

REVIEW



## Safety of *Staphylococcus aureus* four-antigen and three-antigen vaccines in healthy adults: A meta-analysis of randomized controlled trials

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### ABSTRACT

**Purpose:** Two new *Staphylococcus aureus* vaccines, *S. aureus* four-antigen (SA4Ag) and three-antigen (SA3Ag) vaccines, have good immunogenicity and tolerance. However, the safety of these vaccines is worth exploring. Here, we performed a meta-analysis to investigate the safety of SA3Ag and SA4Ag by evaluating systemic and local adverse events.

**Methods:** The Medline, EMBASE, and Cochrane databases were searched for randomized clinical trials confirming the safety of SA4Ag and SA3Ag. Two investigators independently selected suitable trials, assessed trial quality, and extracted data.

**Results:** Three studies comprising a total of 1,148 participants were included in this review. The two *S. aureus* vaccines did not increase systemic adverse events (relative ratio 1.1 [95% confidence interval 0.98, 1.24]), but increased the incidence of local adverse events (2.89 [2.15, 3.90]). However, the incidence of severe local adverse events (4.06 [0.78, 21.24]) did not rise significantly.

**Conclusions:** SA4Ag and SA3Ag have acceptable safety in adults.

### ARTICLE HISTORY

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### KEYWORDS

local adverse events;  
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## Introduction

*Staphylococcus aureus* is a leading cause of infection-related morbidity and mortality<sup>1–4</sup> and the most common cause of postoperative infection.<sup>5–7</sup> Erythromycin, penicillin, gentamicin, and vancomycin are typically used for the treatment of *S. aureus* infection. However, due to the abuse of antibiotics, new multidrug-resistant strains have emerged, thus hindering the management of *S. aureus*. Therefore, the study of *S. aureus* vaccines has once again become a pressing issue.

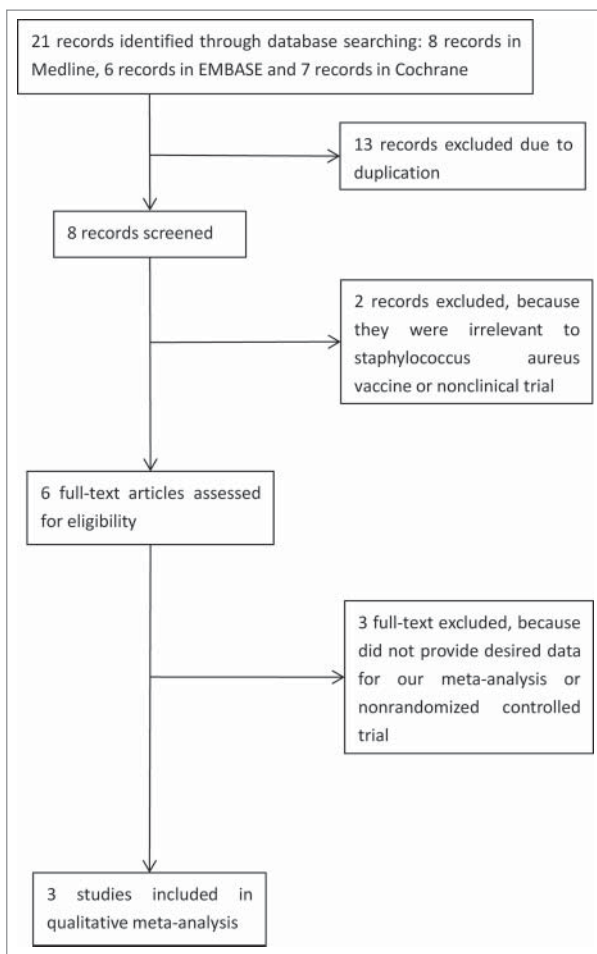
In 1981, Watson et al. created inactivated and live attenuated *S. aureus* vaccines; however, these *S. aureus* vaccines were whole-cell bacterial vaccines that contain many unrelated and toxic components besides the effective antigen.<sup>8</sup> Thus, vaccine toxicity is high and clinical application is limited. The complexity of the virulence mechanism of *S. aureus* poses challenges for the development of prophylactic vaccines. From the experience of previous failures, a successful prophylactic *S. aureus* vaccine must address a combination of well-conserved virulence factors expressed by most strains and generate antibodies that effectively kill the organism.<sup>9–14</sup> Recently, researchers have successfully developed two kinds of multi-antigen vaccine candidates, namely *S. aureus* three-antigen (SA3Ag) and *S. aureus* four-antigen (SA4Ag) vaccines. The former includes bacterial capsular polysaccharide type 5 (CP5), CP8, and clumping factor A (ClfA), and the latter comprises CP5, CP8, ClfA, and recombinant P305A (rP305A).<sup>15–20</sup> SA3Ag and SA4Ag exhibit superior immunogenicity than previous vaccines. Former

