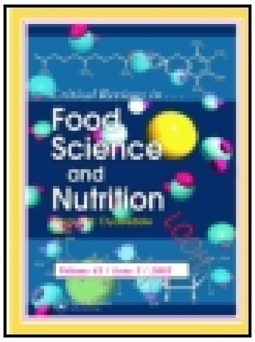

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CRITICAL REVIEWS IN FOOD SCIENCE AND NUTRITION
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# A large meta-analysis of the global prevalence rates of *S. aureus* and MRSA contamination of milk

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**ABSTRACT**
Past reports have indicated a high prevalence of milk contaminated with *Staphylococcus aureus* (*S. aureus*) and methicillin-resistant *Staphylococcus aureus* (MRSA), but the pooled prevalence rates of *S. aureus* and MRSA in pasteurized and boiled cow's milk, raw cow's milk, and raw Caprinae milk (raw sheep's milk and raw goat's milk) and across different periods, continents, economic conditions and purchase locations remain inconsistent. We searched relevant articles published in PubMed, EMBASE, and Web of Science before July 2016. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement was used to evaluate the quality of 93 included studies. We observed that the pooled prevalence rates of *S. aureus* contamination in pasteurized and boiled cow's milk, raw cow's milk, and raw Caprinae milk were 15.4% (95% CI, 6.1–27.5%), 33.5% (95% CI, 29.5–37.7%) and 25.8% (95% CI, 17.5–35.0%), respectively. The pooled prevalence rates of MRSA contamination were 4.9% (95% CI, 0.0–15.7%), 2.3% (95% CI, 1.3–3.6%), and 1.1% (95% CI, 0.5–1.8%), respectively. The prevalence of *S. aureus* contamination in raw cow's milk increased over time. However, the pooled prevalence of raw cow's milk contaminated with *S. aureus* was lowest in European studies. These findings give an indication of the consequence of better milk regulation in Europe. High *S. aureus* prevalence rates in raw milk collected from farms and processing companies pose a potential threat to consumers. The implementation of good hygiene practices, appropriate health knowledge, and food safety principles at the farm level, as well as the prudent use of antibiotics in veterinary medicine and heat treatment before drinking, are necessary to reduce the potential risk of *S. aureus* and MRSA contamination.

**KEYWORDS**
Meta-analysis; milk contamination; *Staphylococcus aureus*; methicillin-resistant *Staphylococcus aureus*

## Introduction

*Staphylococcus aureus* (*S. aureus*) is a spherical, gram-positive, opportunistic pathogen that usually results in clinical infections and bovine mastitis. *Staphylococcal* food poisoning (SFP) is one of the leading causes of foodborne illness due to the widespread presence of *S. aureus* and to the production of enterotoxins by the pathogen (Normanno et al., 2007). *S. aureus* contributes to reduced food quality and food loss. It is also considered a recognized pathogen that has caused outbreaks of food poisoning (Huong et al., 2010; Zhang et al., 2015). Most seriously, ingestion of the enterotoxigenic *S. aureus* could lead to gastroenteritis, diarrhea and vomiting (Madahi et al., 2014).

However, conventional foods including milk and milk products can be potential sources of *S. aureus* because the high level of nutrients provides a suitable growth matrix for it. Many studies have noted that milk and milk products are vulnerable to contamination with enterotoxigenic strains of *S. aureus* (Morandi et al., 2009). Boiling treatment that is stopped after the appearance of foam (i.e., when the milk temperature was approximately 95°C) is the optimal method to ensure the hygienic quality of raw milk (Tremonte et al., 2014). A short, high-temperature pasteurization method (71.6°C for 15 s) or a long, low-temperature pasteurization method (62.7°C for 30 min) can effectively decrease the number of bacteria in milk (Elizondo-Salazar et al., 2010). However, improper handling and poor hygiene result in low-quality milk. The presence of *S. aureus* suggests potentially failed milk (O'Ferrall-Berndt, 2003). Because of the introduction of pathogenic strains into the dairy product supply chain, the emergence of *S. aureus* in raw milk represents a potential risk to human health (Spanu et al., 2013). Furthermore, methicillin-resistant *Staphylococcus aureus* (MRSA) has been detected in a small proportion of raw milk samples (Doyle et al., 2012). The presence and transmission of MRSA in milk samples indicates a significant threat to public health (Pu et al., 2014).

Past studies have also described a slightly higher incidence of *S. aureus* contamination in milk (Chao et al., 2007). However, there are no systematic reviews available on the pooled *S. aureus* and MRSA contamination rates in milk across different periods, continents, economic conditions and purchase

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Authors Ou and Zhou contributed equally to this work.

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locations. Therefore, a meta-analysis of the literature was performed to assess the global prevalence rates of *S. aureus* and MRSA contamination in pasteurized and boiled cow's milk (heat treatment conditions were as previously mentioned), raw cow's milk and raw Caprinae milk (raw sheep's milk and raw goat's milk) sampled from retail markets, farms and processing companies.

## Methods

### Search strategy

This systematic review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher et al., 2010). We performed systematic searches in PubMed, EMBASE and Web of Science. The keywords used as search queries in the databases were "milk" AND ("*Staphylococcus aureus*" OR "*S. aureus*" OR "methicillin resistant *Staphylococcus aureus*" OR "MRSA") AND ("contaminated" OR "contamination" OR "prevalence"). The search was limited to the publication dates from January 2000 to 1 July, 2016.

