Sharan *et al. Annals of Microbiology* (2024) 74:22
https://doi.org/10.1186/s13213-024-01768-5

Annals of Microbiology

UNIVERSITÀ DEGLI STUDI DI MILANO

ORIGINAL ARTICLE

Open Access

Assessment of biofilm-forming capacity and multidrug resistance in *Staphylococcus aureus* isolates from animal-source foods: implications for lactic acid bacteria intervention

Manjeet Sharan[1], Pankaj Dhaka[1*], Jasbir Singh Bedi[1], Nitin Mehta[2] and Randhir Singh[1]

Abstract

Background *Staphylococcus aureus*, a Gram-positive bacterium, poses a significant threat to public health and food safety due to its virulence and its ability to develop antimicrobial resistance (AMR). Moreover, *S. aureus* can form biofilms in food environments, making it difficult to eradicate and pose a major challenge in foodborne illness prevention.

Methods The study aimed to investigate the biofilm-forming capabilities and AMR profiles of 107 *S. aureus* isolates derived from milk, chicken meat, and chicken eggs. Further, the study compared the biofilm formation tendencies between multi-drug resistant (MDR) and non-MDR *S. aureus* isolates. Additionally, the research explored the antibacterial and anti-biofilm properties of *Lactobacillus rhamnosus* and *Lactobacillus casei*, focusing on their aggregation and co-aggregation effects with *S. aureus*.

Results Around 70.10% of *S. aureus* isolates were found to be resistant to at least three antibiotic classes. The biofilm assay revealed that 16.82% isolates were strong biofilm formers. The MDR isolates displayed a strong biofilm-forming ability (i.e., 18.67%) and a higher prevalence of biofilm-associated genes [i.e., *ica*A (53.33%) and *ica*D (44.0%)] compared to non-MDR isolates. The LAB strain, *L. rhamnosus* exhibited a 29.06 mm mean antibacterial inhibition zone, an average reduction of 48.19% in biofilm growth, 55.46% auto-aggregation, and 40.61% co-aggregation with *S. aureus*. Similarly, *L. casei* demonstrated a 21.80 mm mean antibacterial inhibition zone, an average reduction of 31.56% in biofilm growth, 45.23% auto-aggregation, and 36.81% co-aggregation with *S. aureus* isolates.

Conclusion This study provides valuable insights into the biofilm formation of MDR *S. aureus* and underscores the potential of *L. rhamnosus* and *L. casei* as bio-control agents. These findings highlight the necessity for additional research into the mechanisms through which LAB strains inhibit pathogenic biofilms and their potential applications in enhancing food safety.

Keywords Antimicrobial resistance, Biofilm, Chicken meat, Eggs, Milk, Probiotics, *Staphylococcus aureus*

*Correspondence:
Pankaj Dhaka
pankaj.dhaka2@gmail.com
Full list of author information is available at the end of the article

Background

Staphylococcus aureus, a Gram-positive bacterium, can cause illness in both animals and humans. Its ability to survive under a range of environmental conditions and resistance to various sanitizing chemicals make it a formidable pathogen, especially in food processing environments (Tallent et al. 2019). The presence of organism in food or on food processing equipment can lead to food poisoning outbreaks, making it as a serious public health biohazard in the food industry (Kadariya et al. 2014).

S. aureus poses a substantial risk for foodborne illnesses, primarily due to its ability to produce enterotoxins. This bacterium can proliferate on the mucous membranes and skin of food handlers, presenting a major challenge for food processing facilities (Miao et al. 2017). The heat stability of staphylococcal enterotoxins allows them to persist and be released during bacterial growth in food matrices, potentially contaminating the food through contact with handlers or animals (Giaouris et al. 2015; Galié et al. 2018). Furthermore, *S. aureus* demonstrates a high capability in forming biofilms on diverse surfaces within animal environments, further complicating control measures along the food production chain (Ferry et al. 2005; Vergara et al. 2017). Biofilms are complex colonial structures where bacteria aggregate and attach to each other and to surfaces, with genes responsible for extracellular polymeric substances (EPS) or 'slime' production, followed by maturation (Flemming 2016). Biofilm development is particularly concerning, as it increases the bacteria's ability to persist long enough in adverse environment. The *ica*ADBC operon, which encodes *ica*A, *ica*D, *ica*B, and *ica*C core genes, as well as a regulatory gene (*ica*R), initiates the maturation of the biofilm matrix into multi-layered patterns. The co-expression of *ica*A and *ica*D genes facilitates the production of slime/EPS (Atshan et al. 2012). Additionally, diverse environmental stressors during biofilm formation can result in genetic variation and the formation of distinct biofilm communities, making them resistant and challenging to eradicate from the surfaces due to their diversity and intricacy (Sharan et al. 2022).

In addition to biofilms, the antimicrobial resistance (AMR) also poses a serious challenge to counter *S. aureus* as a foodborne pathogen (Sharan et al. 2023). The overuse and misuse of antibiotics have led to the emergence of AMR clones in food animal production systems, resulting in the development of drug-resistant strains of *S. aureus* that are challenging to human and animal health (Kadariya et al. 2014). Once the AMR strains establish biofilms, they render them resistant to antibacterial treatments and tolerant to harsh conditions, making eradication challenging (Sharan et al. 2022). Given the gravity of the problem, it is crucial to implement effective measures to control and prevent the spread of AMR strains of *S. aureus.*

Addressing biofilms remains a formidable challenge at the interface of food industry. Traditional control measures such as physical methods (hot steam, ultrasonication) and use of chemical compounds (sodium hypochlorite, sodium hydroxide solutions, hydrogen peroxide, peracetic acid, etc.) often fall short in effectively eliminating adhered bacteria from processing equipment, underscoring the pressing need for alternative strategies (Galié et al. 2018). Among the promising approaches, the utilization of probiotics stands out as a potential method for biofilm control. The *Lactobacillus* genus, comprising the most commonly used probiotic species, such as *L. rhamnosus, L. casei, L. acidophilus, L. plantarum, L. delbrueckii,* and *L. reuteri*, is the largest among LAB bacteria (Sengupta and Paramasivan 2019). *Lactobacilli* are Generally Recognized as Safe (GRAS) for consumption (Giordani et al. 2021). Furthermore, *Lactobacilli* release bacteriocins, biosurfactants, lactic acids, and exopolysaccharides. These compounds inhibit the growth of other microorganisms by reducing the pH of the surrounding environment and exerting antagonistic effects (Galié et al. 2018; Giordani et al. 2021). These characteristics make *Lactobacilli* a promising candidate for the prevention of biofilm formation, both as a probiotic supplement and as a biocontrol agent.

With this background, the current study was undertaken to examine the biofilm-forming capacity of *S. aureus* strains isolated from different animal-source foods. The objectives were to ascertain the correlation between biofilm formation and multi-drug resistance (MDR) in *S. aureus* and to assess the potential impact of lactic acid bacteria on *S. aureus* biofilm formation.

Methodology

S. aureus isolates from animal-source foods

The study involved the analysis of 116 *S. aureus* isolates obtained from different animal-source foods, including milk ($n=41$), chicken meat ($n=39$), and chicken eggs ($n=36$). The milk and chicken meat isolates were obtained from the repository of the Centre for One Health, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, while the chicken egg isolates were taken from the previous study conducted by Sharan et al. (2023).

Isolation and identification

S. aureus isolates were identified using standard microbiological procedures on Baird-Parker agar (BPA) plates supplemented with egg yolk tellurite, as outlined in the Bacteriological Analytical Manual (Tallent et al. 2019). Additionally, biochemical confirmation was performed

using a Microxpress *Staphylococcus* spp. identification kit (HiMedia, India).

The recovered isolates were further validated by using polymerase chain reaction (PCR) targeting *16S* rRNA and *nuc* genes of *S. aureus*. The PCR protocol was adopted from Zehra et al. (2019). *S. aureus* ATCC 33591 strain was used as a positive control. In brief, the total reaction mixture of 25 µl was made containing 6.5 µl Go Taq green master mix (Promega, U.S.A), 0.5 µl of 10 pmol/µl of forward and reverse primers each, 5 µl of DNA template and nuclease-free water (NFW) to make up the reaction volume. The cycling conditions were as follows: 1 cycle of initial denaturation (94 °C/3 min), 30 cycles each of denaturation (94 °C/30 s), annealing (55 °C/30 s), extension (72 °C/2 min), 1 cycle final extension (72 °C/4 min) and withhold at 4 °C. The amplified PCR products were visualized through the agarose gel electrophoresis and further recorded under Gel documentation system (UVP Gel Seq. Software; Syngene, U.S.A). The details of the primers are listed in Table 1.

