Prevalence of *icaADBC* genes, and correlation with biofilms and antibiotic resistance in *S. aureus*: a systematic review and meta-analysis

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Abstract

We evaluated the gene prevalence of the *icaADBC* operon, its correlation with biofilm formation and antibiotic resistance through a global meta-analysis. We searched for articles that reported the prevalence of *icaADBC* operon, biofilm, and antibiotic resistance in *S. aureus* from 2000 up to 1st March 2024. The search was done in scientific databases such as PubMed, Scopus, Google Scholar, EMBASE, and Web of Science. The MESH keywords were: *icaADBC* operon, biofilm, methicillin-resistant *Staphylococcus aureus*, antibiotic resistance. Comprehensive Meta-Analysis Software was used for data analysis. The estimation of the combined prevalence of each desired variable was performed by depicting a forest plot through the random-effects model with a 95% confidence interval. Data heterogeneity was estimated by Q and I^2 indices, and p-value <0.05 was reflected as statistically significant heterogeneity.

Fifteen articles were eligible for inclusion. The prevalence of *ica* operon genes varied between 28-51.5%. The prevalence of total *ica* operon genes in *S. aureus* was reported at 42.4% (95%CI: 29.4-56.5). Biofilm formation prevalence of *S. aureus* isolates in different studies was reported between 10-100%. The rate of total biofilm in *S. aureus* was 95.8%. The rate of total strong, moderate, and weak biofilm in *S. aureus* was reported at 35.4%, 35.3%, and 23.9%, respectively. Most reviewed studies reported a correlation between *ica* genes and biofilm.

We found that many studies reported a correlation between the high prevalence of *ica* operon genes, phenotypic biofilm production, and antibiotic resistance. Also, regardless of whether the strains were MRSA or not, the high biofilm formation ability was reported at 95.8% by most studies.

Keywords ***Staphylococcus aureus*, biofilms, antibiotic resistance, methicillin-resistant *Staphylococcus aureus*.**

Introduction

Staphylococcus aureus is known as an important pathogen in hospitals. The spread of infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) strains in all health-treatment centers of the world, especially vital units such as the intensive care unit (ICU) and the respiratory unit, has been reported.[1] Today, widespread and increasing resistance to the antibiotics available to treat infections caused by this microorganism has reached its peak, which

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depicts a difficult future for infections caused by MRSA strains.[2]

From the pathogenic aspects of this microorganism, we can mention the extraordinary ability to form biofilm outside and inside the human body in different infections.[3] This high ability to produce biofilm has led to the spread of resistance against current antibiotics and disinfectants.[4,5]

Attachment to host cells is the primary stage necessary for the initiation and establishment of infection. The organization of *Staphylococcus aureus* is facilitated by the expression of multiple proteins and adhesins, notably the microbial surface components that identify adhesive matrix molecules (MSCRAMMs), enabling binding to laminin, fibrinogen, fibronectin, and collagen.[6] Once the initial attachment is established, the biofilm forms and expands through proliferation and extracellular matrix production. Extracellular matrix components such as polysaccharide extracellular adhesions, extracellular DNA, proteins, and teichoic acids contribute to biofilm formation.[7] Also, *S. aureus* provides biofilm thickening through the synthesis of polysuccinyl glucosamine. All these steps are regulated and controlled by *icaADBC* operon specifically *icaD* gene.[8] The *icaA* gene encodes the enzyme N-acetyl-glucosaminyl transferase, which plays a crucial role in the synthesis of N-acetyl-glucosamine oligomers derived from UDP-N-acetyl-glucosamine. The complete phenotypic manifestation of the capsular polysaccharide is associated with the *icaD* gene.[9] The produced polysaccharide wraps around the bacterial cells, functioning as a shield against the immune system of the host and the action of antimicrobial agents.[10]

The involvement of the *icaADBC* operon in biofilm development and its link to antibiotic resistance has been convincingly demonstrated in various research efforts. However, there is currently no extensive review that integrates all these foundational studies. This global systematic review and meta-analysis intends to fill this gap by thoroughly examining the relationship between the *icaADBC* operon, biofilm production, and antibiotic resistance.

Methods

Search plan

The search plan was based on articles that reported the prevalence of the *icaADBC* operon, biofilm, and antibiotic resistance in *S. aureus* from 2000 up to 1st March 2024. The search was done in scientific databases such as PubMed, Scopus, Google Scholar, EMBASE, and Web of Science. The MESH keywords were: *icaADBC* operon, biofilm, biofilm formation, *S. aureus*, and methicillin-resistant *Staphylococcus aureus*, *S. aureus*, MRSA, antibiotic resistance, antimicrobial resistance, *ica* genes.

Eligibility criteria

The studies that reported the prevalence of biofilm-related genes, biofilm, and the relationship with microbial resistance, from clinical samples between 2000 up to 1st March 2024, were included in our analysis. Studies on non-clinical strains (environmental and animal) were excluded from our study. Systematic review studies, case controls, and clinical trials were excluded from the current review.

Quality assessment

To determine the quality of the studies, a checklist of Appraisal tools for Cross-Sectional Studies (AXIS) was used,[11] in which 20 different questions were asked about varied parts of the study, from the title to the conclusion, and finally, studies characterized by weaknesses were omitted (Supplementary 1).

