0.2 (v/v), and in a range between 0.241 and 0.591. In the current study, the results showed that eugenol has remarkable antibiofilm activity and there was a significant decrease in OD values at a 0.39 (v/v) eugenol concentration. This outcome is supported by the work of AZMI et al. (2019).

The results of SEM were in accordance with the studies of researchers GÓMEZ-SEQUEDA et al. (2020) and BAI et al. (2023), which revealed similar changes in the architecture of MRSA biofilms after treatment with eugenol.

Conclusions

MRSA has a tendency to form biofilms and becomes attached to the surface of food and containers. This leads to an increase in the microbial load of food products including dairy products, which may cause economic losses to the dairy industry. Persistent biofilm-related infections are not easy to treat due to the resident multidrug resistant microbes. The low efficiency of various treatments and *in vivo* toxicity of the available antibiotics, disinfectants and sanitizers are driving researchers towards the discovery of effective natural anti-biofilm agents. The findings of the CV- microtitre plate method and SEM in this study show that eugenol possesses considerable antibiofilm activity against MRSA biofilms. Thus, this study reveals that eugenol exerts antibiofilm effects on MRSA biofilms and can be applied to the surfaces of equipment, food containers, utensils and other materials, to prevent and eradicate of MRSA biofilms. The present study revealed that eugenol has antibiofilm effect on established MRSA biofilms and thus this oil can

be applied to the surfaces of equipment, food containers, utensils and other materials to inhibit the formation of biofilm by this food borne pathogen. The outcome of the study may be useful in the natural control of MRSA as well other biofilm-forming food borne pathogens.

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References

ALGHIZZI, M., A. SHAMI (2021): The prevalence of *Staphylococcus aureus* and methicillin resistant *Staphylococcus aureus* in milk and dairy products in Riyadh, Saudi Arabia. Saudi. J. Biol. Sci. 28, 7098-7104.

https://doi.org/10.1016/j.sjbs.2021.08.004

ALIYU, Y., I. O. ABDULLAHI, C. Z. WHONG, B. O. OLALEKAN, R. C. REUBEN (2020): Occurrence and antibiotic susceptibility of methicillin-resistant *Staphylococcus aureus* in fresh milk and milk products in Nasarawa State, North-Central Nigeria. J. Microbiol. Antimicrob. 12, 32-41.

https://doi.org/10.5897/JMA2020.0424

AZMI, K., W. QREI, Z. ABDEEN (2019): Screening of genes encoding adhesion factors and biofilm production in methicillin resistant strains of *Staphylococcus aureus* isolated from Palestinian patients. BMC Genomics 20, 578.

BAI, J., J. LI, Z. CHEN, X. BAI, Z. YANG, Z. WANG, Y. YANG (2023): Antibacterial activity and mechanism of clove essential oil against foodborne pathogens. Food. Sci. Technol. 173, 114249.

https://doi.org/10.1016/j.lwt.2022.114249

BALLAH, F. M., M. S. ISLAM, M. L. RANA, F. B. FERDOUS, R. AHMED, P. K. PRAMANIK, J. KARMOKER, S. IEVY, M. A. SOBUR, M. P. SIDDIQUE, M. M. KHATUN, M. RAHMAN, M. T. RAHMAN (2022): Phenotypic and genotypic detection of biofilm-forming *Staphylococcus aureus* from different food sources in Bangladesh. Biology (Basel) 11, 949.

https://doi.org/10.3390/biology11070949

BAUER, M., W. M. KIRBY, J. C. SHERRIS, M. TURCK (1966): Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. 45, 493-6.

BHATTACHARYA, D., J. BANERJEE, S. BANDYOPADHYAY, B. MONDAL, P. K. NANDA, I. SAMANTA, S. BANDYOPADHYAY (2016): First report on vancomycin-resistant *Staphylococcus aureus* in bovine and caprine milk. Microb. Drug. Resist. 22, 675-681. https://doi.org/ 10.1089/mdr.2015.0330

BRAKSTAD, O. G., K. AASBAKK, J. A. MAELAND (1992): Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the *nuc* gene. J. Clin. Microbiol. 30,1654-1660. https://doi.org/ 10.1128/jcm.30.7.1654-1660.1992

CALO, J. R., P. G. CRANDALL, C. A. O'BRYAN, S. C. RICKE (2015): Essential oils as antimicrobials in food systems – A review. Food. Control. 54, 111-119. https://doi.org/10.1016/j.foodcont.2014.12.040

CHRISTENSEN, G. D., W. A. SIMPSON, J. J. YOUNGER (1985): Adherence of coagulase negative *Staphylococci* to plastic tissue cultures: a quantitative model for the adherence of *staphylococci* to medical devices. J. Clin. Microbiol. 22, 996-1006. https://doi.org/10.1128/jcm.22.6.996-1006.1985

CLSI (2019): Clinical and Laboratory Standards Institute. M100S Performance Standards for Antimicrobial Susceptibility Testing-Approved Standard. 26th ed., Wayne, PA, USA.