### Study selection criteria

The titles and abstracts were screened for relevance. Articles or abstracts were selected based on predefined inclusion and exclusion criteria. Studies were considered eligible for inclusion if they met the following criteria: (1) the samples were tested for *S. aureus* or MRSA contamination; (2) sample size data were provided; (3) the articles were published in the English language. Studies were excluded if they met the following criteria: (1) the articles were reviews, letters, editorial articles or meta-analyses; (2) the milk was not obtained from cows, sheep or goats; (3) the studies were experimental; (4) the samples were from sick animals; (5) the articles were outbreak reports; and (6) the studies were duplicates of included studies. The titles and abstracts of the studies were screened to ascertain the articles' relevance to the meta-analysis. Two researchers independently screened each record. The eligibility was determined by reading the full texts if the selection could not be made based on the screening alone. Disagreements were resolved by discussions among our team members.

### Data extraction and study quality assessment

The most relevant information was extracted from each included study (first author, country, continent, economic condition, study year, sampling location, type of milk, sample size, and sample size that was determined to be *S. aureus*- or MRSA-positive) and considered for the meta-analysis.

The methodological quality of the articles was rated using the Strengthening the Reporting of Observational Studies in Epidemiology Statement (Elm et al., 2014). The following 22 items were assessed to calculate a total quality score: title and abstract (item 1), introduction (items 2 and 3), methods (items 4–12), results (items 13–17), discussion (items 18–21), and other information (item 22 on funding). Answers were scored 0 or 1 for "No" and "Yes." The possible overall scores range from 0 to 22. Studies with scores below 8 were excluded from the meta-analysis. Any disagreement was resolved by discussions within our team.

### Statistical analysis

A meta-analysis was performed for the pooled prevalence rates of *S. aureus* and MRSA contamination in milk and their corresponding 95% confidence intervals (CIs) using Stata v.13.1 software (Stata Corp., College Station, TX). Heterogeneity among studies was computed using the Q test and the $I^2$ statistic. $P < 0.10$ suggests heterogeneity among intervention effects. The $I^2$ statistic was used to quantify the degree of heterogeneity, with values of 25%, 50%, and 75% indicating low, medium and high degrees of heterogeneity, respectively (Johnson and Whisman, 2013). A meta-analysis was performed using a random-effects model when heterogeneity was present. Subgroup analyses were performed to explore the possible sources of heterogeneity based on the predefined variables including sample size, study year, continent, economic condition, and sampling location. Pasteurized cow's milk and boiled cow's milk were included in the same category because they were considered to undergo thermal treatment (conditions were as previously mentioned). Raw sheep's milk and raw goat's milk were classified as raw Caprinae milk, because of the limited number of studies focused on raw sheep's milk and raw goat's milk. Sensitivity analyses served to estimate the influence of a single study on the overall estimate by omitting each study. Begg's test was conducted to assess the probability of publication bias. A $P$-value of less than 0.05 indicated statistical significance.

## Results

### General information about included studies

According to the results of the literature search, 1219 unduplicated articles published between January 2000 and 1 July 2016 met the search criteria. After screening titles and abstracts, we selected 146 potentially relevant articles to read the full texts and included 93 independent studies in the meta-analysis. The study selection process is shown in Fig. 1. Of the included studies, nine, seventy-three and seventeen articles related to the prevalence of *S. aureus* contamination in pasteurized and boiled cow's milk, raw cow's milk and raw Caprinae milk, respectively. Three, twenty-two and four articles focused on screening for MRSA contamination in pasteurized and boiled cow's milk, raw cow's milk and raw Caprinae milk were included. We rated the quality of the included articles, and the mean quality score was 13.60 (range from 10 to 19). Table 1 shows the details of the included studies.

### Overall analysis

Differences in the prevalence rates of *S. aureus* and MRSA in these three types of milk were statistically significant ($P = 0.012$ and $P = 0.031$, respectively). The pooled analysis of eligible studies showed that the pooled prevalence rates of *S. aureus* contamination in pasteurized and boiled cow's milk, raw cow's milk and raw Caprinae milk were 15.4% (95% CI,