Data extraction

In this review, our authors independently used a series of designed forms that contained information such as first author, publication time, first name, year of study, year of publication, type of study, location, sample size, *S. aureus* isolates, *ica* gene prevalence, molecular methods for detecting genes, biofilm formation, methods for measuring biofilm. The extracted data were entered into these forms.

Data analysis

For the purpose of data analysis, Comprehensive Meta-Analysis software (version

3.3.070) was employed. The combined prevalence of the targeted variables was estimated by constructing a forest plot utilizing a random-effects model, which provided a 95% confidence interval (CI). Heterogeneity among the data was measured using the Q and I^2 indices, with a p value of less than 0.05 denoting statistically significant heterogeneity. To assess potential publication bias, both Egger's linear regression test and a funnel plot were utilized.

Results

Among the 417 articles retrieved from the different databases, there were 201 duplicates that were deleted. Records were screened. A series of articles were removed and the rest of the articles were evaluated for retrieval. At this stage again, with the exclusion of several articles, the eligibility of other studies was checked, and finally, due to the reasons mentioned in Figure 1, only 15 articles were eligible to be included in this review. Included studies used standard techniques for biofilm evaluation such as microtiter plate (MTP), Tissue culture plate (TCP), and Congo Red Agar (CRA). All the studies included here, used PCR for the detection of *ica* operon genes (Table 1).

Prevalence of total *ica* operon genes in *S. aureus*

The prevalence of *ica* operon genes varied between 28.0% and 51.5%. The prevalence of total *ica* operon genes in *S. aureus* was reported at 42.4% (95%CI: 29.4-56.5), Z=1.06, p=0.280. The frequencies of *icaA*, *icaB*, *icaD* and *icaA/D* were reported 38.4% (95%CI: 21.0-59.3), 65.5% (95%CI: 57.6-72.6), 67.8% (95%CI: 52.6-79.9), and 47.7% (95%CI: 22.8-73.9), respectively (Supplementary 2a, b, c, d, e and Table 2). The prevalence of *icaA* gene varied (between 3.1% and 80%), *icaB* (between 52.0% and 68.8%), *icaD* (between 38.5% and 91.5%), and *icaA/D* (between 3.8% and 74.2%).

Total biofilm formation rate in *S. aureus* strains

The prevalence of biofilm formation by *S. aureus* isolates in different studies was reported between 10-100% (Table 3). The rate of total biofilm in *S. aureus* was 95.8% (95%CI: 88.2-98.6), Z=5.4, p<0.001. The rates of total strong, moderate, and weak biofilm in *S. aureus* were reported at 35.4% (95%CI: 20.4-53.9), 35.3% (95%CI: 24.6-47.7), and 23.9% (95%CI: 11.7-42.6), respectively (Supplementary 3a, b, c, d and Table 2). The rate of total biofilm in MRSA strains was reported at 95.4% (95%CI: 83.6-98.8), Z=4.23, I^2=90.9, p<0.001 (Tables 2 and 4). The rate of total strong, moderate and weak biofilm in MRSA strains was 26.9% (95%CI: 15.3-42.9), 33.5% (95%CI: 23.2-45.8), and 30.6% (95%CI: 19.0-45.3), respectively (Tables 2 and 4). The rates of total biofilm in MSSA isolates were reported at 25.1% (95%CI: 14.4-40.1), 50.0% (95%CI: 36.9-63.2), and 37.5% (95%CI: 14.4-68.1), respectively (Tables 2 and 4).

Correlation between the prevalence of these genes with biofilm formation and antibiotic resistance

Most studies encompassed in this review have indicated a connection between *ica* genes and biofilm, regardless of the extent of biofilm development. Certain studies have established a link between robust biofilm production and increased antibiotic resistance, whereas other investigations have found no such association. They concluded that the ability to form biofilm, regardless of its degree, can increase antibiotic resistance.

Heterogeneity analysis and publication bias among the studies included

The heterogeneity indices reported for total biofilm were as follows: Q2=180.3, I^2=91.2, and t=5.2, with a significance level of p<0.001. A visual assessment of the funnel plot concerning *ica* operon genes indicated the presence of publication bias, which was further corroborated by the Egger regression test yielding p<0.001. Conversely, the funnel plot analysis for *ica* operon genes also suggested publication bias among the reports (Figure 2a), while the Egger regression test indicated no such bias (p=0.790). Additionally, both the funnel plot and the Egger regression test confirmed the presence of publica-