candidate vaccines have only demonstrated the ability to generate anti-staphylococcal binding antibodies using enzyme-linked immunosorbent assays and bacterial uptake by phagocytic cells, while SA3Ag and SA4Ag can induce high levels of antibodies to kill *S. aureus* in opsonophagocytosis assays.<sup>9,12</sup> However, Frenck et al. observed systematic adverse events (SAE) in participants who had been vaccinated with SA4Ag, including remarkable diarrhoea.<sup>16</sup> Related studies<sup>16–18,20</sup> also showed that participants had significant local adverse events (LAE) after two SA4Ag or SA3Ag inoculations, such as redness, swelling, and injection site pain and some LAE were severe. Therefore, the purpose of the present study was to identify and combine all relevant published clinical randomized controlled trials (RCTs) to investigate the safety of SA4Ag and SA3Ag in healthy adults.

## Results

### Literature search

The literature search identified 8 records of clinical trials in Medline, 6 records in EMBASE, and 7 records in the Cochrane databases (Fig. 1). After checking for duplicates, 8 unique references remained; 2 were excluded for reasons such as irrelevance to *S. aureus* vaccine or describing a nonclinical trial<sup>21,22</sup> and the remaining 6 full texts underwent further evaluation. Among these, 3



**Figure 1.** Flow diagram of study search and selection

articles<sup>15,18,19</sup> were excluded as they did not provide the necessary data for our meta-analysis.

### Types of vaccine injected

Of the three included studies, one [17] involved participants receiving one of three dose levels (low, mid, high) of SA4Ag or one dose level of SA3Ag or placebo. In this study, the same intervention group with multiple doses was recommended for data consolidation; however, to observe potential differences between the two vaccines, the data were equally divided into two subgroups, according to the Cochrane Handbook.<sup>23</sup> One<sup>16</sup> involved participants receiving one of three dose levels (low, mid, high) of SA4Ag or placebo. Data from the same intervention group with multiple doses were

consolidated. Another study<sup>20</sup> established two age strata, each corresponding to one of three dose levels of SA3Ag or placebo. Thus, this study was considered as two results because of the independence of the two participant groups (Table 1).

### S.aureus vaccine protocols

In one study<sup>17</sup>, three dose levels (low, mid, high) of SA4Ag were evaluated; each dose level included 30  $\mu$ g CP5-CRM<sub>197</sub>, 30  $\mu$ g CP8-CRM<sub>197</sub>, and 60  $\mu$ g recombinant surface protein clumping factor A (rmClfA) and one of three dose levels of rP305A (low, 20  $\mu$ g; mid, 60  $\mu$ g; or high, 200  $\mu$ g). The SA3Ag comparator vaccine consisted of the same fixed-dose levels of 30  $\mu$ g CP5-CRM<sub>197</sub>, 30  $\mu$ g CP8-CRM<sub>197</sub>, and 60  $\mu$ g rmClfA without rP305A. Study vaccine or placebo was administered as a single 0.5-mL intramuscular injection into the deltoid muscle. In another study<sup>16</sup>, three dose levels of SA4Ag were evaluated, each containing the same fixed-dose levels of 30  $\mu$ g CP5-CRM<sub>197</sub>, 30  $\mu$ g CP8-CRM<sub>197</sub>, and 60  $\mu$ g rmClfA plus one of three dose levels of rP305A (low, 20  $\mu$ g; mid, 60  $\mu$ g; or high, 200  $\mu$ g) per 0.5-mL dose. SA4Ag was administered as a non-adjuvanted, lyophilized vaccine reconstituted with 60 mM sodium chloride prior to administration. Study vaccine or placebo was administered as a single 0.5-mL intramuscular injection into the deltoid muscle of the non-dominant arm. In another study<sup>20</sup>, SA3Ag was administered as a non-adjuvanted, lyophilized vaccine containing 10  $\mu$ g each of CP5- and CP8-CRM<sub>197</sub> and 20  $\mu$ g of rmClfA (low-dose level), 30  $\mu$ g of CP5- and CP8-CRM<sub>197</sub> and 60  $\mu$ g of rmClfA (mid-dose level), or 100  $\mu$ g of CP5- and CP8-CRM<sub>197</sub> and 200  $\mu$ g of rmClfA (high-dose level), reconstituted with 60 mM sodium chloride.