CUCARELLA, C., M. A. TORMO, C. UBEDA, M. P. TROTONDA, M. MONZÓN, C. PERIS, B. AMORENA, I. LASA, J. R. PENADES (2004): Role of biofilm-associated protein bap in the pathogenesis of bovine *Staphylococcus aureus*. Infect. Immun. 72, 2177-2185. https://doi.org/10.1128/IAI.72.4.2177-2185.2004

CUI, H., C. ZHANG, C. LI, L. LIN (2020): Inhibition mechanism of cardamom essential oil on methicillin-resistant *Staphylococcus aureus* biofilm. Food Sci. Technol. 122, 109057

EL-FAR, A., S. SAMIR, E. EL-GEBALY, N. Y. TAHA, E. M. FAHMY, T. M. DIAB, A. EL-SHENAWY (2021): Assessment of eugenol inhibitory effect on biofilm formation and biofilm gene expression in methicillin resistant *Staphylococcus aureus* clinical isolates in Egypt. Infect. Genet. Evol. 89, 104722.

https://doi.org/ 10.1016/j.meegid.2021.104722

GIRMAY, W., G. GUGSA, H. TADDELE, Y. TSEGAYE, N. AWOL, M. AHMED, A. FELEKE (2020): Isolation and Identification of Methicillin-Resistant *Staphylococcus aureus* (MRSA) from Milk in Shire Dairy Farms, Tigray, Ethiopia. Vet. Med. Int. 15, 8833973. https://doi.org/10.1155/2020/8833973

GÓMEZ-SEQUEDA, N., M. CÁCERES, E. E. STASHENKO, W. HIDALGO, C. ORTIZ (2020): Antimicrobial and Antibiofilm Activities of Essential Oils against *Escherichia coli* O157:H7 and Methicillin-Resistant *Staphylococcus aureus* (MRSA). Antibiotics (Basel) 9, 730.

https://doi.org/10.3390/antibiotics9110730

JAFRI, H., I. AHMAD (2021): *In Vitro* Efficacy of Clove Oil and Eugenol against *Staphylococcus* spp. and *Streptococcus mutans* on Hydrophobicity, Hemolysin production and Biofilms and their syner-

gy with Antibiotics. Adv. Microbiol. 11, 117-143. https://doi.org/10.4236/aim.2021.112009

JHA, N. K., B. RAJKUMAR, A. OMELCHENKO, J. P. MONTIEL, A. M. SHEHATA, M. KHAN (2024): Methicillin-resistant *Staphylococcus aureus* Infections - A Review. J. Surg. Case Rep. 3, 019. https://doi.org/10.59657/2993-1126.brs.24.0019

KAHRAMAN, B. B., R. GECKINLI, S. AK (2024): Detection of enterotoxigenic *Staphylococcus aureus* in raw and pasteurized milk. Vet. arhiv. 94, 33-42. https://doi.org/10.24099/vet.arhiv.2038

KEYVAN, E., O. YURDAKUL, A. DEMIRTAS (2020): Identification of Methicillin-Resistant *Staphylococcus aureus* in Bulk Tank Milk. Food. Sci. Technol. 40, 150-156. https://doi.org/10.1590/fst.35818

KHAIRULLAH, A. R., S. C. KURNIAWAN, S. A. SUDJARWO, A. EFFENDI, A. WIDODO, I. B. MOSES, A. HASIB, R. L. A. Z. ZAHRA, M. A. GELOLODO, D. A. KURNIAWATI (2024): Kinship analysis of *mecA* gene of Methicillin Resistant *Staphylococcus aureus* isolated from milk and risk factors from the farmers in Blitar, Indonesia. Vet. World. 17, 216-225.

https://doi.org/10.14202/vetworld.2024.216-225

KOU, X., H. CAI, S. HUANG, Y. NI, B. LUO, H. QIAN, H. JI, X. WANG (2021): Prevalence and characteristics of *Staphylococcus aureus* isolated from retail raw milk in Northern Xinjiang, China. Front. Microbiol. 12, 705947.