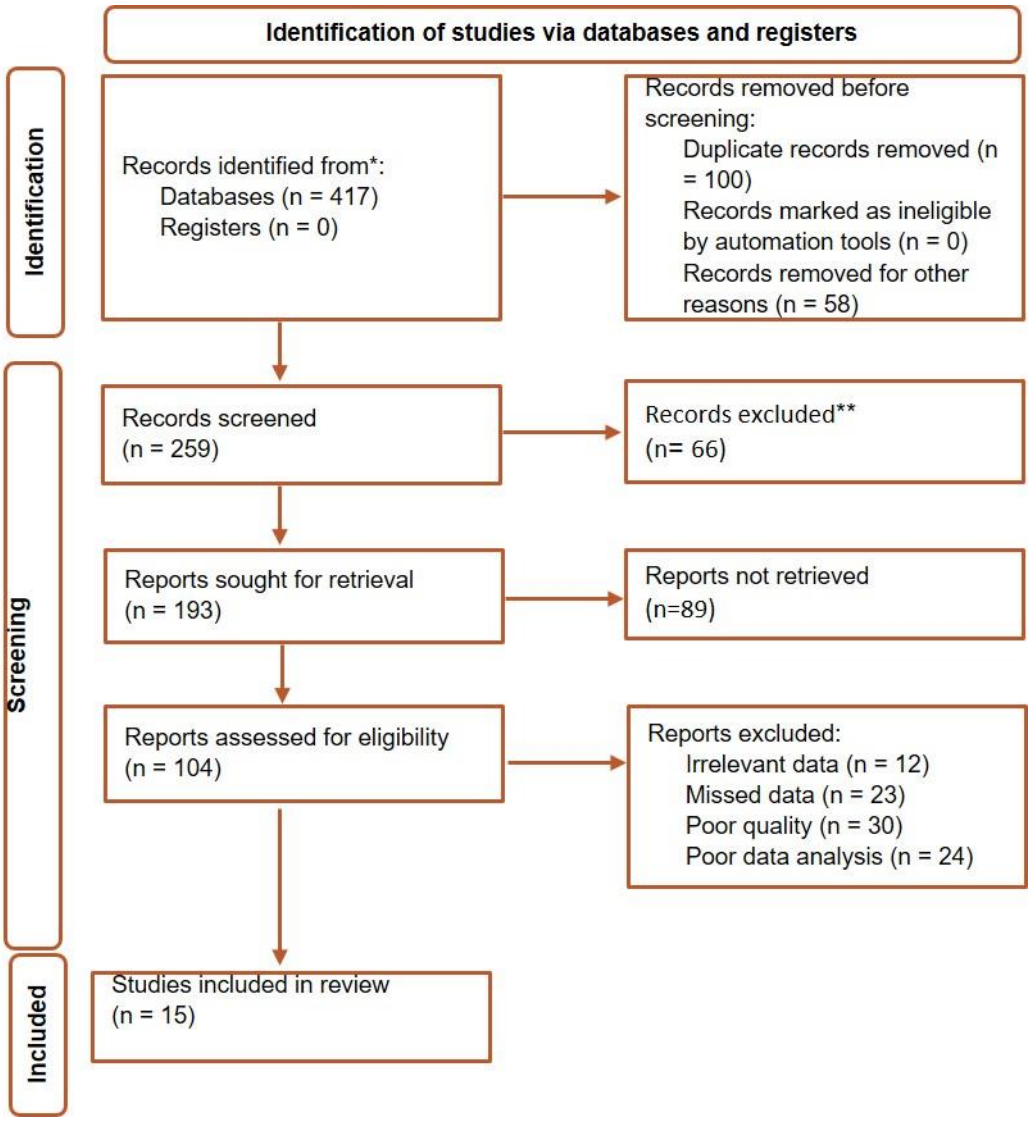

Figure 1. Flow diagram for selection of studies included in this review

Table 1. *Characteristics* of included studies in the present review

Study	Year of study	Publication	Location	Samples	*S. aureus* isolates	Biofilm total (n)	MSSA biofilm (n)	MRSA biofilm (n)	Methods for biofilm	Molecular methods	*ica* genes n/%	*icaA* gene	*icaB* gene	*icaA/D*	*icaD*
Šmitran et al.[12]	2019-2020	2022	Bosnia and Herzegovina	363	60	53	29	24	MTP	PCR	30	-	-	-	-
Shafi et al.[13]	2019-2020	2022	Egypt	66	46	46	-	-	MTP	PCR	-	9	10	-	-
Manandhar et al.[14]	2017-2018	2021	Nepal	375	161	84	14	70	TCP	PCR	45	-	-	-	-
Shivaee et al.[15]	-	2019	Iran	-	100	100	50	50	MTP	Real-time PCR	-	-	-	-	-
Babra et al.[16]	-	2013	Australia	-	31	31	-	-	CRA/TCP	PCR	-	-	-	23	-
El-Mahallawy et al.[17]	-	2009	Egypt	-	10	6	-	-	CRA	-	-	5	-	-	-
Jasińska et al.[6]		2021	Poland	-	26	7	5	2	MTP	PCR	11	-	-	1	10
dos Santos-Goes et al.[18]	2017	2021	Brazil	63	59	59	-	-	CRA	PCR	-	38	-	35	54
Azmi et al.[19]	2015-2018	2019	Palestinian	-	248	248	-	248	MTP	PCR	-	41	-	248	207
Bimanand et al.[20]	2012-2013	2018	Iran	-	96	92	-	92	MTP	PCR	-	15	66	-	66
Piechota et al.[21]	2015-2017	2018	Poland		130	129	57	72	MTP	PCR	67	4	-	20	-
Mollaahmadi et al.[22]	-	2021	Iran	80	39	39	-	39	CRA	PCR	-	25	25	-	25
Yousefi Nojookambari et al.[8]	2015-2016	2018	Iran	-	85	85	-	45	CRA/TCP	PCR	-	45	-	-	45
Oufrid et al.[23]	-	2015	Morocco	-	20	18	-	-	MTP	PCR	-	16	-	-	11
Manandhar et al.[24]	-	2018	Nepal	-	161	161	30	131	CRA, TM, and TCP	PCR	-	-	-	45	-

CRA – *Congo Red Agar*; **MRSA** – methicillin-resistant *Staphylococcus aureus*; **MSSA** – methicillin-susceptible *Staphylococcus aureus*; **MTP** – microtiter plate; **TCP** – tissue culture plate; **TM** – tube method.