### Patient characteristics

The mean ages of included participants ranged from 21.3 to 70.9 years (Table 2), and the percentages of males varied from 30.2% to 51.9%. Whites, including non-Hispanic or non-Latino subjects, accounted for the majority of subjects, with proportions ranging from 73.2% to 94.9% and 64.4% to 99.7%, respectively. The average body mass index ranged from 25.4% to 29.5%, while smoking ranged from 9.0% to 19.1%.

### Primary outcome

The primary outcome was SAE and sSAE. The vaccine group did not exhibit increased incidence of diarrhoea (RR 1.33 [95% CI 0.87, 2.02]) (Fig. 2) compared with the placebo

**Table 1.** Study design characteristics.

Study	Year	Design	Participants	Administering method	Dosing groups
Creech <sup>17</sup>	2017	Prospective, multicentre, randomized, controlled, double-blind	Participants receiving SA3Ag or SA4Ag or placebo	a single 0.5 mL intramuscular injection into the deltoid muscle	SA3Ag, low-dose SA4Ag, mid-dose SA4Ag, high-dose SA4Ag, placebo
Frenck <sup>16</sup>	2017	Prospective, multicentre, randomized, controlled, double-blind	Participants receiving SA4Ag or placebo	a single 0.5 mL intramuscular injection into the deltoid muscle	Low-dose SA4Ag, mid-dose SA4Ag, high-dose SA4Ag, placebo
Nissen <sup>20</sup>	2015	Prospective, multicentre, randomized, controlled, double-blind	Participants in the two age groups receiving SA3Ag or placebo, independently	Not Reported	Low-dose SA3Ag, mid-dose SA3Ag, high-dose SA3Ag, placebo

SA3Ag: *Staphylococcus aureus* three-antigen vaccine; SA4Ag: *Staphylococcus aureus* four-antigen vaccine.

**Table 2.** Patient characteristics.

Study	Year	Number of subjects	Age	Male	Race(White)	Ethnicity (Non-Hispanic and non-Latino)	BMI (kg/m <sup>2</sup> )	Smoking
Crech <sup>17</sup>	2017	284	70.9 ± 4.62	49.2%	91.5%	64.4%	29.4 ± 5.67	10.2%
Frenc <sup>16</sup>	2017	456	45.0 ± 13.13	43.2%	73.2%	73.7%	29.5 ± 6.42	19.1%
Nissen <sup>20</sup> 18-24-year age stratum	2015	96	21.3 ± 1.65	30.2%	91.7%	99.0%	25.4 ± 5.26	17.7%
Nissen <sup>20</sup> 50-85-year age stratum	2015	312	63.6 ± 8.15	51.9%	94.9%	99.7%	28.8 ± 5.82	9.0%

group, and there was no evidence for statistical heterogeneity ( $\chi^2 = 8.06$ ,  $I^2 = 48.7\%$ , and  $P_{\text{heterogeneity}} = 0.099$ ). Joint pain, muscle pain, vomiting, headache, and fatigue had RRs [95% CIs] of 1.10 [0.76, 1.59], 1.25 [0.91, 1.71], 1.11 [0.48, 2.53], 0.96 [0.79, 1.16], and 1.19 [0.95, 1.50], respectively. According to these results, the incidence of SAE was not increased in the vaccine group compared with the placebo group. Overall effects seemed to increase the incidence of SAE (1.1 [0.98, 1.24]) compared to placebo; however, this difference was not significant and no statistical heterogeneity was observed ( $\chi^2 = 18.86$ ,  $I^2 = 0\%$ , and  $P_{\text{heterogeneity}} = 0.896$ ). Additionally, the vaccine group also did not demonstrate increased incidence of sSAE (1.57 [0.74, 3.30]) (Fig. 3) compared to the placebo group, and no statistical heterogeneity was found ( $\chi^2 = 1.25$ ,  $I^2 = 0\%$ , and  $P_{\text{heterogeneity}} = 0.742$ ).

### Secondary outcome

The incidence of redness was significantly increased by vaccine injection (RR 2.97 [95% CI 1.57, 5.62]) (Fig. 4) compared to the placebo group, and no statistical heterogeneity was found ( $\chi^2 = 5.08$ ,  $I^2 = 11.9\%$ , and  $P_{\text{heterogeneity}} = 0.338$ ). The levels of swelling reported in the three studies also showed significant differences between the intervention and placebo groups (3.34 [1.45, 7.67]), with no statistical heterogeneity ( $\chi^2 = 2.69$ ,  $I^2 = 0\%$ , and  $P_{\text{heterogeneity}} = 0.685$ ). The intervention group exhibited increased incidence of injection site pain (2.79 [1.92, 4.04]) compared to the placebo group, with no statistical heterogeneity ( $\chi^2 = 5.23$ ,  $I^2 = 0\%$ , and  $P_{\text{heterogeneity}} = 0.464$ ). Overall effects also showed the intervention group demonstrated increased secondary outcomes (2.89 [2.15, 3.90]), and no statistical heterogeneity was found ( $\chi^2 = 11.78$ ,  $I^2 = 0\%$ , and  $P_{\text{heterogeneity}} = 0.720$ ). However, the vaccine group did not show increased incidence of sLAE (4.06 [0.78, 21.24]) (Fig. 5) compared to the placebo group, and there was no heterogeneity ( $\chi^2 = 0.03$ ,  $I^2 = 0\%$ , and  $P_{\text{heterogeneity}} = 0.985$ ).