Antibiotic sensitivity test

The antibiotic susceptibility was tested against nine different classes of antibiotics viz., aminopenicillin (ampicillin), aminoglycosides (gentamicin), cephalosporins (cefoxitin), chloramphenicol (chloramphenicol), fluoroquinolones (ciprofloxacin), macrolides (erythromycin), oxazolidinones (linezolid), sulphonamides (sulpha-cotrimoxazole), and tetracyclines (tetracycline) as per Kirby-Bauer disc diffusion method (Bauer et al. 1966). The antibiotic selection was carried out with discussions between veterinary academicians and field experts to target the commonly used antibiotics in the region. The results were interpreted as per the guidelines of Clinical and Laboratory Standards Institute (M100 Performance Standards for Antimicrobial Susceptibility Testing, 32rd edition) (CLSI 2022). In brief, the isolates were added to Muller Hinton (MH) broth and incubated for 2–3 h at 37 °C, and the turbidity was adjusted using McFarland standards (0.5). Further, the inoculums were placed on MHA plates and allowed to dry. The discs were placed and incubated for 18–24 h at 37 °C to measure the inhibition zone. The multiple antibiotic resistance (MAR) index was calculated using the formula provided (Krumperman 1983):

$$\mathrm{MAR} = \frac{x}{Y_n}$$

whereas,

X = Number of antibiotics exhibited resistance towards the isolate.

Y_n = Number of tested antibiotics.

The MAR index of more than 0.2 indicates high risk of antibiotics' exposure to the microorganism.

Biofilm evaluation using crystal violet (CV) assay

The biofilm formation was evaluated using the crystal violet (CV) assay as described by Stepanovic et al. (2000) with minor modifications. The overnight test cultures were suspended in 1000 µl of tryptic soy broth (TSB) for 18 h at 37 °C and further diluted to 1:100 in 1% glucose-supplemented TSB. In triplicates, 200 µl of culture adjusted to 10^7–10^8 CFU/mL was added per well and incubated for 48 h at 37 °C, with negative control wells containing no test culture. The plates were washed with phosphate-buffered saline (PBS; pH 7.2), fixed with 200 µl methanol/well for 15 min and stained with 200 µl of 2% crystal violet dye. The plates were then washed and air-dried. Lastly, 200 µl of 33% peracetic acid was added per well, and absorbance (OD) was measured using microtiter plate reader at 570 nm.

The results were interpreted following the guidelines provided by Stepanović et al. (2007) based on the ODc (control), and the isolates were classified into four categories:

Table 1 Details of primers used in the study

Genes		Primer sequence (5′-3′)	Annealing temp	Amplicon size (bp)	Reference
***S. aureus* (genus specific) gene**	*16S* rRNA	F: CAG CTC GTG TCG TGA GAT GT R: AAT CAT TTG TCC CAC CTT CG	55 °C	420	(Strommenger et al. 2003)
***S. aureus* (species specific) gene**	*nuc*	F: GCG ATT GAT GGT GAT ACG GTT R: AGC CAA GCC TTG ACG AAC TAG C	55 °C	279	(Brakstad et al. 1992)
S. aureus biofilm forming gene	*icaA*	F: GAC CTC GAA GTC AAT AGA GGT R: CCC AGT ATA ACG TTG GAT ACC	56 °C	814	(Diemond-Hernández et al. 2010)
	icaD	F: AAA CGT AAG AGA GGT GG R: GGC AAT ATG ATC AAG ATA C	45 °C	381	(Vasudevan et al. 2003)

a) Non-biofilm formers: OD of the test isolate ≤ OD_c
b) Weak biofilm formers: OD of the test isolate between OD_c to 2xOD_c
c) Moderate biofilm formers: OD of the test isolate between 2 to 4xOD_c
d) Strong biofilm formers: OD of the test isolate > 4xOD_c

Molecular detection of biofilm forming genes of *S. aureus*

The detection of biofilm-forming *ica*A and *ica*D genes in *S. aureus* was carried out using polymerase chain reaction (PCR). The 2 µl DNA template of previously identified *S. aureus* isolates (i.e., Isolate ID: SE 12 for *ica*A and Isolate ID: SE169 for *ica*D gene) was used as a positive control (Sharan et al. 2023). The PCR assay for *ica*A and *ica*D genes were adopted from Diemond-Hernández et al. (2010) and Vasudevan et al. (2003), respectively. The details of the primers used are listed in the Table 1. The PCR cycling conditions for the *ica*A gene was: 1 cycle initial denaturation (94 °C/3 min), 30 cycles each of denaturation (94 °C/30 s), annealing (56 °C/30 s), extension (68 °C/30 s), 1 cycle final extension (72 °C/7 min) and withholding at 4 °C. The PCR cycling conditions for *ica*D gene was: 1 cycle initial denaturation (94 °C/3 min), 30 cycles each of denaturation (92 °C/45 s), annealing (45 °C/30 s), extension (72 °C/1 min), 1 cycle final extension (72 °C/7 min) and withholding at 4 °C. The amplified PCR products were visualized through the agarose gel electrophoresis and further recorded under Gel documentation system (UVP Gel Seq. Software; Syngene, U.S.A).

Extracellular polymeric substance (EPS) quantification

The EPS was extracted and quantified by following phenol sulphuric acid method as described by Amrutha et al. (2017) and Dubois et al. (1956), respectively with minor modification. The total EPS was estimated using standard curve for different concentrations (in µg/ml) developed using regression model. The absorbance was obtained with different concentration of working standard glucose solution at 490 nm (Fig. 1) and the regression equation of Y = 0.1546*X—0.006730 was formed to determine the EPS concentration of each isolate. The overnight cultured test isolates were harvested by centrifugation at 8000 rpm for 30 min at 20 °C, and the filtered supernatant was added to three volumes of chilled 100% ethanol and incubated overnight at -20 °C. EPS was collected by centrifugation at 7000 rpm for 30 min at 5 °C. After that, 1 ml of MilliQ water was added to pellet with 0.05 ml of 80% phenol and 5 ml of conc. H_2SO_4 (Al-Shabib et al. 2017). The tubes were placed for 10–20 min in a water bath at 25 °C, and the absorbance was read at 490 nm using microtiter plate reader.

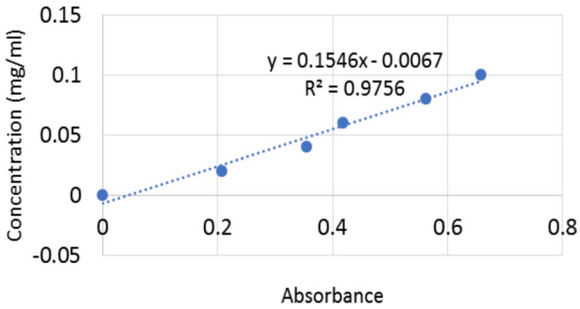


Fig. 1 Standard curve for determination of extracellular polymeric substance (EPS) concentration

Measurement of antibacterial activity using agar spot diffusion assay

The antibacterial activity of two LAB strains, *Lactobacillus rhamnosus* ATCC 53103 and *Lactobacillus casei* ATCC 393, was measured using an agar spot diffusion assay as described by Leite et al. (2015). Overnight cultured LAB strains (10 µl) were spotted on the surface of de Man, Rogosa and Sharpe (MRS) agar and incubated for 24 h at 37 °C. MRS agar was overlaid with 5 ml of tryptic soy agar (TSA) containing 100 µl of test culture and incubated for 24 h at 37 °C. The antibacterial activity was evaluated by measuring the clear zone diameter (mm) around the LAB strain. The results were interpreted as follows:

a) The inhibition zone with no halo: negative (-)
b) The inhibition zone with a halo of 1 mm: positive (+)
c) An inhibition zone between 2 and 5 mm: positive (++)

Effect of LAB strains on in vitro biofilm formation using modified crystal violet assay

The effect of the LAB strains on biofilm production was assessed using the protocols described by Gómez et al. (2016). The overnight test culture of *S. aureus* isolates was diluted 1:100 in TSB supplemented with 1% glucose, while LAB strains were cultured in MRS and turbidity was adjusted to 0.5 MacFarland. A 100 µl culture of *S. aureus* (SA) was inoculated in triplicate with an equal amount of *L. rhamnosus* (LR) and *L. casei* (LC) culture strains, both alone (SA + LR; SA + LC) and in combination (SA + LR + LC). The remaining steps were carried out as described in Sect. "Biofilm evaluation using crystal violet (CV) assay", following the modified crystal violet

assay protocol to evaluate the effect of LAB strains on biofilm formation of *S. aureus*.

Assessment of auto aggregation

The auto-aggregation assay was performed as described by Lee et al. (2021). The overnight cultures of *S. aureus* and LAB cultures were harvested by centrifugation at 10,000 rpm for 5 min at 4 °C. The pellet was washed with 1 ml of 1X PBS (pH 7.2), and the turbidity was adjusted to 0.5 MacFarland. Afterwards, 200 µl of homogenized bacterial suspension was inoculated into a 96 well polystyrene microtiter plate (in triplicate). The OD of the homogenized bacterial suspension was initially measured at 0 h and then re-evaluated after allowing the same suspension to rest for 24 h at 37 °C without agitation. The absorbance was measured at 600 nm, and the data were interpreted using the following formula:

$$\text{Aggregation ability \%} = \left[1 - \frac{OD_t}{OD_i}\right] x100$$

where,

OD_t represents the absorbance of the mixture at 24 h.