Table 2. Subgroups analysis for different variables in the present review

S. aureus	**No. studies**	**Heterogeneity test**			**Egger's test**		**Random model**		
		Prevalence % (95%CI)	Z	p	Q	p	I^2	T	p
Total biofilm	15	95.8 (88.2-98.6)	5.46	<0.001	160.3	<0.001	91.2	5.2	<0.001
Strong biofilm	8	35.4 (20.4-53.9)	1.5	0.120	90.6	<0.001	92.2	0.39	0.700
Moderate biofilm	6	35.3 (24.6-47.7)	2.3	0.021	25.7	<0.001	80.6	0.39	0.780
Weak biofilm	7	23.9 (11.7-42.6)	2.6	0.008	87	<0.001	93.1	2.3	0.060
MSSA isolates									
Total biofilm	6	90.7 (59.3-98.5)	2.3	0.019	43	<0.001	88.4	4.3	0.012
Strong biofilm	5	25.1 (14.4-40.1)	3	0.002	9.6	<0.001	58.6	8.5	0.003
Moderate biofilm	4	50.0 (36.9-63.2)	0.004	0.990	6.2	0.100	51.9	12.29	0.006
Weak biofilm	4	37.5 (14.4-68.1)	0.79	0.430	24.4	<0.001	87.7	1.2	0.320
MRSA isolates									
Total biofilm	10	95.4 (83.6-98.8)	4.23	<0.001	99.1	<0.001	90.9	4.1	0.003
Strong biofilm	9	26.9 (15.3-42.9)	2.7	<0.001	94.9	<0.001	91.5	0.04	0.960
Moderate biofilm	8	33.5 (23.2-45.8)	2.6	0.009	61.1	<0.001	88.5	1.7	0.130
Weak biofilm	8	30.6 (19-45.3)	2.5	0.011	85.1	<0.001	91.7	1	0.320
ica operon genes									
Total *ica* genes	4	42.4 (29.4-56.5)	1.06	0.280	18.89	<0.001	84.1	0.29	0.800
icaA	9	38.4 (21.0-59.3)	1	0.270	138.7	<0.001	94.2	7	0.530
icaB	3	65.5 (57.6-72.6)	3.7	<0.001	1.84	0.390	0.00	1	0.130
icaD	7	67.8 (52.6-79.9)	2.2	0.023	55.7	<0.001	89.2	5	0.510
icaA/D	6	47.7 (22.8-73.9)	0.15	0.870	83.7	<0.001	94	0.91	0.410

MRSA – methicillin-resistant *Staphylococcus aureus*; **MSSA** – methicillin-susceptible *Staphylococcus aureus*.

Table 3. Total biofilm producing isolates of *S. aureus*

Study	***S. aureus* isolates, n**	**Biofilm formation**			
		Strong	**Moderate**	Weak	**Non-biofilm**
S. Abdel-Shafi	46	19	15	12	0
C. Babra	31	14	15	2	0
E. Jasińska	26	2	-	-	24
M. Piechota	130	48	64	17	1
M. Mollaahmadi	39	27	6	6	0
N. Yousefi Nojookambari	85	57	-	28	0
S. Oufrid	20	4	9	5	2
S. Manandhar	161	19	42	100	0
Total	538	35.4%	35.3%	23.9%	3.1%

Table 4. Biofilm production in both MSSA and MRSA isolates

Study	*S. aureus* isolates	Biofilm formation									
		MSSA					MRSA				
		Strong	Moderate	Weak	Non-biofilm	Total biofilm	Strong	Moderate	Weak	Non-biofilm	Total biofilm
A. Smitran	60	0	15	14	1	30	1	5	18	6	30
A. Shivaee	100	16	23	7	-	56	19	26	9	0	54
E. Jasińska	26	5	-	-	-	5	2	-	-	-	2
K. Azmi	248	-	-	-	-	-	52	115	81	-	248
L. Bimanand	96	-	-	-	-	-	4	54	34	4	92
M. Piechota	130	21	26	10	0	57	29	35	8	1	73
M. Mollaahmadi	39	-	-	-	-	-	27	6	6	0	41
N. Yousefi Nojookambari	85	-	-	-	-	-	27	8	10	0	45
S. Manandhar	161	1	15	14	-	30	18	27	86	-	131

MRSA – methicillin-resistant *Staphylococcus aureus*; **MSSA** – methicillin-susceptible *Staphylococcus aureus*.