### Quality of studies

One study performed randomization by computer<sup>20</sup> and was considered to be low risk (Fig. 6). However, the other two studies<sup>16,17</sup> did not specify the randomization method and thus were considered to have unclear risk. In one study<sup>20</sup>, allocation concealment was achieved by a computer, which was defined as low risk of bias, whereas the two remaining studies<sup>16,17</sup> did not give explicit concealment methods. Although all studies were double-blinded (participant- and investigator-blind), there was insufficient information on the effectiveness of the blinding, so performance bias was defined as unclear risk in all studies. Additionally, insufficient information was available to

determine whether effective outcome assessments were carried out. All studies exhibited withdrawal bias, but missing data did not affect the analysis; thus these studies were defined as low risk. No selectivity bias or other bias was found in all studies.

### Publication bias

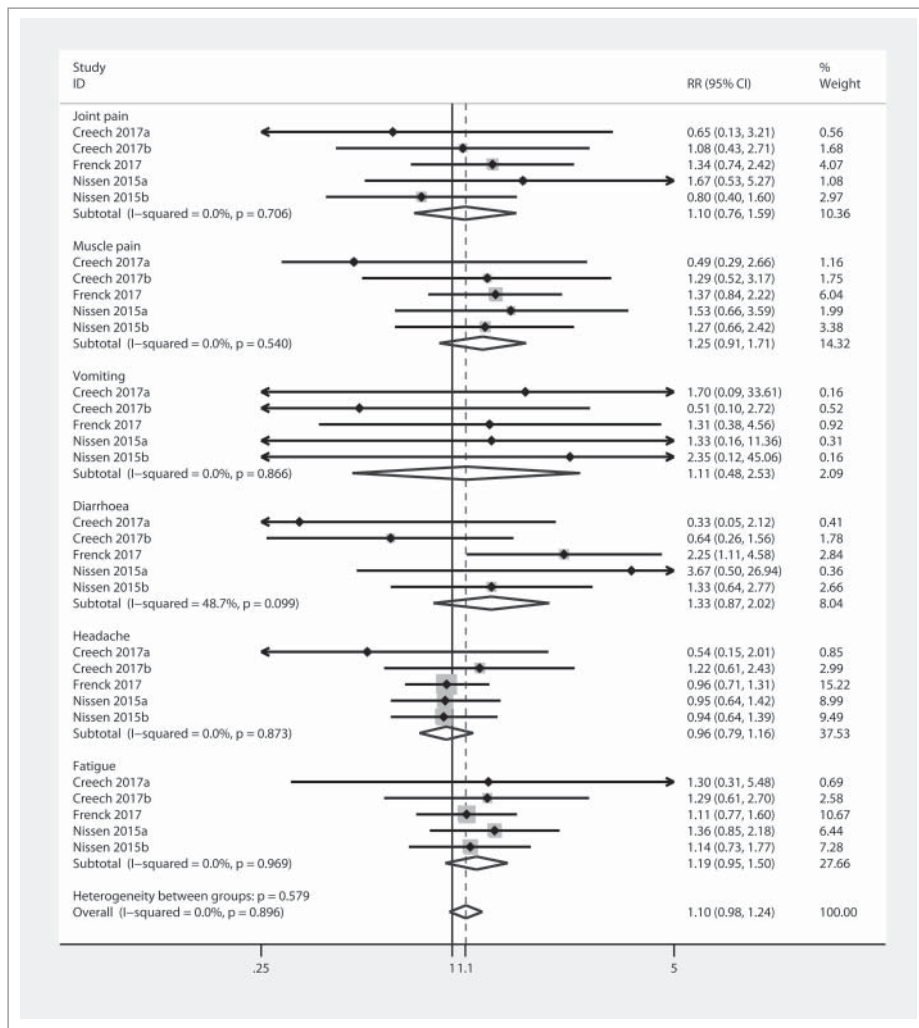
Funnel plots of the study were visually symmetric and a statistical analysis of funnel plots also suggested that no publication bias was present (Harbord's test,  $P = 0.779$ ) (Fig. 7).