OD_i is absorbance at 0 hr

Assessment of co-aggregation assay with *S. aureus*

The preparation of the homogenized bacterial strain suspension followed the procedure outlined in the auto-aggregation assay described in Sect. "Assessment of auto aggregation". The LAB strain suspensions of *L. rhamnosus* and *L. casei* were mixed with equal volumes (100 µl) of the test culture alone (SA + LR; SA + LC) and in combination (SA + LR + LC) per well (in triplicate).

OD was assessed as described in the auto-aggregation assay in Sect. "Assessment of auto aggregation". The percentage of co-aggregation was calculated as described by Gómez et al. (2016) using the formula:

$$\text{Coaggregation ability \%} = \left[1 - \frac{(OD_{mix})}{(OD_p + OD_L)/2}\right] \times 100$$

where OD_p and OD_L represent the absorbance in the tubes containing only the test or LAB strain, respectively, measured at 0 h, while OD_{mix} represents the absorbance of the mixture for both pathogen and LAB strains at 24 h. The calculations were carried out by adjusting the formula as per the input parameters.

Statistical analysis

The data obtained from the experiments were recorded in a Microsoft® Office Excel 2019 spreadsheet. The normal distribution of data was assessed using the Kolmogorov–Smirnov test, which was performed using IBM SPSS Statistics (version 26.0; IBM Corporation, Armonk, New York, USA). The statistical analysis was conducted as necessary, employing Fisher's exact test, chi-squared test, Spearman's correlation analysis, and Wilcoxon signed rank test. The figures were generated using GraphPad Prism 14.0 and Microsoft® Office Excel 2019.

Results

Isolation and molecular identification of *S. aureus*

A total of 107 *S. aureus* isolates, comprising 35 from milk, 36 from chicken meat, and 36 from chicken eggs, were revived on Baird Parker agar. These isolates were subjected to biochemical characterization and were further confirmed by the presence of the *16S rRNA* (genus-specific) and *nuc* (species-specific) genes, specifically identifying them as *Staphylococcus aureus* within the *Staphylococcus* genus.

Phenotypic AMR profile

A total of 70.10% isolates were resistant for ≥ 3 tested antibiotics with 77.57% of isolates showing MAR index > 0.2 (Table 2). The *S. aureus* isolates from milk showed the highest resistance against ampicillin and erythromycin (88.57%) followed by cefoxitin (85.71%), tetracycline (45.71%), linezolid (31.42%), gentamicin (17.14%), sulpha-cotrimoxazole (14.28%), and ciprofloxacin (8.57%), with none demonstrating resistance to chloramphenicol. Similarly, among chicken meat isolates, highest resistance was observed for cefoxitin (100%) followed by tetracycline (88.88%), erythromycin (86.11%), ampicillin (83.33%), linezolid (80.55%), sulpha-cotrimoxazole (77.77%), gentamicin (61.11%), ciprofloxacin (55.55%), and chloramphenicol (50%). Among egg isolates, the highest resistance was observed for cefoxitin (100%), followed by erythromycin (97.22%), ampicillin (86.11%), tetracycline

Table 2 Multidrug resistance (MDR) pattern of *S. aureus* isolates from milk, chicken meat and chicken egg isolates

Source	MDR (resistant to > 3 classes of antibiotics)	MAR index (> 0.2)	Resistance to all antibiotics	Resistance > 7 antibiotics	Resistance 5–7 antibiotics	Resistance 3–5 antibiotics	Resistance to < 3 antibiotics	Sensitive to all antibiotics
Milk	68.60% (24/35)	74.28% (26/35)	0	0	9	14	10	1
Chicken meat	72.23% (26/36)	77.78% (28/36)	1	7	11	8	10	0
Chicken eggs	69.44% (25/36)	80.56% (29/36)	0	0	15	10	11	0

(77.77%), sulpha-cotrimoxazole (55.55%), chloramphenicol (33.33%), gentamicin (33.33%), ciprofloxacin (25%), and linezolid (19.44%) (Fig. 2).

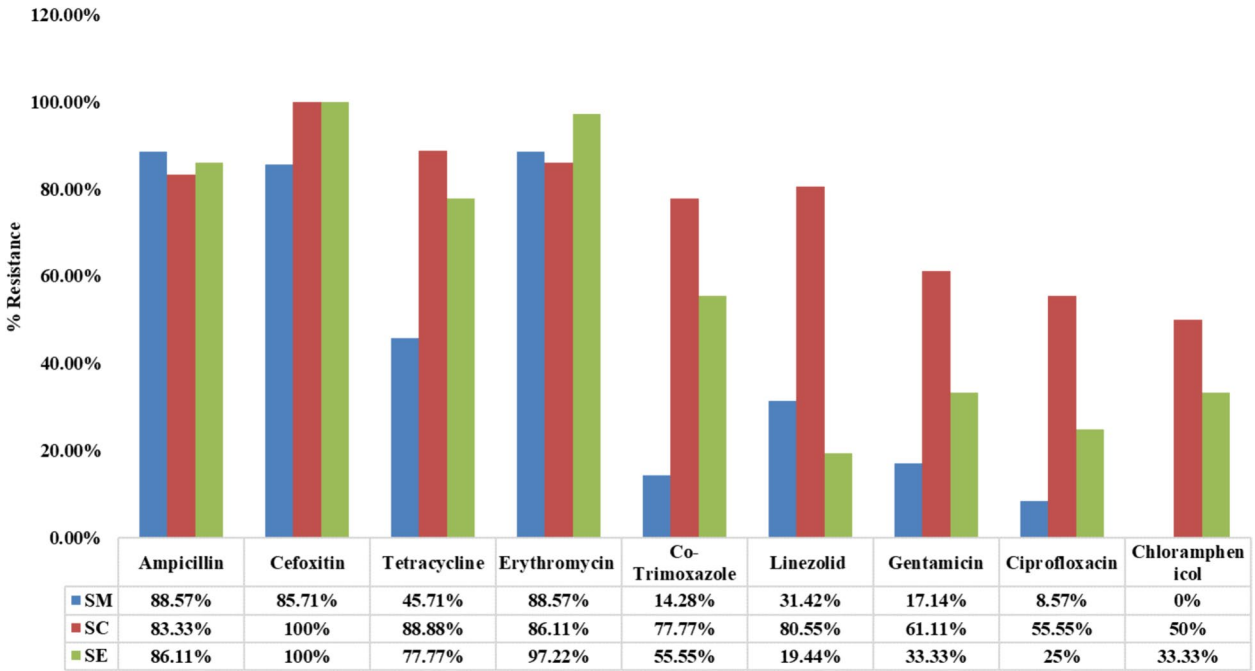
Biofilm forming ability

In present study, 16.82% (18/107) isolates were strong biofilm former, 13.1% (14/107) were moderate, and 55.14% (59/107) were weak biofilm former, whereas 14.95% (16/107) were non-biofilm producers. Among 35 milk isolates, 17.14% (6/35) were strong, 20.0% (7/35) were moderate and 48.57% (17/35) were weak biofilm producers, whereas 14.28% (5/35) isolates were non-biofilm producer. In 36 chicken meat isolates, 11.11% (4/36) were strong, 8.33% (3/36) as moderate, and 72.22% (26/36) were weak biofilm producers, whereas 8.33% (3/36) of the isolates were non biofilm producers. Among 36 egg isolates, 22.22% (8/36) were strong, 11.11% (4/36) were moderate, and 44.44% (16/36) were weak biofilm producers, whereas, 22.22% (8/36) of the isolates were non-biofilm producer. The comparison of the ability of *S. aureus* biofilm formation from different foods are presented as Fig. 3.. On statistical analysis using Kruskal–Wallis's test, a non-significant association (p-value > 0.05; 95% CI) was observed between the mean OD values of different isolates sources (i.e., milk, meat and eggs).

Correlation between biofilm forming ability and antimicrobial resistance

Out of all 107 isolates, 75 were found to be MDR and among them 18.67% (14/75) were strong, 13.33% (10/75) were moderate and 53.33% (40/75) were weak biofilm formers, while 14.67% (11/75) were non-biofilm producer. Among 32 non-MDR isolates, 12.50% (4/32) were strong, 12.50% (4/32) were moderate, and 59.38% (19/32) were weak biofilm former, while 15.63% (5/32) were non-biofilm producer. The relationship between biofilm formation ability and antimicrobial resistance was analysed (Fig. 4). The plot was drawn by converting the isolate number falling into each grade into percentage. The darker colour in the Fig. 4 denotes higher proportion of isolates. The statistical analysis revealed non-significant difference (p value: > 0.05; 95% CI) between biofilm forming ability of MDR and non-MDR isolates.