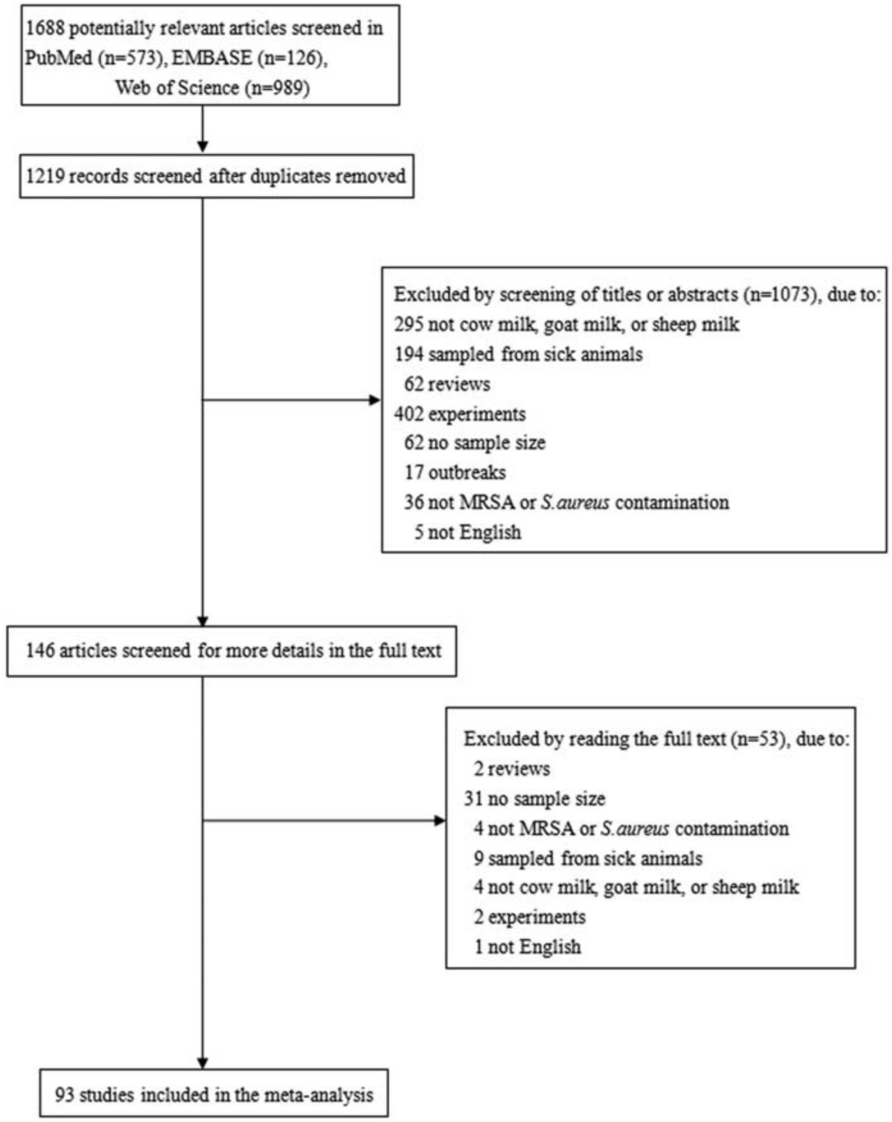


**Figure 1.** Flow diagram of the selection process of the included studies.

6.1–27.5%), 33.5% (95% CI, 29.5–37.7%), and 25.8% (95% CI, 17.5–35.0%), respectively. However, there was substantial heterogeneity across eligible studies in the prevalence rates of pasteurized and boiled cow's milk ($\chi^2 = 102.28$; $P = 0.00$; $I^2 = 90.2\%$), raw cow's milk ($\chi^2 = 12732.88$; $P = 0.00$; $I^2 = 99.4\%$) and raw Caprinae milk ($\chi^2 = 1212.70$; $P = 0.00$; $I^2 = 98.7\%$) contamination. The pooled prevalence rates of MRSA contamination in pasteurized and boiled cow's milk, raw cow's milk and raw Caprinae milk were 4.9% (95% CI, 0.0–15.7%), 2.3% (95% CI, 1.3–3.6%) and 1.1% (95% CI, 0.5–1.8%), respectively. Moreover, significant heterogeneity was observed across eligible studies in the prevalence rates of pasteurized and boiled cow's milk ($\chi^2 = 6.25$; $P = 0.04$; $I^2 = 68.0\%$) and raw cow's milk ($\chi^2 = 175.49$; $P = 0.00$; $I^2 = 86.9\%$), but significant heterogeneity was not observed across studies in the prevalence rates of raw Caprinae milk ($\chi^2 = 2.14$; $P = 0.54$; $I^2 = 0.0\%$). The pooled results of *S. aureus* and MRSA contamination in pasteurized and boiled cow's milk, raw cow's milk, and raw Caprinae milk are shown in Figs. 2–7.

### Subgroup analyses

Heterogeneity between the studies was considerable; in order to perform a secondary analysis, subgroup analyses were conducted to identify probable sources of heterogeneity. In response to the significant heterogeneity across the studies, the pooled prevalence rates for each subgroup were estimated using a random-effects model, except for the pooled prevalence rates of raw Caprinae milk contamination with MRSA. Subgroup analyses were based on sample size, study year, continent, economic condition and sampling location (Table 2).

The prevalence of *S. aureus* contamination in raw cow's milk was significantly higher ($P < 0.05$) in studies with smaller sample sizes (44.0%; 95% CI,31.3–57.1%) than studies with larger sample sizes (28.1%; 95% CI, 23.6–32.8%), while the subgroup of studies with larger sample sizes reported a lower prevalence ($P < 0.01$) of *S. aureus* contamination in raw Caprinae milk (14.6%; 95% CI, 8.0–22.7%).

Significant differences were found in the prevalence of raw cow's milk ($P < 0.01$) contaminated by *S. aureus* by year, with

Table 1. Main characteristics of included studies.