### Discussion

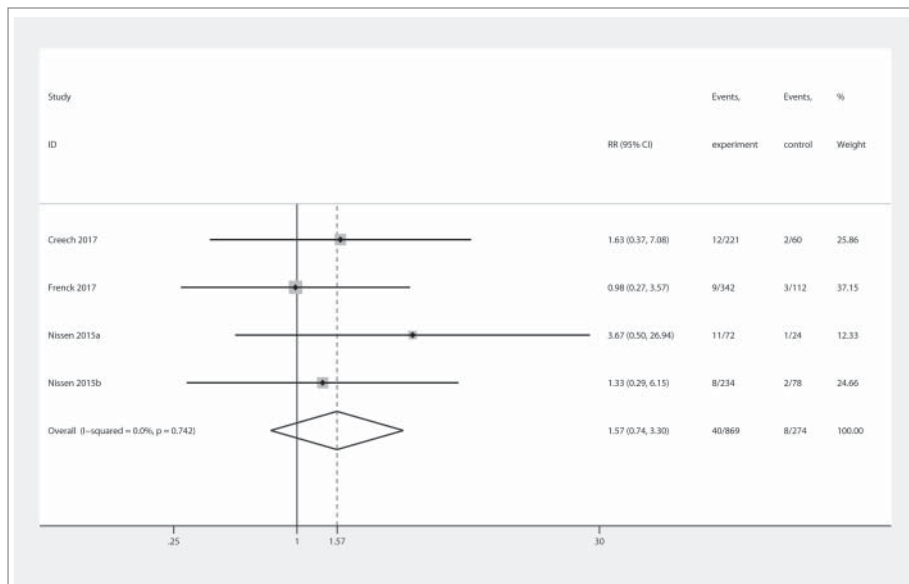
The complexity of the virulence mechanism of *S. aureus* poses challenges for the development of prophylactic vaccines. Previous candidate vaccines, despite generating immune responses, have not proved successful in preventing *S. aureus* infections<sup>12,24,25</sup>, and increasing vaccine dose is bound to cause increased side effects. The reason for the lack of efficacy with previous vaccine candidates is not clear, but may be related to the target of a single virulence pathway.<sup>24,25</sup> Therefore, a successful prophylactic *S. aureus* vaccine must address a combination of well-conserved virulence factors expressed by most strains and generate antibodies that effectively kill the organism. A multi-antigen vaccine candidate targeting *S. aureus* CP5, CP8, ClfA, and rP305A, developed from a lipoprotein manganese transporter C, is under development. *S. aureus* produces capsular polysaccharides to evade the host's immune system<sup>26</sup>, and all *S. aureus* strains have the genetic pathway for synthesis of either CP5 or CP8.<sup>27</sup> ClfA is a highly conserved surface antigen that binds fibrinogen, complement proteins, and platelets, which promotes *S. aureus* infection and mediates adhesion to host tissue.<sup>28</sup> RP305A, the recombinant, nonlipidated form of the manganese transporter C protein, is not expressed *in vitro* by *S. aureus*, but it is rapidly upregulated and expressed *in vivo* early in the infectious process.<sup>29</sup> Moreover, deletion of manganese transporter C results in loss of bacterial viability in assays that mimic the neutrophil's intracellular environment<sup>30</sup>, confirming that it is a virulence factor. While both SA3Ag and SA4Ag have good immunogenicity and tolerability, the safety of these vaccines is worth exploring.

In this meta-analysis, we conducted a review of the side effects of SA3Ag and SA4Ag in healthy adults using data from three RCTs comprising a total of 1,148 patients. The objective of our study was to confirm whether these new *S. aureus* vaccines have good safety. The main finding of this study was that SA3Ag and SA4Ag did not significantly increase SAE. In addition, although LAE were increased in the vaccine group, the incidence of sLAE was not statistically different between the vaccine and placebo groups.