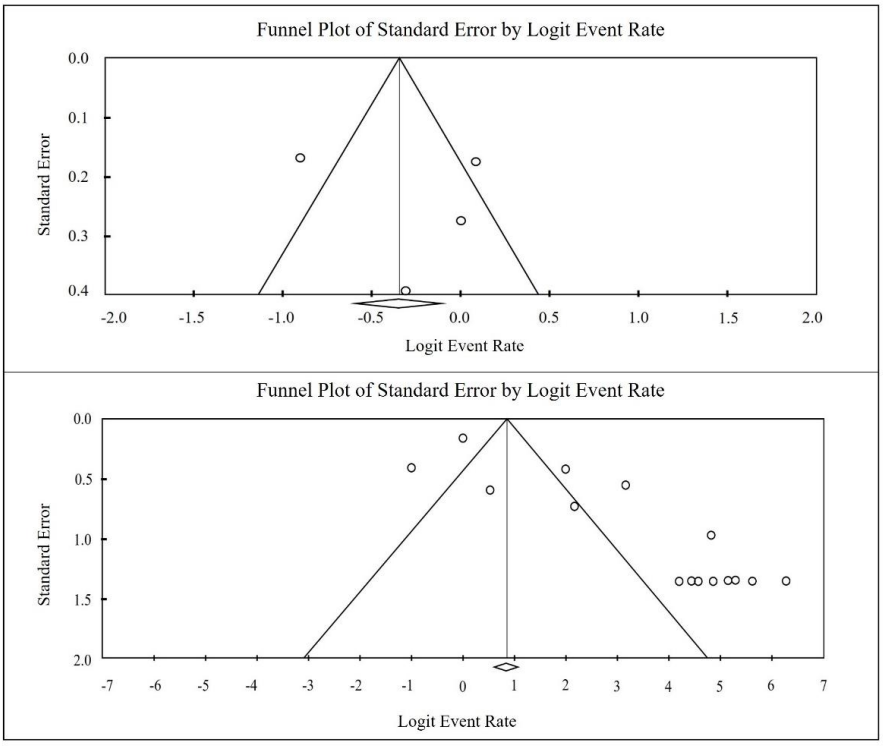

Figure 2. Funnel plot of meta-analysis on the *ica* gene prevalence (top image), and biofilm formation (below image) in S. *aureus* isolated from clinical samples

tion bias related to biofilms (Fig. 2b), with a significance level of $p<0.001$.

Sensitivity analyses

To perform this test, the studies with both the highest and lowest sample sizes were omitted from the analysis, and a new meta-analysis was conducted. The results indicated that the original findings remained consistent.

Discussion

Data obtained from this review showed that the prevalence of *ica* operon genes varied between 28.0-51.5%, while the prevalence of total *ica*

operon genes in *S. aureus* was reported at 42.4%. The combined prevalence of around 42.0% of *ica* genes indicates that the biofilm formed by *S. aureus* is not only dependent on the presence of these genes but other mechanisms independent of these genes are involved. In line with this fact, Piechota et al.[21] reported that although the prevalence of *icaABCD* genes was in a small percentage of MSSA isolates, there was no significant correlation between the existence of strong, moderate, poor biofilm types, and the strains that harbored these genes.

Biofilm prevalence of *S. aureus* isolates in different studies was reported between 10%-100%, while the rate of combined biofilm rate in *S. aureus* strains was 95.8%. This high rate of combined biofilm production in either MSSA or MRSA strains shows the importance of biofilm in the pathogenesis of *S. aureus*, even though methicillin-resistant strains use this biological-biofilm layer to prevent antibiotic penetration inside bacteria.

An interesting finding obtained from these studies was that apart from whether the strains were resistant to methicillin (MRSA) or not, the high biofilm formation ability has been reported by most reports except the study conducted by Jasińska et al.[6] It should be mentioned that the reports of some studies indicated a high rate of biofilm production in methicillin-sensitive strains (MSSA) compared to MRSA strains.[12,24] Furthermore, some research findings have shown that there is not a notable difference in biofilm when comparing MSSA and MRSA,[8,14,24] but this was the opposite regarding the prevalence of strains containing *ica* genes, so MRSA strains showed a higher rate.[24] S. Oufrid et al.[23] reported that 100% of the strains with *ica* genes were also biofilm producers. Similar data has been presented by dos Santos-Goes et al.,[18] where using PCR technique, more than 91% of the isolates had *icaD* gene and the simultaneous presence of *icaD* and *icaA* genes was reported in about 59%, which showed the role of these genes for creating biofilm because 100% of them were biofilm producers. Furthermore, findings from a study carried out by Azmi et al.[19] indicated that all strains harboring the *icaD/icaA* genes were associated with biofilm formation, which indicates a correlation between the existence of these genes and phenotypic biofilm. Manandhar et al.[24] showed that MRSA strains were resistant to most antibiotics, and the biofilm was also higher in these isolates. Meanwhile, strong biofilm producers displayed more drug resistance. Besides, the biofilm-producing strains that harbor *icaAD* genes recorded higher drug resistance compared to the planktonic counterparts.[24] El-Mahallawy et al.[17] reported that 92% of the *ica*-gene-positive strains were biofilm positive and 88% of the *ica*-gene-negatives were biofilm-producing negative.

In contrary, Jasińska et al.[6] have shown that a small number of biofilm producers harbored *ica* gene, and no noteworthy correlation was observed between *ica* gene, biofilm, and methicillin resistance, but Bimanand et at.[20] reported a substantial relationship between *ica* gene and biofilm, but this was not significant between the biofilm and drug resistance. Shivaee et al.[15] pointed out the high expression of *icaA/D* genes in both MSSA and MRSA. Reports from Shafi et al.[13] and Babra et al.[16] highlight that, apart from the type of biofilm (strong, moderate, and weak), biofilm producers have a higher level of resistance.

Of course, it is worth mentioning that this difference in the prevalence of the *ica* operon genes and the amount of biofilm formed by the isolates depends on the type of clinical samples (source), geographical region, molecular techniques used for gene detection, phenotypic method of biofilm detection and many other factors.