| Studies | Country | Continent | Economic condition | Study year | Sampling location | Milk type | *S. aureus* samples[a] | MRSA samples[b] | Sample size | Quality score |
|---|---|---|---|---|---|---|---|---|---|---|
| (Tondo et al., 2000) | Brazil | Americas | developing | 1997–1999 | processing companies | raw cow's milk | 19 | — | 21 | 13 |
| (Schlegelova et al., 2002) | Czech Republic | Europe | developed | — | farms | raw cow's milk | 38 | — | 111 | 11.5 |
| (Vautor et al., 2003) | France | Europe | developed | 2001–2002 | farms | raw goat's milk | 50 | — | 909 | 12 |
| (Muehlherr et al., 2003) | Switzerland | Europe | developed | 2002 | farms | raw goat's & sheep's milk | 130 | — | 407 | 14.5 |
| (Phuektesa et al., 2003) | Australia | Oceania | developed | — | farms | raw cow's milk | 28 | — | 176 | 13 |
| (O'Ferrall-Berndt, 2003) | South Africa | Africa | developing | 1998 | retailers | raw cow's milk | 54 | — | 214 | 14 |
| (Chye et al., 2004) | Malaysia | Asia | developing | — | farms | raw cow's milk | 565 | — | 930 | 13 |
| (Hariharan et al., 2004) | Scotland | Europe | developed | — | farms | raw goat's milk | 1 | — | 492 | 12 |
| (De Reu et al., 2004) | Belgium | Europe | developed | 2002 | farms | raw cow's milk | 143 | — | 143 | 13 |
| (Jørgensen et al., 2005) | Norway | Europe | developed | 2003 | farms | raw cow's milk | 9 | — | 42 | 14 |
| (Østerås et al., 2006) | Norway | Europe | developed | 1998 | farms | raw cow's milk | 1157 | — | 14152 | 19 |
| (Howard, 2006) | New Zealand | Oceania | developed | 2004 | farms | raw cow's milk | 35 | — | 35 | 11.5 |
| (Tenhagen et al., 2006) | Germany | Europe | developed | 2001–2002 | farms | raw cow's milk | 569 | — | 9910 | 17.5 |
| (Kivaria et al., 2006) | Tanzania | Africa | developing | 2003 | retailers | raw cow's milk | 8 | — | 128 | 13 |
| (Aragon-Alegro et al., 2007) | Brazil | Americas | developing | — | retailers | raw cow's milk | 4 | — | 4 | 12.5 |
| (Tsegmed et al., 2007) | Mongolia | Asia | developing | 2000 | farms | raw cow's milk | 22 | — | 97 | 11.5 |
| (Chao et al., 2007) | China | Asia | developing | 2003–2005 | farms | raw cow's milk | 34 | — | 209 | 13.5 |
| (D'Amico et al., 2008) | USA | Americas | developed | 2006 | farms | raw cow's milk | 17 | — | 62 | 15.5 |
| | USA | Americas | developed | 2006 | farms | raw goat's & sheep's milk | 27 | — | 70 | 15.5 |
| (Zouharova and Rysanek, 2008) | Czech Republic | Europe | developed | 2005 | farms | raw cow's milk | 70 | — | 440 | 12 |
| (Tenhagen et al., 2009) | Germany | Europe | developed | 2005–2006 | farms | raw cow's milk | 277 | — | 6915 | 17 |
| (Virgin et al., 2009) | USA | Americas | developed | 2007 | farms | raw cow's milk | 218 | — | 542 | 11 |
| (Moret-Stalder et al., 2009) | Switzerland | Europe | developed | 2004–2005 | farms | raw cow's milk | 48 | — | 2388 | 17 |
| (Ateba et al., 2010) | South Africa | Africa | developing | — | farms | raw cow's milk | 28 | — | 28 | 14 |
| (D'Amico and Donnelly, 2010) | USA | Americas | developed | 2008 | farms | raw cow's milk | 13 | — | 45 | 14 |
| | USA | Americas | developed | 2008 | farms | raw goat's milk | 19 | — | 40 | 14 |
| (Guven et al., 2010) | Turkey | Asia | developing | 2004–2006 | retailers | raw cow's milk | 12 | — | 36 | 13.5 |
| (Riekerink et al., 2010) | Canada | Americas | developed | 2003–2005 | farms | raw cow's milk | 363 | — | 904 | 16.5 |
| (Ortolani et al., 2010) | Brazil | Americas | developing | — | farms | raw cow's milk | 5 | — | 36 | 12.5 |
| (Murphy et al., 2010) | Ireland | Europe | developed | 2005–2006 | farms | raw cow's milk | 37 | — | 145 | 11.5 |
| | Ireland | Europe | developed | 2005–2006 | farms | raw goat's & sheep's milk | 11 | — | 39 | 11.5 |
| (Schwarz et al., 2010) | Germany | Europe | developed | 2000–2003 | farms | raw cow's milk | 7262 | — | 145065 | 18 |
| (Mørk et al., 2010) | Norway | Europe | developed | 2005–2006 | farms | raw goat's milk | 353 | — | 5671 | 14 |
| (Koluman et al., 2011) | Turkey | Asia | developing | — | retailers | pasteurized cow's milk | 2 | 0 | 25 | 13.5 |
| (Mirzaei et al., 2011) | Iran | Asia | developing | 2010–2011 | retailers | raw cow's milk | 22 | 0 | 50 | 14 |
| | Iran | Asia | developing | 2010–2011 | retailers | pasteurized cow's milk | 4 | 2 | 50 | 14 |
| (Aydin et al., 2011) | Turkey | Asia | developing | 2007–2008 | retailers | raw cow's milk | 64 | — | 303 | 14.5 |
| (Persson and Olofsson, 2011) | Sweden | Europe | developed | 2008 | farms | raw goat's milk | 9 | — | 222 | 12 |
| (Jakobsen et al., 2011) | Norway | Europe | developed | — | processing companies | raw goat's milk | 45 | — | 49 | 13.5 |
| | Norway | Europe | developed | — | processing companies | raw cow's milk | 34 | — | 73 | 13.5 |
| (Fotou et al., 2011) | Greece | Europe | developed | — | farms | raw sheep's milk | 58 | — | 240 | 13.5 |
| (Sasidharan et al., 2011) | Malaysia | Asia | developing | 2007 | farms & retailers | raw cow's milk | 4 | — | 12 | 12 |