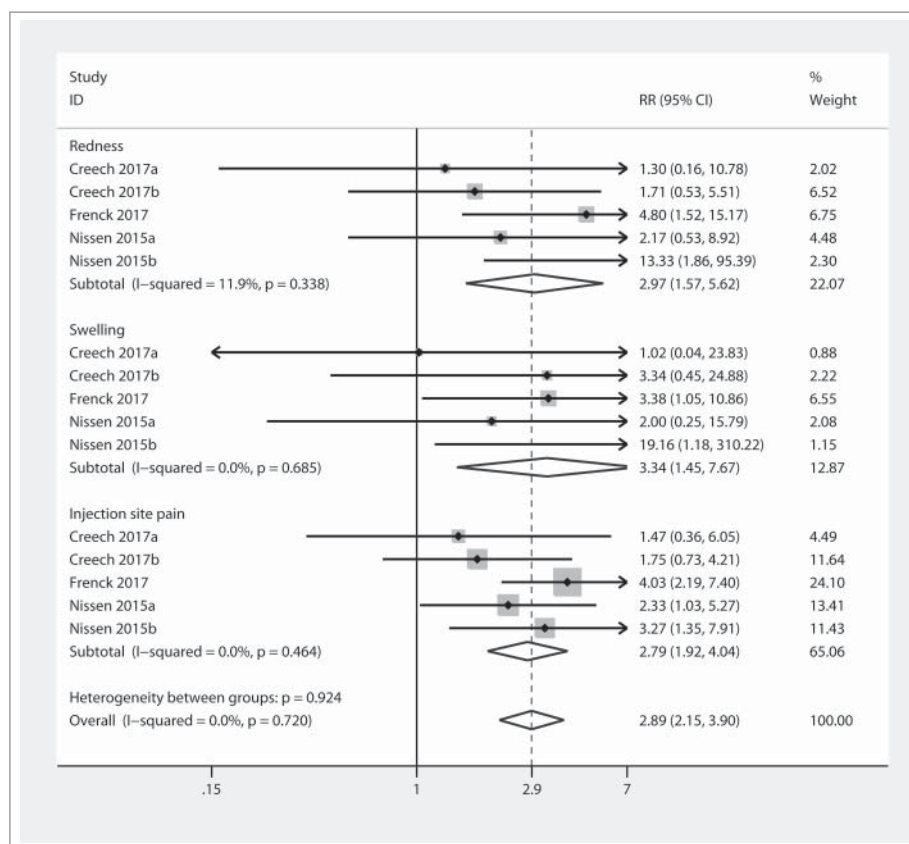
SAE is a key index for evaluating vaccine safety. In this meta-analysis, we included some of the common adverse



**Figure 2.** Forest plot showing RRs for the incidence of systemic events following vaccination with *Staphylococcus aureus* four- and three-antigen vaccines. A significant effect of *S. aureus* vaccines was assumed if the 95% CI did not include the value 1 for RR. CI confidence interval, RR relative ratio



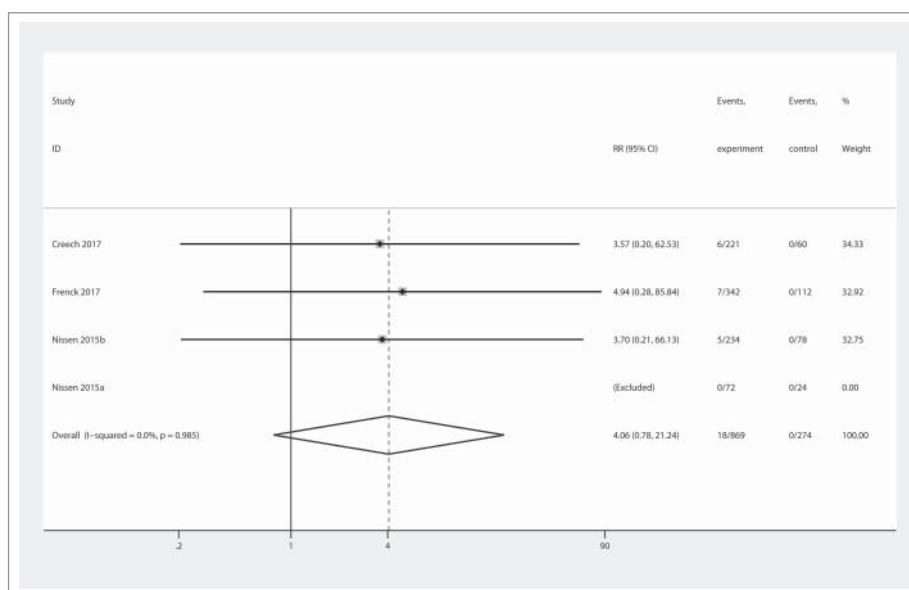
**Figure 3.** Forest plot showing RRs for the incidence of severe systemic events following vaccination with *Staphylococcus aureus* four- and three-antigen vaccines. A significant effect of *S. aureus* vaccines was assumed if the 95% CI did not include the value 1 for RR. CI confidence interval, RR relative ratio