The analysis of the studies incorporated in this meta-analysis revealed a notable correlation between biofilm-associated genes and both biofilm development and antibiotic resistance. This finding underscores the critical role of biofilms in fostering resistance, as they create a protective biological layer that hinders the effective penetration of antibiotics and disinfectants into the microorganisms. Microorganisms residing within biofilms exhibit distinct characteristics compared to their planktonic forms;[25] they typically demonstrate

enhanced resistance against adverse conditions, including exposure to chemical biocides, bacteriophages, antibiotics, and antibodies.[26] Consequently, it is imperative to implement various strategies aimed at inhibiting biofilm formation, such as modifying the properties of abiotic surfaces to deter biofilm establishment, manipulating signaling pathways to suppress biofilm development and promote dispersal, and employing external forces to eliminate existing biofilms.[27]

Regardless of the MRSA status of the strains surveyed or their capacity for biofilm production (whether strong, moderate, or weak), biofilm-producing strains consistently exhibited greater antibiotic resistance. Thus, prioritizing the eradication and prevention of biofilm formation is essential in managing clinical microbial infections. Future research should delve into the interplay between biofilm formation, the *ica* operon, and its related genes across various bacterial species. Additionally, investigations should be conducted to explore the connections between strains harboring these genes and biofilm-forming strains that exhibit resistance to critical antibiotics, such as vancomycin and teicoplanin, which are commonly employed against multidrug-resistant (MDR) and MRSA infections.

Heterogeneity occurs when a collection of studies that are closely related or conceptually aligned on a specific topic produce results that significantly diverge from what would typically be expected due to sampling error. This situation suggests that the observed differences are not simply random variations; rather, they point to fundamental discrepancies in the data, methodologies, participant characteristics, interventions, and outcomes utilized across the studies. In light of this heterogeneity in our study, a random-effects meta-analysis was employed to synthesize the findings. In the context of heterogeneous studies, a random-effects meta-analysis assigns greater weight to smaller studies compared to a fixed-effect meta-analysis. This approach is justified because smaller studies provide more valuable insights into the variability of effects across the research landscape, rather than merely contributing to an assumption of a uniform intervention effect. Consequently, this method allows for a more nuanced understanding of the data.

To further explore the sources of heterogeneity, publication bias was assessed using funnel plots and the Egger regression test, which corroborated the presence of heterogeneity among the studies included in the analysis. To mitigate the impact of this heterogeneity on the results of the meta-analysis, subgroup analyses were conducted, categorizing studies based on the strength of biofilm formation-strong, moderate, and weak. This stratification aimed to clarify the effects observed and enhance the robustness of the findings.

Findings from this review indicated a notable presence of publication bias among the studies included. To assess this further, a visual examination of the funnel plot was conducted alongside the Egger regression test. The existence of publication bias can skew the results of meta-analyses by exaggerating effect sizes, thus highlighting the importance of its identification and correction. Funnel plots and Egger's Test are effective in detecting such biases, while the trim-and-fill method is used for their correction; nonetheless, these methods have inherent limitations, making sensitivity analyses imperative. In order to validate the results, a sensitivity analysis was performed, revealing no significant changes in the findings of this meta-analysis.

Conclusions

Our findings showed that many studies reported a correlation between the high prevalence of *ica* operon genes, phenotypic biofilm production, and antibiotic resistance. Also, regardless of whether the strains were MRSA or not, the high biofilm formation ability was reported at about 95.8% by most studies. Although some reported that certain MRSA strains exhibit a notably high capacity for biofilm formation, others did not consider such a difference between MRSA and MSSA.

Author contributions statement: KB, MP and RHA undertook the collection and analysis of the data and

completed the background literature review for the manuscript. The laboratory experiments, statistical analyses, and manuscript drafting were executed by MRJA, ZSF, ZG, RM and AK. All authors have read and approved the final manuscript version.

Conflicts of interest: All authors – none to declare.

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Availability of data: The data supporting the findings of this study are available within the article [Figures/Tables and supplementary materials].

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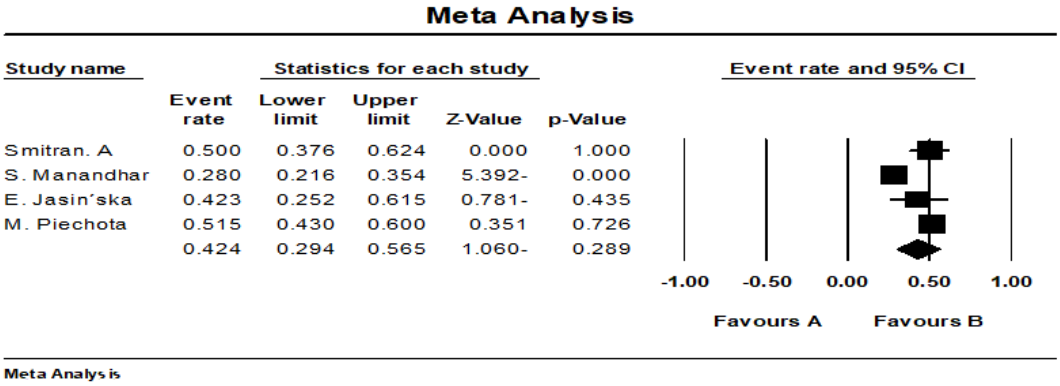
Supplementary 1. The Joanna Briggs Institute Prevalence Critical Appraisal Tool