| | Malaysia | Asia | developing | 2007 | farms & retailers | pasteurized cow's milk | — | 1 | 13 | 12 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| (Kreausukon et al., 2012) | Germany | Europe | developed | 2009–2010 | farms | raw cow's milk | — | 28 | 635 | 15 |
| (Desissa et al., 2012) | Ethiopia | Africa | developing | 2009–2010 | farms | raw cow's milk | 74 | — | 170 | 14.5 |
| | Ethiopia | Africa | developing | 2009–2010 | retailers | raw cow's milk | 18 | — | 25 | 14.5 |
| | Ethiopia | Africa | developing | 2009–2010 | retailers | pasteurized cow's milk | 0 | — | 20 | 14.5 |
| (Haran et al., 2012) | USA | Americas | developed | 2009 | farms | raw cow's milk | 93 | 2 | 150 | 14.5 |
| (Lee et al., 2012) | Brazil | Americas | developing | 2010–2011 | farms | raw cow's milk | 38 | — | 340 | 14.5 |
| (Wang et al., 2012) | China | Asia | developing | 2009 | farms | raw cow's milk | 17 | 0 | 58 | 14.5 |
| (Daka et al., 2012) | Ethiopia | Africa | developing | 2011–2012 | farms | raw cow's milk | 20 | — | 32 | 11.5 |
| | Ethiopia | Africa | developing | 2011–2012 | farms | pasteurized cow's milk | 6 | — | 32 | 11.5 |
| (Sobrinho et al., 2012) | Brazil | Americas | developing | 2009–2010 | farms | raw cow's milk | 145 | — | 154 | 15 |
| (Mork et al., 2012) | Norway | Europe | developed | 2003–2004 | farms | raw cow's milk | 166 | — | 3982 | 12 |
| (Gücükoğlu et al., 2012) | Turkey | Asia | developing | 2012 | retailers | raw cow's milk | 45 | — | 60 | 10.5 |
| (Hill et al., 2012) | New Zealand | Ocean | developed | 2007–2008 | farms | raw cow's milk | 235 | — | 297 | 11 |
| (Kouamé-Sina et al., 2012) | Côte d'Ivoire | Africa | developing | 2008–2010 | farms | raw cow's milk | 21 | — | 118 | 13.5 |
| (Stastkova et al., 2012) | Czech Republic | Europe | developed | 2008–2010 | farms | raw cow's milk | 199 | — | 511 | 14 |
| (Cervinkova et al., 2013) | Czech Republic | Europe | developed | 2012 | farms | raw cow's milk | 60 | — | 669 | 15.5 |
| (Rahimi and Alian, 2013) | Iran | Asia | developing | 2010–2011 | farms | raw cow's milk | 15 | — | 90 | 12 |
| | Iran | Asia | developing | 2010–2011 | farms | raw goat's & sheep's milk | 7 | — | 80 | 12 |
| (de Garnica et al., 2013) | Spain | Europe | developed | 2011 | farms | raw sheep's milk | 199 | — | 752 | 14 |
| (Vahedi et al., 2013) | Iran | Asia | developing | 2011 | retailers | pasteurized cow's milk | 22 | — | 100 | 16.5 |
| | Iran | Asia | developing | 2011 | farms | raw cow's milk | 2 | — | 100 | 16.5 |
| (Mullen et al., 2013) | USA | Americas | developed | 2010 | farms | raw cow's milk | 185 | — | 4660 | 13.5 |
| (He et al., 2013) | China | Asia | developing | 2010 | farms | raw cow's milk | 4 | 0 | 30 | 16 |
| (Cicconi-Hogan et al., 2013) | USA | Americas | developed | 2009–2011 | farms | raw cow's milk | 159 | — | 290 | 17 |
| (Kamal et al., 2013) | Egypt | Africa | developing | 2011–2012 | farms & retailers | raw cow's milk | 33 | 3 | 35 | 12 |
| (Spanu et al., 2013) | Italy | Europe | developed | — | farms | raw goat's milk | 60 | 0 | 78 | 11.5 |
| (Hu et al., 2013) | China | Asia | developing | 2011 | farms | raw cow's milk | 12 | 0 | 40 | 12 |
| (Lim et al., 2013) | Korea | Asia | developed | 2008 | farms | raw cow's milk | — | 35 | 559 | 14.5 |
| (Antoci et al., 2013) | Italy | Europe | developed | 2010 | farms | raw cow's milk | — | 21 | 48 | 16 |
| (Gargouri et al., 2014) | Tunisia | Africa | developing | 2012 | farms | raw cow's milk | 80 | — | 80 | 13.5 |
| (Wang et al., 2014b) | China | Asia | developing | 2011–2012 | farms | raw cow's milk | 6 | — | 67 | 14.5 |
| (Walcher et al., 2014) | Austria | Europe | developed | — | farms | raw cow's milk | 18 | — | 35 | 12.5 |
| (Wang et al., 2014a) | China | Asia | developing | 2008–2009 | farms | raw cow's milk | 43 | 4 | 295 | 14.5 |
| (Rall et al., 2014) | Brazil | Americas | developing | — | farms | raw cow's milk | 1 | — | 280 | 12 |
| (Kamana et al., 2014) | Rwanda | Africa | developing | 2012 | farms | raw cow's milk | 23 | — | 54 | 13.5 |
| | Rwanda | Africa | developing | 2012 | processing companies | raw cow's milk | 67 | — | 120 | 13.5 |
| | Rwanda | Africa | developing | 2012 | processing companies | pasteurized cow's milk | 5 | — | 27 | 13.5 |
| | Rwanda | Africa | developing | 2012 | wholesale | pasteurized cow's milk | 5 | — | 12 | 13.5 |
| | Rwanda | Africa | developing | 2012 | retailers | pasteurized & boiled cow's milk | 45 | — | 78 | 13.5 |
| (McAuley et al., 2014) | Australia | Ocean | developed | 2013–2014 | farms | raw cow's milk | 5 | — | 15 | 12.5 |
| (Gundogan and Avci, 2014) | Turkey | Asia | developing | 2012 | processing companies | raw cow's milk | 28 | — | 50 | 14.5 |
| (Cicconi-Hogan et al., 2014) | USA | Americas | developed | 2009–2011 | farms | raw cow's milk | — | 1 | 288 | 11 |
| (Paterson et al., 2014) | UK | Europe | developed | 2011–2012 | farms | raw cow's milk | — | 11 | 465 | 15 |
| (Tenhagen et al., 2014) | Germany | Europe | developed | 2009–2010 | farms | raw cow's milk | — | 31 | 665 | 16 |