Among 35 milk isolates, 24 were MDR and among them 12.5% (3/24) were strong, 25.0% (6/24) were moderate, and 50% (12/24) were weak biofilm producers whereas, 12.5% (3/24) were non biofilm producer. Among 11 non-MDR isolates, 27.27% (3/11) were strong, 9.1% (1/11) were moderate, 45.45% (5/11) were weak biofilm producers, whereas 18.18% (2/11) were non-biofilm producers. Out of 36 chicken meat isolates, 26 were MDR and among them 15.38% (4/26) were strong, 7.69% (2/26) were moderate, and 73.07% (19/26) were weak biofilm



	Ampicillin	Cefoxitin	Tetracycline	Erythromycin	Co-Trimoxazole	Linezolid	Gentamicin	Ciprofloxacin	Chloramphenicol
SM	88.57%	85.71%	45.71%	88.57%	14.28%	31.42%	17.14%	8.57%	0%
SC	83.33%	100%	88.88%	86.11%	77.77%	80.55%	61.11%	55.55%	50%
SE	86.11%	100%	77.77%	97.22%	55.55%	19.44%	33.33%	25%	33.33%

Fig. 2 Phenotypic antibiogram profile (in percentage) of *S. aureus* isolates (SM: Milk *S. aureus* isolates; SC: Chicken meat *S. aureus* isolates; SE: Chicken eggs *S. aureus* isolates)

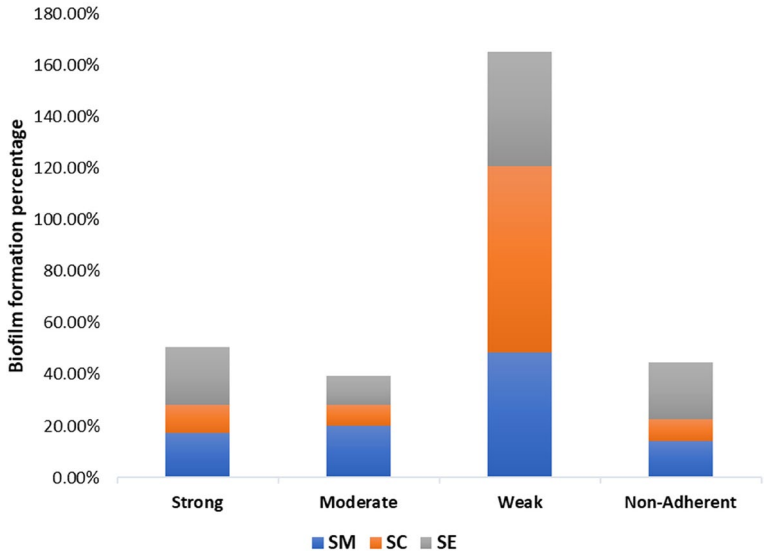


Fig. 3 Comparison of the biofilm formation ability of *S. aureus* from different animal-source foods (SM: Milk *S. aureus*; SC: Chicken meat *S. aureus*; SE: Chicken eggs *S. aureus*)

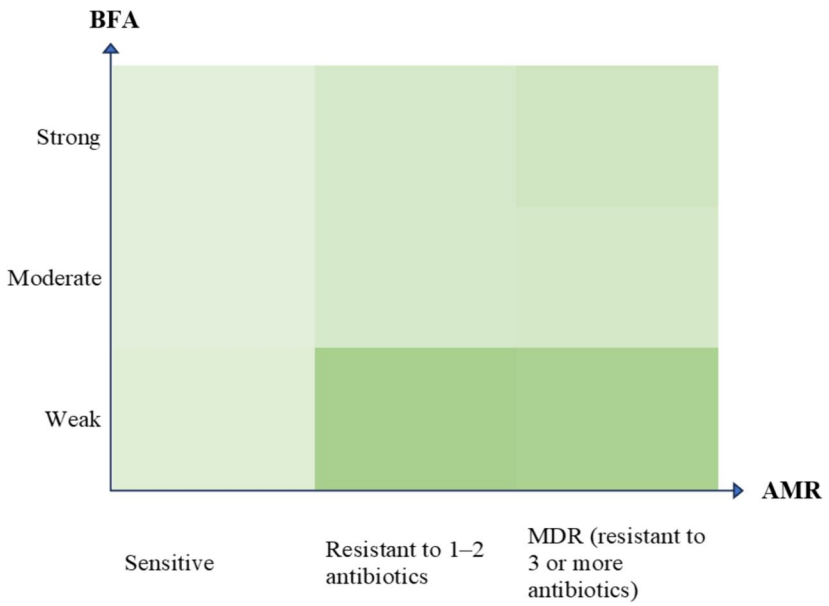


Fig. 4 Relationship between biofilm formation ability (BFA) and antimicrobial resistance (AMR) of *S. aureus* isolates

former, whereas a single isolate was non biofilm producer. Among, 10 non-MDR isolates, 10.0% (1/10) were moderate, 70.0% (7/10) were weak and 20.0% (2/10) were non biofilm producers. Out of 36 egg isolates, 25 were MDR and among them, 28.0% (7/25) were strong, 8.0% (2/25) were moderate and 36.0% (9/25) were weak biofilm former, whereas 28.0% (7/25) of the isolates were non biofilm former. Among 11 non-MDR isolates, 9.10% (1/11) were strong, 18.18% (2/11) were moderate, and 63.64% (7/11) were weak biofilm formers, whereas a single isolate was non biofilm former. On statistical analysis, non-significant difference (p value: > 0.05; 95% CI) was observed between biofilm forming ability of MDR and non-MDR isolates among all three animal-source foods.

Detection of biofilm forming *ica*A and *ica*D genes

The presence of the *icaA* gene was detected in 45.80% (49/107) of all *S. aureus* isolates. Among MDR and

non-MDR isolates, positivity rates were 53.33% (40/75) and 28.12% (9/32), respectively. Among 35 milk isolates, 45.71% (16/35) carried the *ica*A gene wherein among MDR and non-MDR, 50.0% (12/24) and 36.36% (4/11) isolates were positive, respectively. Among 36 chicken meat isolates, 33.33% (12/36) isolates were positive wherein among MDR and non-MDR isolates, 38.46% (10/26) and 20.0% (2/10) positivity were observed, respectively. Among 36 egg isolates, 58.33% (21/36) isolates carried the *ica*A gene wherein among MDR and non-MDR, 72.0% (18/25) and 27.27% (3/11) isolates were positive, respectively.

The presence of the *ica*D gene was observed in 38.31% (41/107) of all isolates, with positivity rates of 44.0% (33/75) among MDR isolates and 25.0% (8/32) among non-MDR isolates. Among 35 milk isolates, 42.85% (15/35) isolates carried the *ica*D gene wherein among MDR and non-MDR, 47.83% (11/23) and 36.36% (4/11) of were positive, respectively. Moreover, among 36 chicken meat isolates, 25.0% (9/36) isolates were positive for *ica*D gene, whereas among MDR and non-MDR, 30.77% (8/26) and 10.0% (1/10) of isolates were positive, respectively. Among 36 egg isolates, 47.22% (17/36) isolates were positive wherein among MDR and non-MDR, 56.0% (14/25) and 27.27% (3/11) of the isolates were positive, respectively.

Quantification of extracellular polymeric substance (EPS)

The EPS yield obtained from *S. aureus* isolates was ranging from 3.11 to 59.64 mg/ml (mean: 14.10 mg/ml). Among the milk *S. aureus* isolate, the EPS concentration obtained was 3.90 to 59.64 mg/ml (mean: 15.21 mg/ml). Among chicken meat isolates, the EPS concentration ranged from 3.11 to 44.83 mg/ml (mean: 11.42 mg/ml), wherein among egg isolates the EPS concentration lies between 3.38 to 46.51 mg/ml (mean: 15.67 mg/ml). Three isolates, one each from milk, meat and egg produced a significantly higher EPS with the concentration 59.64 mg/ml, 44.83 mg/ml and 46.51 mg/ml, respectively (Fig. 5). The difference between the EPS production among various source isolates were found to be non-significant (p value: > 0.05; 95% CI). Overall, the highest average EPS yield was observed among the *S. aureus* isolates of egg, followed by milk and chicken meat.

Measurement of anti-bacterial activity of LAB strains on *S. aureus* isolates using agar spot diffusion assay

All the *S. aureus* isolates showed a halo inhibition zone of diameter > 2 mm around *L. rhamnosus* and *L. casei* strains (Fig. 6). Overall, the inhibition zone diameter range observed was 15 to 34 mm (Fig. 7).