https://doi.org/10.3389/fmicb.2021.705947

KRONING, I. S., M. A. IGLESIAS, C. P. SEHN, T. K. V. GANDRA, M. M. MATA, W. P. DA SILVA (2016): *Staphylococcus aureus* isolated from handmade sweets: Biofilm formation, enterotoxigenicity and antimicrobial resistance. Food. Microbiol. 58, 105-111. https://doi.org/10.1016/j.fm.2016.04.001

LAKHANPAL, P., A. K. PANDA, R. CHAHOTA, S. CHOUDHARY, S. D. THAKUR (2019): Incidence and antimicrobial susceptibility of *Staphylococcus aureus* isolated from ready-to-eat foods of animal origin from tourist destinations of North-western Himalayas, Himachal Pradesh, India. J. Food Sci. Technol. 56, 1078-1083. https://doi.org/10.1007/s13197-018-03556-x

MARQUES, V. F., C. C. D. MOTTA, B. D. S. SOARES, D. A. D. MELO, S. D. M. D. O. COELHO, I. D. S. COELHO, M. M. S. D. SOUZA (2017): Biofilm production and beta-lactamic resistance in Brazilian *Staphylococcus aureus* isolates from bovine mastitis. Braz. J. Microbiol. 48, 118-124.

https://doi.org/10.1016/j.bjm.2016.10.001

MASTOOR, S., F. NAZIM, S. RIZWAN-UL-HASAN, A. K. KHAN, S. N. ALI, S. H. ABIDI (2022): Analysis of the Antimicrobial and Anti-Biofilm Activity of Natural Compounds and Their Analogues against *Staphylococcus aureus* Isolates. Molecules 27, 6874. https://doi.org/10.3390/molecules27206874

MILLEZI, A. F., K. A. D. COSTA, J. M. OLIVEIRA, S. P. LOPES, M. O. PEREIRA, R. H. PICCOLI (2019): Antibacterial and anti-biofilm activity of cinnamon essential oil and eugenol. Ciênc. Rural. 49.

https://doi.org/10.1590/0103-8478cr20180314

MISHRA, R., A. K. PANDA, S. D. MANDAL, M. SHAKEEL, S. S. BISHT, J. KHAN (2020): Natural Anti-biofilm Agents: Strategies to Control Biofilm-Forming Pathogens. Front. Microbiol. 24, 195-204.

https://doi.org/10.3389/fmicb.2020.566325

MOGHADDAM-GHASEMZADEH, H., A. AZIMIAN, G. BAYANI, V. DASHTI, S. NOJOOMI, N. SHIRAZI, A. SOLATI, A. V. BELKUM (2021): High prevalence and expression of antiseptic resistance genes among infectious t037/ST239 methicillin-resistant *Staphylococcus aureus* (MRSA) strains in North Khorasan Province, Iran. Iran. J. Basic Med. Sci. 25, 775-780.

https://doi.org/10.22038/IJBMS.2022.63780.14055

MOSSELHY, D. A., M. ASSAD, T. SIRONEN, M. ELBAHRI (2021): Nanotheranostics: A Possible Solution for Drug-Resistant *Staphylococcus aureus* and their Biofilms? Nanomaterials (Basel) 11, 82.

https://doi.org/10.3390/nano11010082

NOTCOVICH, S., G. DENICOLO, S. H. FLINT, N. B. WILLIAMSON, K. GEDYE, A. GRINBERG, N. LOPEZ-VILLALOBOS (2018): Biofilm-Forming potential of *Staphylococcus aureus* isolated from clinical mastitis cases in New Zealand. Vet. Sci. 5, 8.

https://doi.org/10.3390/vetsci5010008

OMARA, S. T., A. S. HAKIM, M. A. BAKRY (2021): Phenotypic and Genotypic Characterization of Methicillin, Vancomycin, and Erythromycin-resistant *Staphylococcus aureus* Isolated from Milk and Dairy Products. World. Vet. J. 11, 642-657. https://doi.org/10.54203/scil.2021.wvj81

PANDA, P. S., U. CHAUDHARY, S. K. DUBE (2016): Comparison of four different methods for detection of biofilm formation by uropathogens. Indian. J. Pathol. Microbiol. 59, 177-179.

https://doi.org/10.4103/0377-4929.182013

PATEL, H., S. RAWAT (2023): A genetic regulatory see-saw of biofilm and virulence in MRSA pathogenesis. Front. Microbiol. 14, 1204428.

https://doi.org/10.3389/fmicb.2023.1204428

PICOZZI, C., D. ANTONIANI, I. VIGENTINI, R. FOSCHINO, W. KNEIFEL (2017): Genotypic characterization and biofilm formation of Shiga toxin-producing *Escherichia coli*. FEMS Microbiol. Lett. 364.