(Continued on next page)

Table 1. (*Continued*)

| Studies | Country | Continent | Economic condition | Study year | Sampling location | Milk type | *S. aureus* samples[a] | MRSA samples[b] | Sample size | Quality score |
|---|---|---|---|---|---|---|---|---|---|---|
| (Rahbar Saadat et al., 2014) | Iran | Asia | developing | — | proessing companies & retailers | raw cow's milk | 9 | — | 100 | 10 |
| (Oliveira et al., 2015) | Brazil | Americas | developing | — | farms | raw cow's milk | 65 | 21 | 552 | 12 |
| (Jamali et al., 2015) | Iran | Asia | developing | 2006–2013 | retailers | raw cow's milk | 162 | 21 | 1035 | 14.5 |
| | Iran | Asia | developing | 2006–2013 | retailers | raw sheep's milk | 86 | 11 | 895 | 14.5 |
| (Ngasala et al., 2015) | Tanzania | Africa | developing | 2012 | farms & retailers | raw cow's milk | 35 | — | 105 | 12.5 |
| (Tigabu et al., 2015) | Ethiopia | Africa | developing | — | processing companies | raw cow's milk | 120 | — | 477 | 14.5 |
| (Akindolire et al., 2015) | South Africa | Africa | developing | 2012–2013 | farms & retailers | raw cow's milk | 55 | — | 125 | 13.5 |
| | South Africa | Africa | developing | 2012–2013 | farms & retailers | pasteurized cow's milk | 10 | — | 75 | 13.5 |
| (Traversa et al., 2015) | Italy | Europe | developed | 2008 | retailers | raw cow's milk | 46 | — | 453 | 14 |
| | Italy | Europe | developed | 2008 | — | raw cow's milk | 107 | — | 261 | 14 |
| (Cortimiglia et al., 2015) | Italy | Europe | developed | 2012 | farms | raw goat's milk | 85 | 4 | 197 | 11.5 |
| (Riva et al., 2015) | Italy | Europe | developed | 2012 | farms | raw cow's milk | 30 | — | 282 | 12.5 |
| | Italy | Europe | developed | 2012 | retailers | raw cow's milk | 5 | — | 101 | 12.5 |
| (Rola et al., 2015) | Poland | Europe | developed | 2009–2013 | farms | raw cow's milk | 69 | 0 | 115 | 10.5 |
| (Antonios et al., 2015) | Greece | Europe | developed | 2011–2013 | farms | raw sheep's milk | 79 | — | 342 | 14 |
| (Zeinhom et al., 2015) | Egypt | Africa | developing | — | retailers | raw cow's milk | 12 | — | 100 | 10.5 |
| (Cortimiglia et al., 2016) | Italy | Europe | developed | 2012–2013 | farms | raw cow's milk | 398 | 32 | 844 | 16 |
| (Bao et al., 2016) | China | Asia | developing | — | farms | raw cow's milk | 52 | 5 | 121 | 15 |
| (Bogdanovicova et al., 2016) | Czech Republic | Europe | developed | 2012–2014 | farms | raw cow's milk | 47 | — | 175 | 15 |
| | Czech Republic | Europe | developed | 2012–2014 | farms | raw goat's & sheep's milk | 20 | — | 55 | 15 |
| (Caruso et al., 2016) | Italy | Europe | developed | — | farms | raw goat's & sheep's milk | — | 2 | 162 | 14 |
| (Rola et al., 2016) | Poland | Europe | developed | 2011–2013 | farms | raw cow's milk | 12 | — | 26 | 12 |
| (Parisi et al., 2016) | Italy | Europe | developed | 2012–2013 | farms | raw cow's milk | — | 12 | 486 | 12.5 |
| (Bi et al., 2016) | China | Asia | developing | 2012–2013 | farms | raw cow's milk | 448 | — | 894 | 14.5 |
| (Song et al., 2016) | China | Asia | developing | 2009–2014 | retailers | pasteurized cow's milk | 3 | — | 100 | 13 |
| (da Costa et al., 2016) | USA | Americas | developed | 2015 | farms | raw cow's milk | 146 | — | 307 | 14 |

[a]No. of *S. aureus*-positive samples;
[b]No. of methicillin-resistant *S. aureus* (MRSA) positive-samples.