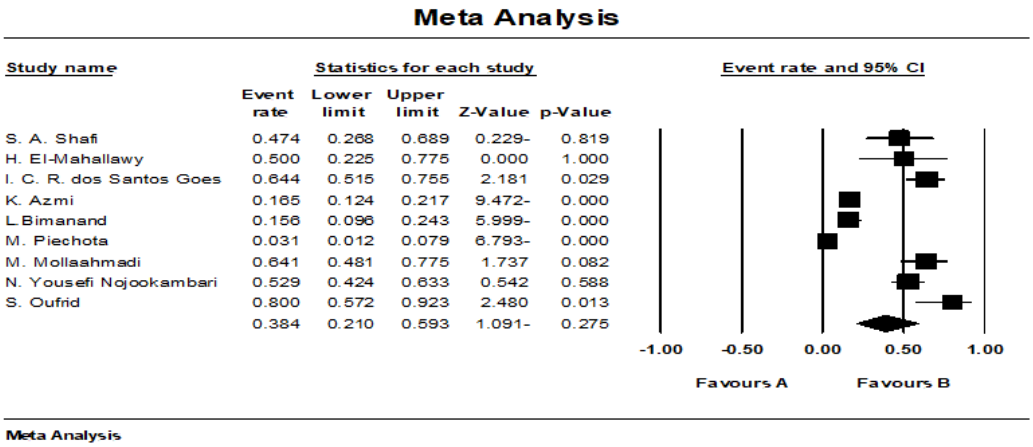
Study	1. Was the sample frame appropriate to address the target population?	2. Were study participants sampled in an appropriate way?	3. Was the sample size adequate?	4. Were the study subjects and the setting described in detail?	5. Was the data analysis conducted with sufficient coverage of the identified sample?	6. Were valid methods used for the identification of the condition?	7. Was the condition measured in a standard, reliable way for all participants?	8. Was there appropriate statistical analysis?	9. Was the response rate adequate, and if not, was the low response rate managed appropriately?	Overall
Šmitran. A	y	Y	Y	Y	Y	Y	Y	Y	Y	Included
S. A. Shafi	y	Y	Y	Y	Y	Y	Y	Y	Y	Included
S. Manandhar	y	Y	Y	Y	Y	Y	Y	Y	Y	Included
A. Shivaee	y	Y	Y	Y	Y	Y	Y	Y	Y	Included
C. Babra	y	Y	Y	Y	Y	Y	Y	Y	Y	Included
H. El-Mahallawy	y	Y	Y	Y	Y	Y	Y	Y	Y	Included
E. Jasin´ska	y	Y	Y	Y	Y	Y	Y	Y	Y	Included
I. C. R. dos Santos Goes	y	Y	Y	Y	Y	Y	Y	Y	Y	Included
K. Azmi	y	Y	Y	Y	Y	Y	Y	Y	Y	Included
L. Bimanand	y	Y	N	Y	N	Y	Y	Y	Y	Included
M. Piechota	y	Y	Y	Y	Y	Y	Y	Y	Y	Included
M. Mollaahmadi	y	Y	Y	Y	Y	Y	Y	Y	Y	Included
N. Yousefi Nojookambari	Y	Y	N	Y	Y	Y	Y	Y	Y	Included
S. Oufrid	y	Y	Y	Y	Y	Y	Y	Y	Y	Included
S. Manandhar	y	Y	Y	Y	Y	Y	Y	Y	Y	Included

Y – yes; **N** – no; **U** – unclear; **N/A** – not applicable.

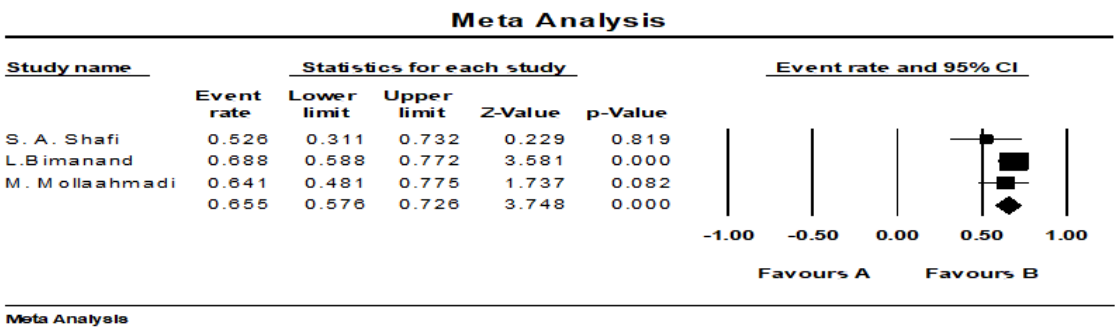
Supplementary 2a. Forest plot of the meta-analysis of the combined *ica* gene prevalence in *S. aureus* retrieved from clinical samples



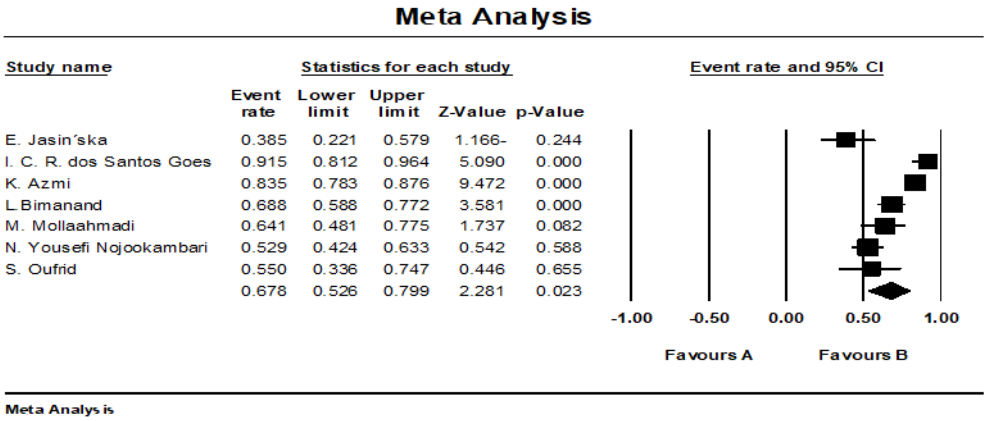
Supplementary 2b. Forest plot of the meta-analysis of the *icaA* gene prevalence in *S. aureus* retrieved from clinical samples