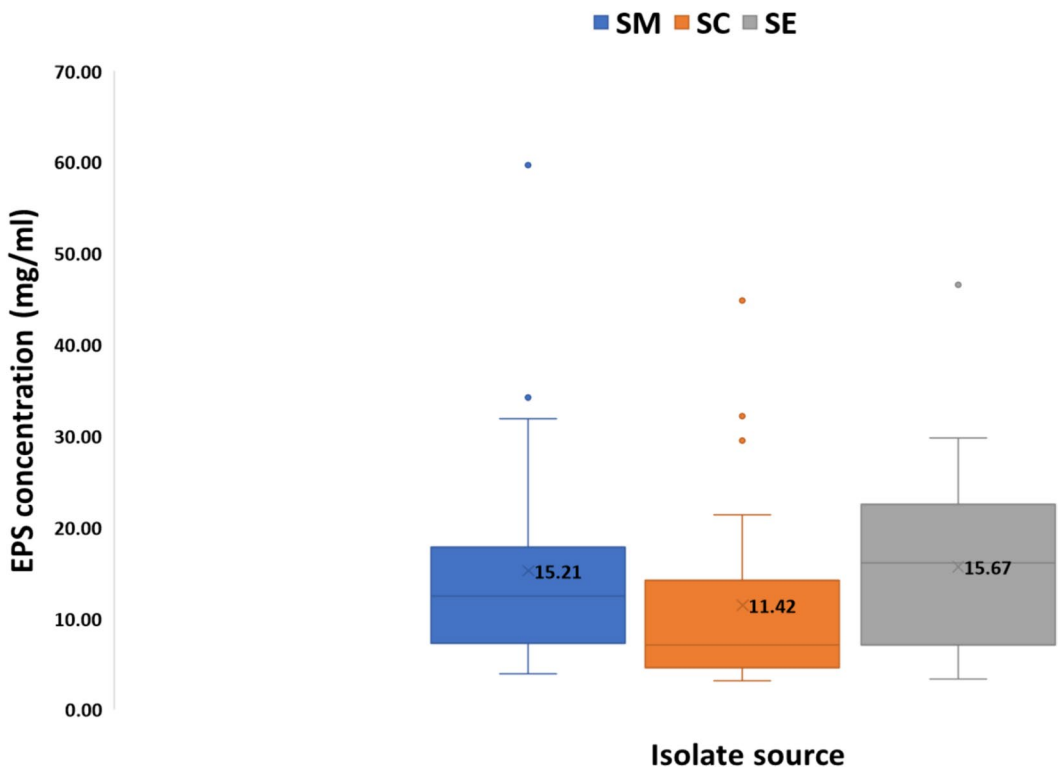


Fig. 5 Boxplot depicting the distribution of extracellular polymeric substance (EPS) concentration (in mg/ml) among *S. aureus* isolates (SM: Milk *S. aureus* isolates; SC: Chicken meat *S. aureus* isolates; SE: Chicken eggs *S. aureus* isolates)

Fig. 6 Anti-bacterial inhibition zone formation by *Lactobacillus rhamnosus* (LR) and *Lactobacillus casei* (LC) on a *S. aureus* isolate

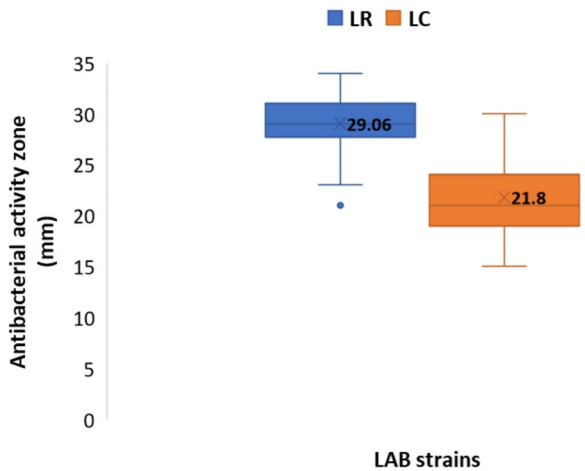


Fig. 7 Boxplot for antibacterial activity mean inhibition zone (in mm) by *Lactobacillus rhamnosus* (LR) and *Lactobacillus casei* (LC) for *S. aureus*

The *L. rhamnosus* strain showed an inhibition zone within the range 19 to 34 mm (mean: 29.06 mm). The inhibition range in milk was 19 to 34 mm (mean: 28.76 mm), in chicken meat was 24 to 34 mm (mean: 28.96 mm) and in chicken egg was 26 to 33 mm (mean: 29.58 mm). Similarly, the *L. casei* strain showed an inhibition zone within the range 15 to 31 mm (mean: 21.80 mm). The inhibition zone range in milk was 16 to 29 mm (mean: 21.48 mm), in chicken meat was 17 to 31 mm (mean: 22.15 mm) and in chicken egg was 15 to 28 mm (mean: 21.74 mm).

Determination of LAB strains effect on biofilm formation of *S. aureus* isolates

All the isolates were subjected to crystal violet (CV) assay with *L. rhamnosus* and *L. casei*, both individually and in combination. The results showed a marked reduction in absorbance when the isolates were co-incubated with *L. rhamnosus* and *L. casei* strains (Fig. 8a, b, c). The *L. rhamnosus* showed average reduction in absorbance by 48.19% to the absorbance of *S. aureus* isolates biofilm. On co-culture with *L. rhamnosus,* an average reduction of 48.08%, 47.76% and 48.93% was observed in *S. aureus* isolates of milk, chicken meat and chicken eggs, respectively. Similarly, the *L. casei* strain reduced the biofilm formation of *S. aureus* isolates by 31.56%. An average reduction of 29.02% was observed in milk isolates, 33.35% in chicken meat isolates, and 32.43% in chicken egg isolates, on co-culture of *S. aureus* with *L. casei.* Furthermore, on combined treatment (i.e., with LR and LC strains), an average reduction of 38.51% in absorbance was observed. A reduction of 34.57% was observed in milk isolates, 43.26% in chicken meat isolates, and 37.19% in chicken egg isolates, on co-culture of *S. aureus* with *L. rhamnosus* and *L. casei*. Further, the absorbance value of *S. aureus* isolates was compared with the absorbance value obtained after co-incubating with the LAB strains (alone and in combination) using Wilcoxon signed rank test. On analysis, the results were found statistically significant (p-value: < 0.05; 95% CI) among all combinations (SA + LR; SA + LC; SA + LR + LC).

Assessment of auto-aggregation

The highest auto-aggregation percentage was observed after 24 h of incubation. The aggregation abilities of *L. rhamnosus* and *L. casei* was observed to be 55.46% and 45.23%, respectively. Overall, the *S. aureus* isolates ($n = 107$) was observed to have auto-aggregation percentage between 11.24% and 50.90% (mean: 25.59%). Further, the aggregation in milk isolates was observed to be 11.24% to 50.90% (mean: 26.30%), in chicken meat, 16.28% to 38.95% (mean: 24.79%), wherein among egg isolates, 14.53% to 42.62% (mean: 25.77%).

Assessment of co-aggregation in between *S. aureus* and LAB strains (*L. rhamnosus* and *L. casei)*

Overall, the co-aggregation percentage of *L. rhamnosus* with *S. aureus* isolates was observed in the range of 27.19% to 55.15% (mean: 40.61%). The range in milk, chicken meat and chicken eggs were observed as 27.19% to 55.15% (mean: 40.30%), 31.59% to 52.12% (mean: 39.93%) and 30.70% to 51.22% (mean: 41.60%),