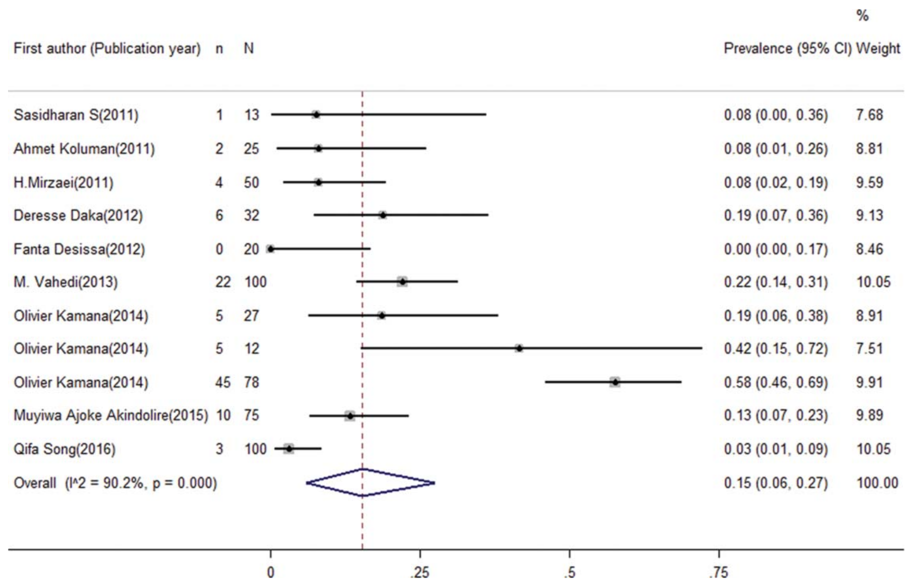


**Figure 2.** Forest plot of the prevalence of *Staphylococcus aureus* (*S. aureus*) and its 95% confidence interval (CI) in pasteurized and boiled cow's milk. **Label**: The combined prevalence was calculated using a random-effects model; $^{n}$*S. aureus*-positive sample size; $^{N}$total sample size; repeated first authors' names and publication years represent the same study but different sampling locations.

prevalence rates of 23.0% (95% CI, 19.1–27.2%) in 1998–2006 and 38.1% (95% CI, 29.3–47.3%) in 2007–2016. No significant differences were found in the prevalence rates of pasteurized and boiled cow's milk and raw Caprinae milk contaminated with *S. aureus*, nor in the prevalence rates of MRSA contamination in the three types of milk. The trends of *S. aureus* contamination prevalence rates and their 95% confidence interval (CI) in raw cow's milk over time are shown in Fig. 8.

The pooled prevalence rate of *S. aureus* contamination in raw cow's milk reported in Oceanian studies (62.3%; 95% CI, 17.1–97.3%) was the highest ($P < 0.01$), followed by the African studies (50.4%; 95% CI, 34.7–66.0%), North and South American studies (38.0%; 95% CI, 21.8–55.6%), Asian studies (27.8%; 95% CI, 18.5–38.1%) and European studies (22.8%; 95% CI, 19.0–27.0%). However, the pooled prevalence rate of *S. aureus* contamination in raw Caprinae milk was highest ($P = 0.00$) in North and South American studies (41.8%; 95% CI, 32.6–51.2%), followed by European studies (26.6%; 95% CI, 16.5–38.1%) and Asian studies (9.3%; 95% CI, 7.6–11.3%). There were no African or Oceanian studies available. In terms of MRSA contamination in pasteurized and boiled cow's milk, it was highest in African studies (15.6%; 95% CI, 4.8–30.6%), followed by Asian studies (2.0%; 95% CI, 0.0–7.3%). However, no significant differences were observed in the rates of *S. aureus* contamination in pasteurized and boiled cow's milk or MRSA contamination in raw cow's milk and raw Caprinae milk on different continents ($P > 0.05$).

Significant differences were also observed in the prevalence rates of raw Caprinae milk contamination with *S. aureus* in developed and developing nations ($P = 0.00$). The rate was 28.6% (95% CI, 18.7–39.6%) in developed nations but 9.3% (95% CI, 7.6–11.3%) in developing nations. No significant differences were found in the prevalence rates of *S. aureus* contamination in raw cow's milk or in the prevalence rates of MRSA contamination in raw cow's milk and raw Caprinae milk in different economic conditions ($P > 0.05$).

The pooled prevalence rate of *S. aureus* contamination in raw cow's milk samples obtained from processing companies (54.0%; 95% CI, 33.4–74.0%) was highest ($P < 0.01$), followed by those obtained from farms (32.3%; 95% CI, 27.7–36.9%) and those obtained from retailers (27.0%; 95% CI, 18.1–36.9%). However, the pooled prevalence rate of *S. aureus* contamination in raw Caprinae milk obtained from processing companies was higher than that of samples obtained from farms or retailers (91.8%, 23.1%, and 9.6%, $P = 0.00$). The prevalence rate of MRSA contamination in pasteurized and boiled cow's milk samples obtained from farms was higher than in those obtained from retailers ($P < 0.05$).

## *Assessment of publication bias*

No evidence in Begg's test suggested the presence of publication bias in the studies evaluating *S. aureus* contamination in pasteurized and boiled cow's milk ($P = 1.00$), raw cow's milk ($P = 0.65$) or raw Caprinae milk ($P = 0.20$). The $P$ values for publication bias in the studies evaluating MRSA contamination in pasteurized and boiled cow's milk, raw cow's milk, and raw Caprinae milk were 1.00, 0.71, and 0.73, respectively.