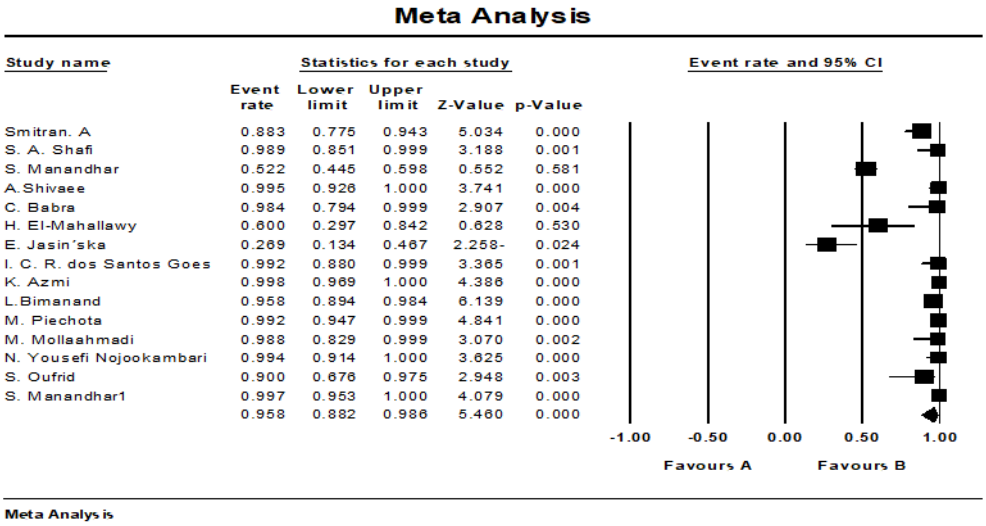
Supplementary 2c. Forest plot of the meta-analysis of the *ica*B gene prevalence in *S. aureus* retrieved from clinical samples



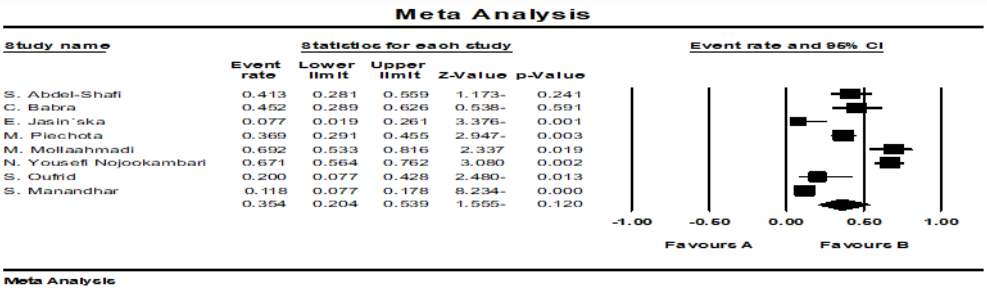
Supplementary 2d. Forest plot of the meta-analysis of the *ica*D gene prevalence in *S. aureus* retrieved from clinical samples



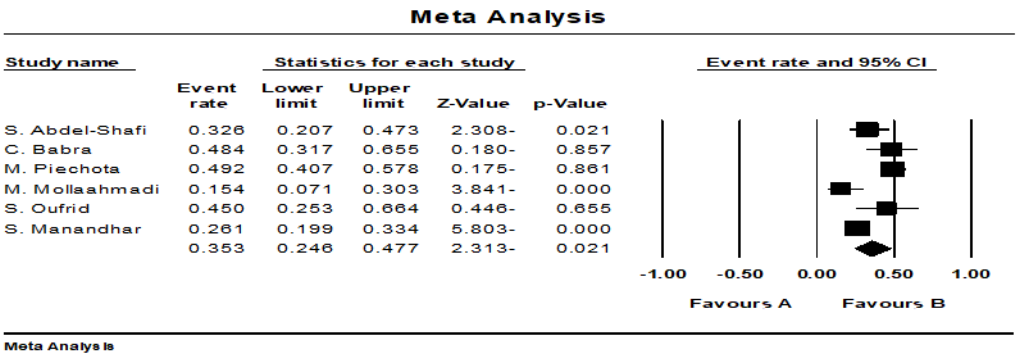
Supplementary 3a. Forest plot of the meta-analysis of the total biofilms in *S. aureus* isolated from clinical samples



Supplementary 3b. Forest plot of the meta-analysis of the total strong biofilm in *S. aureus* isolated from clinical samples



Supplementary 3c. Forest plot of the meta-analysis of the Total moderate biofilm in *S. aureus* isolated from clinical samples



Supplementary 3d. Forest plot of the meta-analysis of the total weak biofilm in *S. aureus* isolated from clinical samples