**Figure 4.** Forest plot showing RRs for the incidence of local reactions following vaccination with *Staphylococcus aureus* four- and three-antigen vaccines. A significant effect of *S. aureus* vaccines was assumed if the 95% CI did not include the value 1 for RR. CI confidence interval, RR relative ratio

reactions, such as joint pain, muscle pain, vomiting, diarrhoea, headache, and fatigue. Fever was not included because of the lack of data. Frenck et al.<sup>16</sup> found that SA4Ag can increase the incidence of diarrhoea. Our results showed that the vaccine group seemed to exhibit an increased incidence of diarrhoea compared to the placebo group; however, the difference was not significant. In addition, the incidence of joint pain, muscle

pain, vomiting, and fatigue events tended to increase in the vaccine group, but there was no statistical difference. It is worth mentioning that if the subgroup of headache was removed, the combined effects of the primary end point were statistically significant, suggesting that vaccination may have an impact on SAE. However, the incidence of sSAE in the vaccine group did not increase, suggesting that vaccine safety was acceptable.



**Figure 5.** Forest plot showing RRs for the incidence of severe local reactions following vaccination with *Staphylococcus aureus* four- and three-antigen vaccines. A significant effect of *S. aureus* vaccines was assumed if the 95% CI did not include the value 1 for RR. CI confidence interval, RR relative ratio

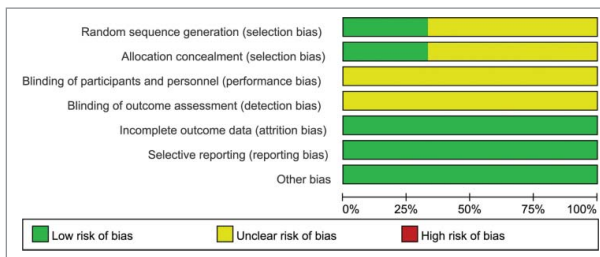


Figure 6. Risk of bias summary

LAE are very common after vaccination. In this meta-analysis, we incorporated three of the most common adverse reactions: redness, swelling, and pain. Predictably, our results showed a marked increase in LAE in the vaccine group, but sLAE were not statistically significant, which also showed that the two vaccines had good safety. Moreover, there was no statistically significant difference in sSAE or sLAE according to vaccine subgroup analysis.

There are some limitations associated with the present study. First, the RCTs included in the meta-analysis did not evaluate all SAE, such as fever, which could change the outcome of the combined effect. Although our results showed that the vaccine group did not exhibit increased sSAE and sLAE, we used the definition applied by the investigators of the respective studies when examining the endpoint events. This means that varying severities of events could have been classified as identical, and we may have erroneously pooled these events in the meta-analysis. Second, most of these studies had an inadequate blinding design for performance and outcome assessment, which is seemingly more difficult to achieve in RCTs than in trials investigating a pharmacological agent. In this meta-analysis, all included studies had bias in the performance and outcome evaluation design.

Overall, our meta-analysis showed that these two novel *S. aureus* vaccines, SA3Ag and SA4Ag, had acceptable safety according to the incidence of SAE and LAE. However, the study was based on a small number of subjects and participants were predominantly white. Moreover, the inadequate blinding design may also have led to false positives in the data. Therefore, additional, large-scale RCTs must be carried out to further

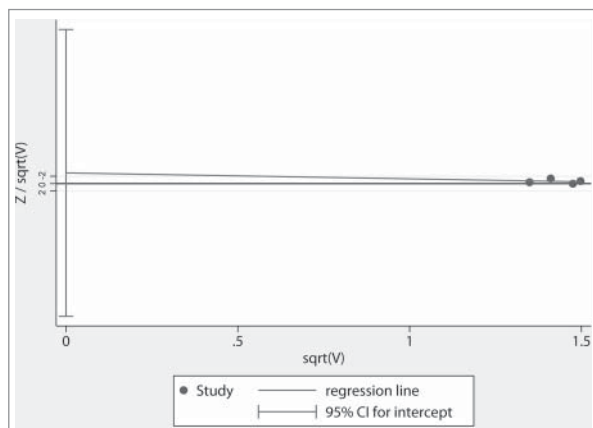


Figure 7. Harbord's funnel plot of all studies included in the meta-analysis

determine the immunogenicity, tolerance, and safety of SA3Ag and SA4Ag.

## Methods

### Data sources

The Medline, EMBASE, and Cochrane databases and the reference lists found in original and review articles were searched independently by two reviewers (XX, HZ) using medical subject heading terms, key words, titles, and abstracts. The search terms were “*Staphylococcus aureus* four-antigen vaccine,” “SA4Ag,” “*Staphylococcus aureus* three-antigen vaccine,” and “SA3Ag.” All historical literature was searched up until July 2017.