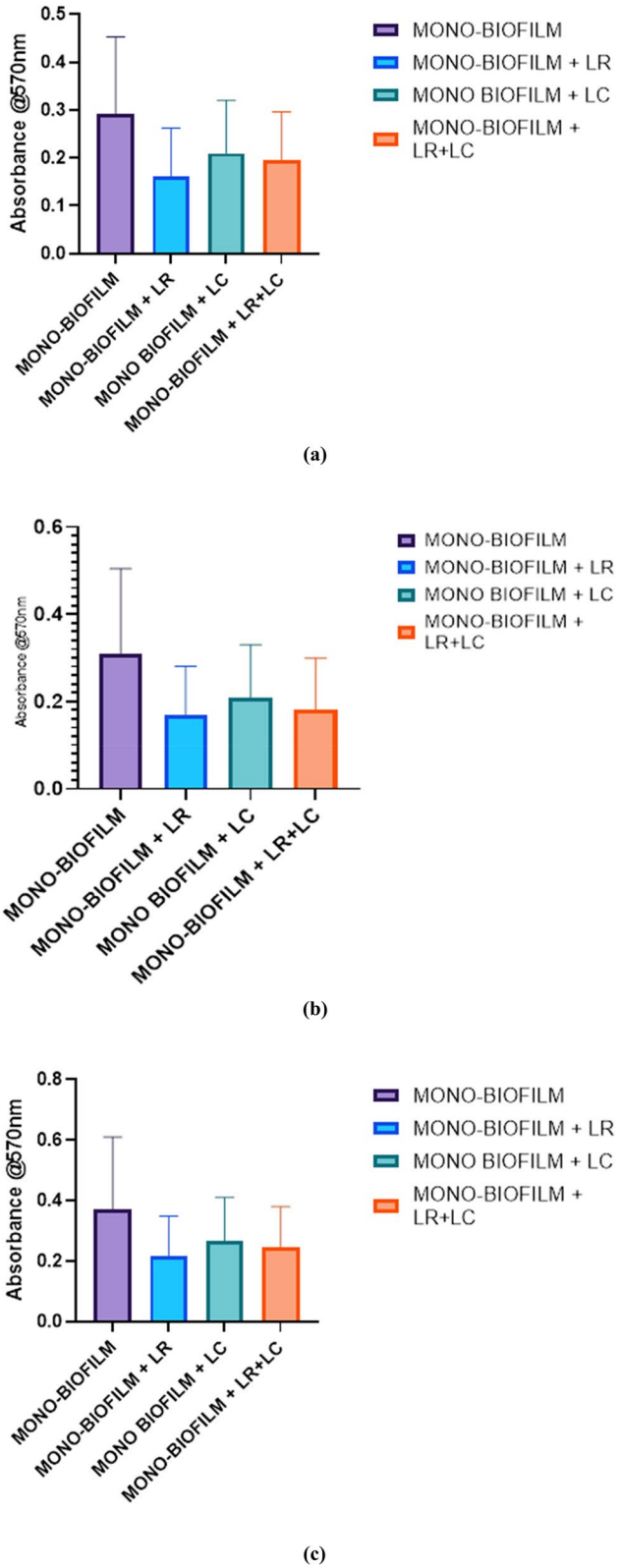
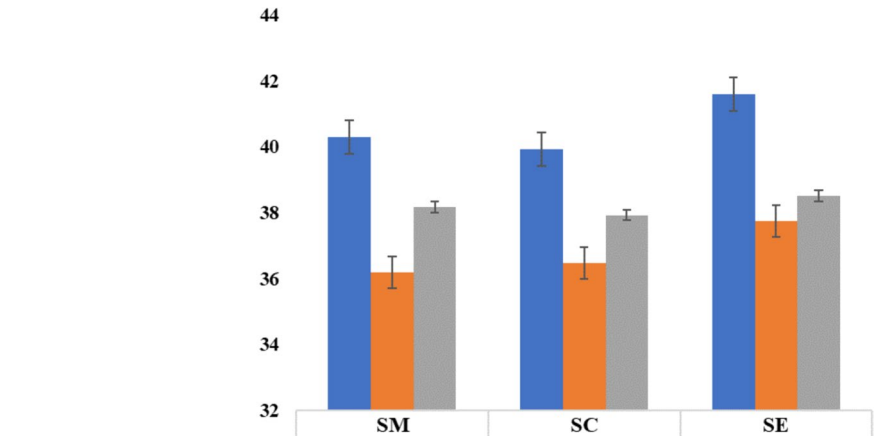


Fig. 8 Effect of *Lactobacillus rhamnosus* (LR) and *Lactobacillus casei* (LC) strains on biofilm formation of (**a**) Milk *S. aureus* isolates, (**b**) Chicken meat *S. aureus* isolates and (**c**) Chicken egg *S. aureus* isolates

respectively. Similarly, the co-aggregation of *L. casei* with *S. aureus* isolates was observed in the range of 25.28% to 50.12% (mean: 36.81%). Among milk isolates, the range were observed to be 25.83% to 50.12% (mean: 36.20%) and in chicken meat isolates was 27.57% to 47.89% (mean: 36.48%), and in chicken egg isolates was 25.28% to 49.87% (mean: 37.75%). In addition, the co-aggregation of *L. rhamnosus* and *L. casei* strains with *S. aureus* isolates were also assessed, where cell to cell interaction was observed to be in the range of 25.0% to 52.28% (mean: 38.21). Among milk isolates, the range was 27.04% to 52.28% (mean: 38.18%), in chicken meat isolates, the range was 25.20% to 51.39% (mean: 37.93%), and in chicken egg isolates, the range was 24.42% to 52.28% (mean: 38.51%). The co-aggregation abilities between LAB strains and pathogen are present in Fig. 9.

Discussion

The multi-drug resistance (MDR) has been identified as a major public health concern around the world. We observed a high MDR in *S. aureus* isolates from chicken meat (72.23%) followed by chicken eggs (69.44%) and milk (68.60%). The observed high resistance in poultry products might be due to overuse of antibiotics for therapeutic and prophylactic purpose in poultry sector. Similar observations, indicating high MDR prevalence in the poultry sector, were also reported by Rahman et al. (2018). Among the MDR isolates, the highest MDR resistance was observed in *S. aureus* isolates from eggs (90.91%), followed by chicken meat (53.85%), and milk (12.0%). It is widely recognized that the misuse or overuse of antibiotics creates selection pressure, which promotes the development of resistance in microbes (Bissong and Ateba 2020). Furthermore, the present study also assessed the potential relationship between AMR and the ability to form biofilm among both MDR and non-MDR isolates. As shown in Fig. 4, most of the isolates with drug-resistance characteristics have a strong biofilm formation ability. This indicates a relationship between biofilm formation ability and AMR. Notably, among the isolates forming biofilms, the proportion of MDR isolates forming weak biofilms was slightly lower (53.33%) than that of non-MDR isolates (59.38%). However, assuming that isolates with strong biofilm formation inherently display high levels of drug resistance poses a challenge, as the underlying mechanisms, despite the observed correlation, remain unclear. Various factors such as bacterial species or strain, type of antimicrobial agent, stage of biofilm development, and growth conditions are reported to influence biofilm formation and associated antimicrobial resistance in many studies (Barros et al. 2017; Al-Shabib et al. 2017; Bissong and Ateba 2020; Idrees et al. 2021).



	SM	SC	SE
Co-aggregation % with LR	40.3	39.93	41.6
Co-aggregation % with LC	36.2	36.48	37.75
Co-aggregation % with LR + LC	38.18	37.93	38.51

Fig. 9 Co-aggregation percentage of *S. aureus* and LAB strains (*L. rhamnosus* and *L. casei)* (SM: Milk *S. aureus* isolates; SC: Chicken meat *S. aureus* isolates; SE: Chicken eggs *S. aureus* isolates)

The findings of the present study revealed that *S. aureus* isolates from various animal-origin foods exhibit diverse levels of biofilm-forming ability. Isolates from chicken eggs showed the highest rate of strong biofilm formation (22.22%), followed by those from milk (17.14%) and chicken meat (11.11%) (Fig. 3). Additionally, the mean values of biofilm formation ability (@OD570) for *S. aureus* derived from different sources were found to be similar. Similarly, a study by Ou et al. (2020) demonstrated comparable mean biofilm-forming abilities of *S. aureus* from various animal-origin food sources (pork, chicken, beef, duck, lamb, aquatic products, egg, and milk). The findings suggests that the shared characteristics of food substrates, such as surface properties and viscosity plays a more pivotal role in facilitating the successful colonization of *S. aureus* than variations in food surface properties and bacterial species. In essence, animal-source food serves as an excellent adhesive medium and reservoir for *S. aureus* biofilm growth (Koohestani et al. 2018).

The development of biofilm of *S. aureus* is regulated by the *ica*ADBC operon in which *ica*A and *ica*D genes play significant role in their adherence to surfaces during early stages of biofilm development (Idrees et al. 2021). In the present study, nearly half of the biofilm-forming isolates possessed either the *ica*A or *ica*D gene, which play a major role in surface adherence. None of the non-biofilm producer isolates were positive for either *ica*A or *ica*D gene. The past studies demonstrated that in addition to the role of the *ica*A and *ica*D genes in bacteria's biofilm formation, there are many additional bacterial and environmental factors that also influence the adhesion properties (O'Gara 2007; Kroning et al. 2016). The varying prevalence rates of these genes are likely due to distinct gene expression exhibited by *S. aureus* when exposed to different temperatures and contact surfaces over varying periods (Atshan et al. 2012; Kroning et al. 2016). A study by O'Gara (2007) highlighted the existence of *ica* independent biofilm mechanisms in *S. aureus.* However, the understanding of the regulatory mechanisms governing the *ica* locus in the present study remains incomplete and requires further exploration in future research.

In addition to the regulation of the *ica*ADBC operon, microbial surface adherence is facilitated by extracellular polymeric substances (EPS) (Flemming 2016). The carbohydrate content of EPS was assessed in the present study. It was found that all isolates exhibiting biofilm activity produced a significantly varied concentration of EPS (mean: 14.1 mg/ml). Strong biofilm formers exhibited a higher concentration of EPS compared to weak biofilm formers. Furthermore, no significant difference in EPS production was observed among isolates from different sources, such as milk, chicken meat, and chicken eggs. Earlier studies have reported varied concentrations of EPS from different microbial strains. For example, Amrutha et al. (2017) observed EPS yields ranging from 35 to 100 mg/ml among *Salmonella* isolates, which is significantly higher than the findings of our study. In contrast, Patel et al. (2012) reported an EPS yield of 0.5 mg/ml from *Weissella* sp. isolated from fermented food. Thereby, the variation in the production of EPS can be specifically

strain dependent and vary among different environmental conditions (Flemming and Wingender 2010). The EPS matrix is extremely complex and dynamic structure that warrants detailed exploration in future studies (Flemming 2016).