https://doi.org/10.1093/femsle/fnw291

RAHIMI, F., M. KATOULI, S. KARIMI (2016): Biofilm production among methicillin resistant *Staphylococcus au-*

reus strains isolated from catheterized patients with urinary tract infection. Microb. Pathog. 98, 69-76.

https://doi.org/10.1016/j.micpath.2016.06.031

RODRIGUEZ- LAZARO, D., C. ALONSO-CALLEJA, E. A. ONICIUC, R. CAPITA, D. GALLEGO, C. GONZÁLEZ-MACHADO, M. WAGNER, V. BARBU, J. M. EIROS-BOUZA, A. I. NICOLAU, M. HERNÁNDEZ (2018): Characterisation of biofilms formed by Methicillin Resistant *S. aureus*. Front. Microbiol. 9, 3004.

SAYED, A., A. HUSSEIN (2010): Surveillance of methicillin resistant *Staphylococcus aureus* (MRSA) in raw milk and milk handlers with *nuc* gene sequencing of the isolated strain. Assiut Vet. Med. J. 56, 1-12.

https://doi.org/10.21608/avmj.2010.166830

SHAHID, A. H., K. H. M. N. H. NAZIR, M. E. EL ZOWALATY, A. KABIR, S. A. SHAHJAHAN, M. P. SIDDIQUE, H. M. ASHOUR (2021): Molecular detection of vancomycin and methicillin resistance in *Staphylococcus aureus* isolated from food processing environments. One Health 13, 100276.

https://doi.org/10.1016/j.onehlt.2021.100276

SHARIFIMEHR, S., N. SOLTANIZADEH, S. A. H. GOLI (2019): Physicochemical properties of fried shrimp coated with bio-nano-coating containing eugenol and Aloe vera. Food. Sci. Technol. 109, 33-39.

https://doi.org/10.1016/j.lwt.2019.03.084

SINGH, A., M. GUPTA, A. AGARWAL, P. GUPTA, M. SINGH. (2013): Prevalence of Methicillin-Resistant *Staphylococcus aureus* colonisation and its antibiotic susceptibility profile among healthcare personnel in a tertiary care setup of northern India. Int. J. Curr. Microbiol. Appl. Sci. 2, 293-299.

SOLTAN, D. M. M., R. M. N. FARD, M. K. SHARIFI-YAZDI (2018): Prevalence of sea, seb, tsst and mecA genes in *Staphylococcus aureus* isolated from shrimps sold in seafood retailers in Tehran, Iran. J. Food Qual. Haz. Cont. 5, 72-76.

https://doi.org/10.29252/jfqhc.5.2.7

SWETHA, C. S., R. A. SUPRIYA, S. S. GOUD, A. J. BABU, T. M. RAO (2017): A study on the prevalence of zoonotic important methicillin resistant and vancomycin resistant *Staphylococcus aureus* (MRSA & VRSA) and coagulase negative *Staphylococci* (MR-CNS & VR-CNS) in raw milk samples of Tirupati, Andhra Pradesh. J. Pharm. Innov. 6, 17-24.

THAKER, H. C., M. N. BRAHMBHATT, J. B. NAYAK (2013): Isolation and identification of *Staphylococcus aureus* from milk and milk products and their drug resistance patterns in Anand, Gujarat. Vet. World. 5, 10.

https://doi.org/10.5455/vetworld.2013.10-13

TIBEBU, L., Y. BELETE, E. TIGABU, W. TSEGAYE (2021): Prevalence of *Staphylococcus aureus*, Methicillin-Resistant *Staphylococcus aureus* and Potential Risk Factors in Selected Dairy Farms at the Interface of Animal and Human in Bishoftu, Ethiopia. Vet. Med. 12, 241-251.

https://doi.org/10.2147/VMRR.S331968

TIWARI, H. K., M. R. SEN (2006): Emergence of vancomycin resistant *Staphylococcus aureus* (VRSA) from a tertiary care hospital from northern part of India. Infect. Dis. 6, 156. https://doi.org/10.1186/1471-2334-6-156

TORMO, M. Á., E. KNECHT, F. GÖTZ, I. LASA, J. R. PENADÉS (2005): Bap-dependent biofilm formation by pathogenic species of *Staphylococcus*: evidence of horizontal gene transfer? Microbiology (Reading) 151, 2465-2475.

https://doi.org/10.1099/mic.0.27865-0

VERMA, S. K., B. PARUL SHARMA, U. JAIN, A. SHARMA, M. GOSWAMI, P. SINGH, A. A. RATHEE, I. AGNIHOTRI (2023): Detection of biofilm forming *Staphylococcus aureus* from mastitic milk. Haryana Vet. 62, 42-45.