### Study selection

An initial eligibility screen of all retrieved titles and abstracts was conducted, and original studies were included in our meta-analysis if they met the following criteria: (1) human subjects; (2) participants in general good health, including those with stable pre-existing chronic medical conditions; (3) participants were randomly assigned to receive either prophylactic *S. aureus* vaccine or placebo; and (4) sufficient data on SAE and LAE were available. Full manuscripts were obtained for all selected articles based on the initial assessment of abstracts. Only fully published trials were included (abstracts and congress presentations were not included). The primary outcome was the combined endpoint, SAE, including joint pain, muscle pain, vomiting, diarrhoea, headache, and fatigue. The secondary outcome was LAE, including redness, swelling, and injection site pain. Trials not reporting any of these parameters were excluded from the review. Two investigators (XX, HZ) independently reviewed all full-text articles that potentially met the inclusion criteria according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement<sup>31</sup> and the Cochrane Handbook guidelines.<sup>23</sup> In cases of disagreement, a consensus was obtained by discussion with the third author (HL).

### Data extraction

All selected papers were reviewed by two reviewers (XX, HZ), who independently extracted data to a data sheet. Data extraction included year of publication, study design, sample size, patient characteristics, inclusion and exclusion criteria, control and intervention protocols, randomization, blinding, and follow-up, as well as the outcome parameters described above. SAE and severe systemic adverse events (sSAE) were the primary endpoints; the former included all systemic adverse events, whereas the latter was only included severe side effects. The secondary outcome was also classified into LAE and severe local adverse events (sLAE), defined similarly. For a study involving multiple intervention groups and one control group, data for different doses of intervention groups were combined, while data for intervention groups with different vaccine types were equally segmented, according to the Cochrane Handbook guidelines.<sup>23</sup> Where data were presented in a graph but not in

the text, we requested the data from the corresponding author of the paper. If the data were not provided, we extrapolated them from the graph using a charting digital tool (GetData Graph Digitizer, <http://getdata-graph-digitizer.com>). Following the extraction of relevant data by two of the authors, data were examined for possible inconsistencies, which were then resolved by discussion. If a consensus could not be reached, then the third author was consulted (HL). Studies were not directly conducted on humans and ethical approval was therefore unnecessary.

### Quality assessment

Two authors (XX, HZ) used the seven domains of the Cochrane risk of bias tool to evaluate the quality of the included studies, using the following criteria: randomization sequence generation, concealment of randomization sequence, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other bias. Studies were classified as having low risk, high risk, or unclear risk of bias for each item, as suggested in the Cochrane Handbook.<sup>23</sup>

### Statistical analysis

The verified data were analysed using Stata software (version 13.0; Stata Corporation, College Station, TX, USA) and REV-MAN software (version 5.2; Cochrane Collaboration, Oxford, UK). One author (XX) entered the data and a second author (HZ) verified data entry. The relative ratio (RR) and corresponding 95% confidence intervals (CIs) were calculated for dichotomous data. A fixed effects model was used to analyse data with values higher than 0.05 by heterogeneity testing ( $\chi^2$ -based Q-test), while a random effects model was used when values were less than 0.05. The magnitude of heterogeneity was assessed by  $I^2$  test ( $I^2 = 0$ –25%, no heterogeneity;  $I^2 = 25$ –50%, moderate heterogeneity;  $I^2 = 50$ –75%, large heterogeneity;  $I^2 = 75$ –100%, extreme heterogeneity). An intervention was assumed to have had a significant effect if the 95% CIs did not include the value 1 for RR. In the analysis for small-study effects, publication bias was assessed using funnel plot techniques and Harbord's test.<sup>32</sup>

### Abbreviations

CI	confidence interval
ClfA	clumping factor A
CP5	CP5 capsular polysaccharide type 5
CP8	capsular polysaccharide type 8
LAE	local adverse events
RCT	randomized controlled trial
rP305A	recombinant P305A
RR	relative ratio
SA3Ag	<i>Staphylococcus aureus</i> three-antigen vaccine
SA4Ag	<i>Staphylococcus aureus</i> four-antigen vaccine
SAE	systematic adverse events
sLAE	severe local adverse events
sSAE	severe systematic adverse events

### Disclosure of potential conflicts of interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and publication of this article.

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### Author contributions

Designed the study: Huoyang Lv and Xiaoqun Xu. Performed the study: Xiaoqun Xu and Houyong Zhu. Analysed the data: Xiaoqun Xu, Houyong Zhu, and Huoyang Lv. Wrote the paper: Xiaoqun Xu and Houyong Zhu. Revised the manuscript: Huoyang Lv.

### Ethical approval

Studies were not directly conducted on humans and ethical approval was therefore unnecessary.

### Informed consent

Studies were not directly conducted on humans and informed consent was therefore unnecessary.

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