The present study investigated the effectiveness of both LAB strains' antibacterial activity, anti-biofilm activity, auto-aggregation and coaggregation ability against *S. aureus. L. rhamnosus* showed better antibacterial property in comparison to *L. casei* strains against all *S. aureus* isolates, regardless of their source of origin. The results revealed that antibacterial activity produced an inhibition zone with a diameter greater than 12 mm against all *S. aureus* strains tested. Consequently, both LAB strains were further tested for their efficacy against biofilm forming ability of *S. aureus* isolates. The LAB strains (alone and in combination) removed the biofilm growth of *S. aureus*. The observed high anti-biofilm activity of LAB strains against food associated *S. aureus* might be due to production of anti-biofilm compounds such as lactic acid as it alters the pH and disrupts the bacterial viability (Gerbaldo et al. 2012; Van Der Weerden et al. 2013). Moreover, LAB provides competitive conditions to microbial cells by inhibiting their adhesions and by adapting competitive exclusion approach towards bacteria to prevent it from colonizing (Giaouris 2020). When comparing both LAB strains, the *L. rhamnosus* were observed to be more effective in biofilm removal than *L. casei* (alone and in combination). Furthermore, several investigations have made similar observations and concluded that the anti-biofilm activity of LAB strains is a species-dependent phenomenon (Song and Lee 2017; Koohestani et al. 2018). The findings of present study observed that both the LAB strains, *L. rhamnosus* and *L. casei* (alone and in combination), possess a potent biofilm removal potential against *S. aureus* biofilm. Therefore, these strains can be used as biocontrol agents in food production, either alone and in combination to reduce the risk of microbial contamination and improving food safety in sustainable way.

Furthermore, both LAB strains exhibited weak biofilm formation, but demonstrated higher auto-aggregation ability compared to *S. aureus*. The majority of *S. aureus* isolates were found to have low aggregation ability, potentially contributing to the formation of weaker biofilms. In comparison, *L. rhamnosus* exhibited higher aggregation ability than the *L. casei* strain, consistent with a study conducted by Woo and Ahn (2013), which also reported higher auto-aggregation ability in *L. rhamnosus* compared to *L. casei* and *L. acidophilus* strains. In addition, when determining the co-aggregation ability of both LAB strains, the results were consistent with those of auto-aggregation, with *L. rhamnosus* exhibiting higher co-aggregation than *L. casei*. The results suggests that an aggregative phenotype can be one criterion to screen the strains with co-aggregative properties. In general, probiotics inhibit pathogen colonization and biofilm formation by binding pathogens into co-aggregates, altering their microenvironment, competing for nutrients and adherence sites, and releasing inhibitory substances (García-Cayuela et al. 2014; Monteagudo-Mera et al. 2019).

A notable limitation of our study is that the experiments were conducted in vitro within a controlled laboratory setting rather than in vivo. While we aimed to assess various parameters associated with biofilm and intervention by LAB, conducting experiments in food matrices and under food processing conditions would provide more accurate evidence regarding the impacts of probiotics. Also, additional investigation is required to elucidate the precise mechanisms by which LAB and their active compounds impede the growth of foodborne pathogens.

Conclusion

In conclusion, the present study demonstrates that *S. aureus* isolates from animal-source foods display a high resistance to more than three classes of antibiotics. Additionally, these isolates were found to be capable of forming biofilms, which could act as a persistent source of foodborne contamination. Also, surface properties of various food samples act as an important factor in determining the colonisation of *S. aureus* strains. The study also revealed that MDR isolates have a higher potential to form strong biofilms and possess a greater percentage of biofilm genes than non-MDR isolates. Further, the study investigated the use of LAB strains (*L. rhamnosus* and *L. casei*), both individually and in combination, for their ability to combat *S. aureus* biofilms. The findings suggest that both strains are effective in removing biofilm growth, with *L. rhamnosus* proving to be more effective than *L. casei.* Thereby, probiotic-based intervention strategies may provide a promising approach to reduce the establishment of both MDR and non-MDR biofilms.

Abbreviations

AMR	Antimicrobial resistance
EPS	Extracellular polymeric substance
LAB	Lactic acid bacteria
LR	*Lactobacillus rhamnosus*
LC	*Lactobacillus casei*
MDR	Multidrug Resistant
MHA	Mueller Hinton Agar
OD	Optical density
SA	*Staphylococcus aureus*

Acknowledgements

Authors are thankful to the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana for providing necessary help to carry out the research work.

Authors' contributions
Manjeet Sharan: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Software; Writing – original draft; Pankaj Dhaka: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Supervision; Visualization; Writing – original draft; Writing – review & editing; Jasbir Singh Bedi: Conceptualization; Funding acquisition; Methodology; Project administration; Supervision; Validation; Writing – review & editing; Nitin Mehta: Conceptualization; Methodology; Resources; Supervision; Writing – review & editing; Randhir Singh: Conceptualization; Methodology; Resources; Supervision; Writing – review & editing.

Funding
The financial support received from the project of 'Rashtriya Krishi Vikas Yojana (RKVY), India' is thankfully acknowledged.

Availability of data and materials
All data are incorporated into the article and its online supplementary material.

Declarations

Competing interests
The authors have declared that no competing interests exist.

Author details
[1]Centre for One Health, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana 141004, India. [2]Department of Livestock Products Technology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana 141004, India.

Received: 22 February 2024 Accepted: 6 June 2024
Published online: 16 July 2024

References

Al-Shabib NA, Husain FM, Ahmad I, Khan MS, Khan RA, Khan JM (2017) Rutin inhibits mono and multi-species biofilm formation by foodborne drug resistant *Escherichia coli* and *Staphylococcus aureus*. Food Control 79:325–332. https://doi.org/10.1016/j.foodcont.2017.03.004

Amrutha B, Sundar K, Shetty PH (2017) Study on *E. coli* and *Salmonella* biofilms from fresh fruits and vegetables. J Food Sci Technol 54:1091–1097. https://doi.org/10.1007/s13197-017-2555-2

Atshan SS, Nor Shamsudin M, Sekawi Z, Lung LTT, Hamat RA, Karunanidhi A, Mateg Ali A, Ghaznavi-Rad E, Ghasemzadeh-Moghaddam H, Chong Seng JS, Nathan JJ, Pei Pei C (2012) Prevalence of adhesion and regulation of biofilm-related genes in different clones of *Staphylococcus aureus*. J Biomed Biotechnol 2012:1–10. https://doi.org/10.1155/2012/976972

Barros J, Grenho L, Fontenente S, Manuel CM, Nunes OC, Melo LF, Monteiro FJ, Ferraz MP (2017) *Staphylococcus aureus* and *Escherichia coli* dual-species biofilms on nanohydroxyapatite loaded with CHX or ZnO nanoparticles. J Biomed Mater Res A 105:491–497. https://doi.org/10.1002/jbm.a.35925

Bauer AW, Kirby WM, Sherris JC, Turck M (1966) Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 45:493–496

Bissong MEA, Ateba CN (2020) Genotypic and phenotypic evaluation of biofilm production and antimicrobial resistance in *Staphylococcus aureus* isolated from milk, North West Province, South Africa. Antibiotics 9. https://doi.org/10.3390/antibiotics9040156

Brakstad OG, Aasbakk K, Maeland JA (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

CLSI (2022) Performance Standards for Antimicrobial Susceptibility Testing, 32rd Edition, Clinical and Laboratory Standards Institute (CLSI) antimicrobial susceptibility testing standards M100. https://clsi.org/standards/products/microbiology/documents/m100/. Accessed 20 Jun 2022

Diemond-Hernández B, Solórzano-Santos F, Leaños-Miranda B, Peregrino-Bejarano L, Miranda-Novales G (2010) Production of *ica*ADBC-encoded polysaccharide intercellular adhesin and therapeutic failure in pediatric patients with staphylococcal device-related infections. BMC Infect Dis 10:68. https://doi.org/10.1186/1471-2334-10-68

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28:350–356. https://doi.org/10.1021/ac60111a017

Ferry T, Perpoint T, Vandenesch F, Etienne J (2005) Virulence determinants in *Staphylococcus aureus* and their involvement in clinical syndromes. Curr Infect Dis Rep 7:420–428. https://doi.org/10.1007/s11908-005-0043-8

Flemming HC (2016) Eps—then and now. Microorganisms 4

Flemming H-C, Wingender J (2010) The biofilm matrix. Nat Rev Microbiol 8:623–633. https://doi.org/10.1038/nrmicro2415

Galié S, García-Gutiérrez C, Miguélez EM, Villar CJ, Lombó F (2018) Biofilms in the food industry: Health aspects and control methods. Front Microbiol 9