WORMANN, M. E., J. PECH, F. REICH, B. A. TENHAGEN, H. WICHMANN-SCHAUER, T. LIENEN (2023): Growth of Methicillin Resistant *Staphylococcus aureus* during raw milk soft cheese-production and the inhibitory effect of starter cultures. Food. Microbiol. 119,104451.

https://doi.org/10.1016/j.fm.2023.104451

YADAV, M. K., S. W. CHAE, G. J. IM, J. W. CHUNG, J. J. SONG (2015): Eugenol: a phyto-compound effective against Methicillin Resistant and Methicillin Sensitive *Staphylococcus aureus* clinical strain biofilms. PLoS One. 17**,** 10.

https://doi.org/10.1371/journal.pone.0119564

YOUNIS, W., H. SAMIR, S. SULTAN, M. W. ABD EL-AZEEM (2021): Detection of Biofilm and some Enterotoxins of *Staphylococcus aureus* Isolates in Ice Cream. J. Adv. Vet. Res. 11, 230-236.

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SAŽETAK

Ukupno je 315 uzoraka sirovog mlijeka, tradicionalnih slatkiša, zamrznutih mliječnih porizvoda i brisova ruku prodavača prikupljeno u maloprodajnim trgovinama u okrugu Mathura, u pokrajini Uttar Pradesh, u Indiji. Prevalencija zlatnog stafilokoka (*Staphylococcus aureus*) u uzorcima sirovog mlijeka bila je 78,0%, tradicionalnih slatkiša 21,3%, zamrznutih mliječnih porizvoda 15,0% i brisova ruku prodavača 32,7%, a prevalencija zlatnog stafilokoka rezistentnog na meticilin (engl. *methicillin resistant S. aureus*, MRSA) 24,0 % u uzorcima sirovog mlijeka, 6,6% u uzorcima tradicionalnih slatkiša, 5,0% u uzorcima zamrznutih mliječnih proizvoda i 16,3% u uzorcima brisova ruku prodavača. Ukupna je prevalencija zlatnog stafilokoka i MRSA bila 31,1% i 10,7% u svim analiziranim uzorcima. U izolatima u kojima je potvrđena MRSA analizirana je sposobnost stvaranja biofilma trima različitim metodama te je metodom kulture tkiva (engl. *tissue culture plate*, TCP) utvrđena sposobnost stvaranja biofilma u 61,7% uzoraka, metodom epruvete (engl. *tube method*, TM) u 38,2% uzoraka, a metodom bojenja kongo crvenilom (engl. *Congo red agar*, CRA) u 20,5% uzoraka. Ustanovljeno je da je metoda kulture tkiva najosjetljivija metoda među navedenima. Mikrodilucijskom metodom sojevi MRSA koji su formirali najjači biofilm (n=21) analizirani su kako bi se ustanovila minimalna inhibicijska koncentracija (MIK) protiv eugenola. Vrijednosti MIK-a eugenola bile su od 3,125 do 0,190 (v/v) za sojeve MRSA. Učinak antibiofilma eugenola na utvrđene biofilmove sojeva MRSA procijenjen je nakon izlaganja minimalnoj inhibicijskoj koncentraciji eugenola od 0,19 i dvostrukoj MIK koncentraciji od 0,39 (v/v). Nakon tretmana eugenolom u koncentraciji od 0,19 (v/v), vrijednosti optičke gustoće (OD) smanjile su se i bile u rasponu od 0,612 do 0,912, dok su se pri koncentraciji od 0,39 (v/v), OD vrijednosti smanjile i bile od 0,301 do 0,663. Pretražnom elektronskom mikrokoskopijom (SEM) uočeno je da su biofilmovi MRSA tretirani MIK-om i dvostrukim MIK-om eugenola pokazali gubitak povezanosti među stanicama, oštećenje organiziranih struktura biofilma i gubitak uredne morfologije, površina je postala hrapava, a bakterijske su stanice propale, vjerojatno zbog gubitka sadržaja. Rezultati su pokazali da eugenol ima znatan antibiofilmni učinak na biofilm sojeva MRSA. Zaključeno je da se uljni ekstrakti eugenola mogu nanijeti na površinu opreme, spremnika za hranu, pribor i druge materijale kako bi se spriječio razvoj biofilma sojeva MRSA.

Ključne riječi: MRSA; eugenol; antibiofilmna aktivnost; sirovo mlijeko