García-Cayuela T, Korany AM, Bustos I, P. Gómez de Cadiñanos L, Requena T, Peláez C, Martínez-Cuesta MC (2014) Adhesion abilities of dairy *Lactobacillus plantarum* strains showing an aggregation phenotype. Food Res Int 57:44–50. https://doi.org/10.1016/j.foodres.2014.01.010

Gerbaldo GA, Barberis C, Pascual L, Dalcero A, Barberis L (2012) Antifungal activity of two *Lactobacillus* strains with potential probiotic properties. FEMS Microbiol Lett 332:27–33. https://doi.org/10.1111/J.1574-6968.2012.02570.X

Giaouris E (2020) Application of lactic acid bacteria and their metabolites against foodborne pathogenic bacterial biofilms. In: Simoes M, Borges A, Simoes LC (eds) Recent Trends in Biofilm Science and Technology, 1st edn. Academic Press, pp 205–232

Giaouris E, Heir E, Desvaux M, Hébraud M, Møretrø T, Langsrud S, Doulgeraki A, Nychas G-J, Kačániová M, Czaczyk K, Ölmez H, Simões M (2015) Intra- and inter-species interactions within biofilms of important foodborne bacterial pathogens. Front Microbiol 6. https://doi.org/10.3389/fmicb.2015.00841

Giordani B, Parolin C, Vitali B (2021) Lactobacilli as anti-biofilm strategy in oral infectious diseases: A mini-review. Front Med Technol 3. https://doi.org/10.3389/fmedt.2021.769172

Gómez NC, Ramiro JMP, Quecan BXV, de Melo Franco BDG (2016) Use of potential probiotic lactic acid bacteria (LAB) biofilms for the control of *Listeria monocytogenes*, *Salmonella* Typhimurium, and *Escherichia coli* O157: H7 biofilms formation. Front Microbiol 7. https://doi.org/10.3389/fmicb.2016.00863

Idrees M, Sawant S, Karodia N, Rahman A (2021) *Staphylococcus aureus* Biofilm: morphology, genetics, pathogenesis and treatment strategies. Int J Environ Res Public Health 18:7602. https://doi.org/10.3390/ijerph18147602

Kadariya J, Smith TC, Thapaliya D (2014) *Staphylococcus aureus* and Staphylococcal food-borne disease: An ongoing challenge in public health. Biomed Res Int 2014. https://doi.org/10.1155/2014/827965

Koohestani M, Moradi M, Tajik H, Badali A (2018) Effects of cell-free supernatant of *Lactobacillus acidophilus* LA5 and *Lactobacillus casei* 431 against planktonic form and biofilm of *Staphylococcus aureus*. Vet Res Forum 9:301–306. https://doi.org/10.30466/vrf.2018.33086

Kroning IS, Iglesias MA, Sehn CP, Valente Gandra TK, Mata MM, da Silva WP (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

Krumperman PH (1983) Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of faecal contamination of foods. Appl Environ Microbiol 46:165–170. https://doi.org/10.1128/aem.46.1.165-170.1983

Lee JE, Lee NK, Paik HD (2021) Antimicrobial and anti-biofilm effects of probiotic *Lactobacillus plantarum* KU200656 isolated from kimchi. Food Sci Biotechnol 30:97–106. https://doi.org/10.1007/s10068-020-00837-0

Leite AMO, Miguel MAL, Peixoto RS, Ruas-Madiedo P, Paschoalin VMF, Mayo B, Delgado S (2015) Probiotic potential of selected lactic acid bacteria strains isolated from Brazilian kefir grains. J Dairy Sci 98:3622–3632. https://doi.org/10.3168/jds.2014-9265

Miao J, Liang Y, Chen L, Wang W, Wang J, Li B, Li L, Chen D, Xu Z (2017) Formation and development of *Staphylococcus* biofilm: With focus on food safety. J Food Saf 37. https://doi.org/10.1111/jfs.12358

Monteagudo-Mera A, Rastall RA, Gibson GR, Charalampopoulos D, Chatzifragkou A (2019) Adhesion mechanisms mediated by probiotics and prebiotics and their potential impact on human health. Appl Microbiol Biotechnol 103:6463–6472. https://doi.org/10.1007/s00253-019-09978-7

O'Gara JP (2007) ica and beyond: Biofilm mechanisms and regulation in *Staphylococcus epidermidis* and *Staphylococcus aureus*. FEMS Microbiol Lett 270:179–188

Ou C, Shang D, Yang J, Chen B, Chang J, Jin F, Shi C (2020) Prevalence of multidrug-resistant *Staphylococcus aureus* isolates with strong biofilm formation ability among animal-based food in Shanghai. Food Control 112:107106. https://doi.org/10.1016/j.foodcont.2020.107106

Patel A, Lindström C, Patel A, Prajapati JB, Holst O (2012) Probiotic properties of exopolysaccharide producing lactic acid bacteria isolated from vegetables and traditional Indian fermented foods. Int J Fermented Foods 1:87–101

Rahman MA, Rahman AA, Islam MA, Alam MM (2018) Multi–drug resistant *Staphylococcus aureus* isolated from milk, chicken meat, beef and egg in Bangladesh. Res Agric Livest Fish 5:175–183. https://doi.org/10.3329/ralf.v5i2.38055

Sengupta K, Paramasivan P (2019) Probiotics and Biofilms. biofilms in human diseases: treatment and control. Springer International Publishing, Cham, pp 263–271

Sharan M, Dhaka P, Bedi JS, Singh R, Mehta N (2023) Characterization of chicken eggs associated *Escherichia coli* and *Staphylococcus aureus* for biofilm production and antimicrobial resistance traits. Anim Biotechnol 34:3533–3544. https://doi.org/10.1080/10495398.2023.2171423

Sharan M, Vijay D, Dhaka P, Bedi JS, Gill JPS (2022) Biofilms as a microbial hazard in the food industry: A scoping review. J Appl Microbiol 133. https://doi.org/10.1111/jam.15766

Song YG, Lee SH (2017) Inhibitory effects of *Lactobacillus rhamnosus* and *Lactobacillus casei* on *Candida* biofilm of denture surface. Arch Oral Biol 76:1–6. https://doi.org/10.1016/j.archoralbio.2016.12.014

Stepanovic S, Vukovic D, Dakic I, Savic B, Svabic-Vlahovic M (2000) A Modified Microtiter-Plate Test for Quantification of Staphylococcal Biofilm Formation. J Microbiol Methods 40:175–179. https://doi.org/10.1016/S0167-7012(00)00122-6

Stepanović S, Vuković D, Hola V, Di BG, Djukić S, Ćirković I, Ruzicka F (2007) Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. APMIS 115:891–899. https://doi.org/10.1111/j.1600-0463.2007.apm_630.x

Strommenger B, Kettlitz C, Werner G, Witte W (2003) Multiplex PCR assay for simultaneous detection of nine clinically relevant antibiotic resistance genes in *Staphylococcus aureus*. J Clin Microbiol 41:4089–4094. https://doi.org/10.1128/JCM.41.9.4089-4094.2003

Tallent S, Hait J, W. Bennett R, A. Lancette G (2019) BAM Chapter 12: *Staphylococcus aureus*. In: Bacteriological Analytical Manual (BAM), 8th edn. https://www.fda.gov/food/laboratory-methods-food/bam-chapter-12-staphylococcus-aureus. Accessed 7 June 2022.

van der Weerden NL, Bleackley MR, Anderson MA (2013) Properties and mechanisms of action of naturally occurring antifungal peptides. Cell Mol Life Sci 70:3545–3570. https://doi.org/10.1007/s00018-013-1260-1

Vasudevan P, Nair MKM, Annamalai T, Venkitanarayanan KS (2003) Phenotypic and genotypic characterization of bovine mastitis isolates of *Staphylococcus aureus* for biofilm formation. Vet Microbiol 92:179–185. https://doi.org/10.1016/S0378-1135(02)00360-7

Vergara A, Normanno G, Di Ciccio P, Pedonese F, Nuvoloni R, Parisi A, Santagada G, Colagiorgi A, Zanardi E, Ghidini S, Ianieri A (2017) Biofilm formation and its relationship with the molecular characteristics of food-related methicillin-resistant *Staphylococcus aureus* (MRSA). J Food Sci 82:2364–2370. https://doi.org/10.1111/1750-3841.13846

Woo J, Ahn J (2013) Probiotic-mediated competition, exclusion and displacement in biofilm formation by food-borne pathogens. Lett Appl Microbiol 56:307–313. https://doi.org/10.1111/lam.12051

Zehra A, Gulzar M, Singh R, Kaur S, Gill JPS (2019) Prevalence, multidrug resistance and molecular typing of methicillin-resistant *Staphylococcus aureus* (MRSA) in retail meat from Punjab, India. J Glob Antimicrob Resist 16:152–158. https://doi.org/10.1016/j.jgar.2018.10.005

Publisher's Note