## *Sensitivity analysis*

To evaluate the robustness of the meta-analysis, we carried out a sensitivity analysis by recalculating the pooled results of the primary analysis by excluding each single study in turn. The sensitivity analysis noted that none of the single studies greatly influenced the overall pooled prevalence of *S. aureus* and MRSA contamination. The leave-one-out prevalence ranged from 0.73 (0.54–0.92) to 0.89 (0.60–1.18), 1.21 (1.13–1.30) to 1.26 (1.15–1.37), and 0.98 (0.79–1.17) to 1.13 (0.93–1.33) for *S. aureus* contamination in pasteurized and boiled cow's milk, raw cow's milk and raw Caprinae milk, respectively. However, the leave-one-out prevalence ranged from 0.36 (0.13–0.59) to

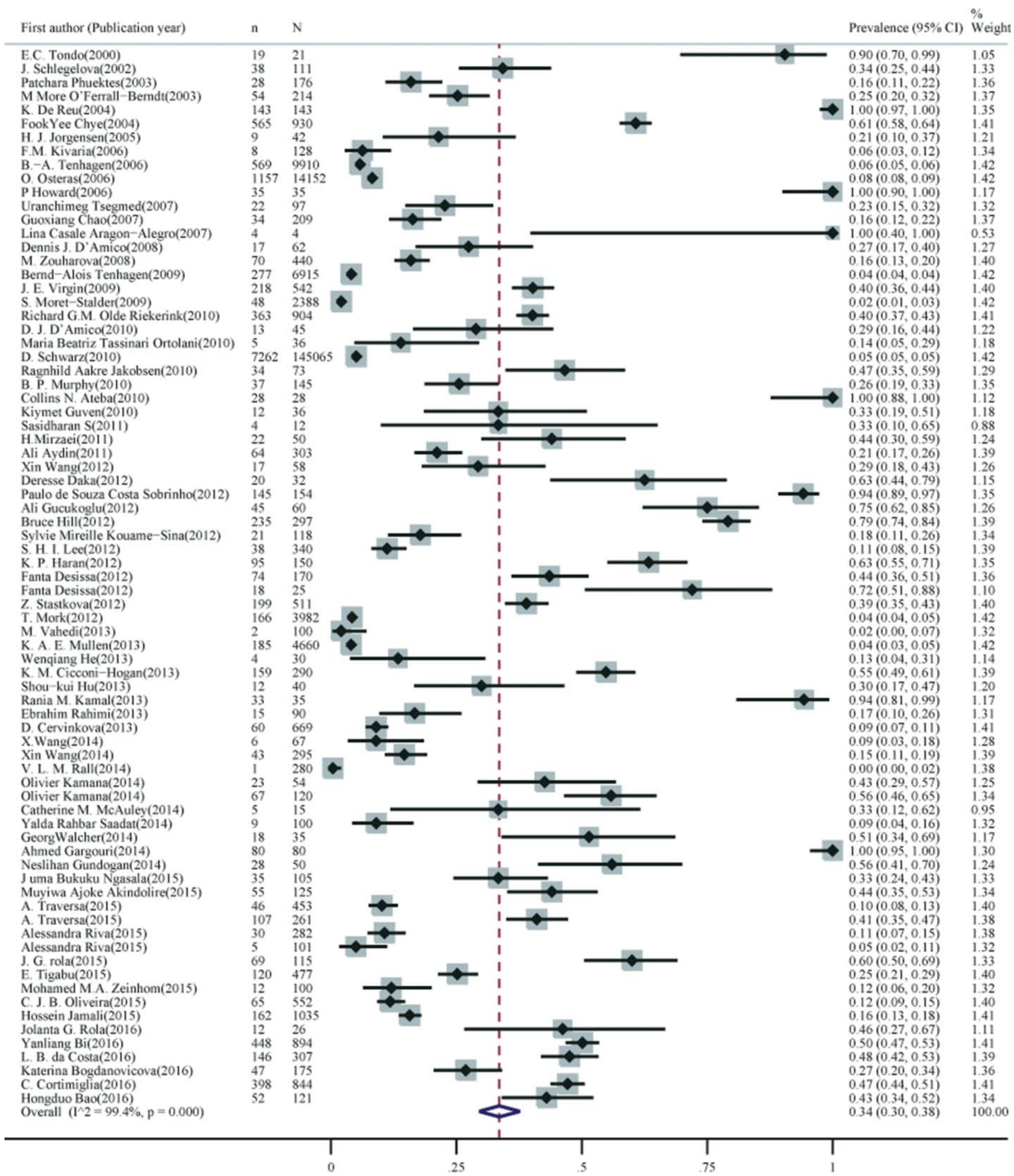

Figure 3. Forest plot of the prevalence of *Staphylococcus aureus* (*S. aureus*) and its 95% confidence interval (CI) in raw cow's milk. **Label**: The combined prevalence was calculated using a random-effects model; [n]*S. aureus*-positive sample size; [N]total sample size; repeated first authors' names and publication years represent the same study but different sampling locations.

0.63 (0.24–1.02), 0.30 (0.25–0.36) to 0.35 (0.29–0.41) and 0.22 (0.16–0.28) to 0.25 (0.15–0.34) for MRSA contamination in pasteurized and boiled cow's milk, raw cow's milk and raw Caprinae milk, respectively.

## Discussion

In this meta-analysis, 93 published studies from PubMed, EMBASE and Web of Science were included. We demonstrated that differences in the prevalence rates of *S. aureus* and MRSA contamination in three types of milk were statistically significant. The pooled prevalence rates of *S. aureus* contamination in pasteurized and boiled cow's milk, raw cow's milk and raw Caprinae milk were 15.4% (95% CI, 6.1–27.5%), 33.5% (95% CI, 29.5–37.7%) and 25.8% (95% CI, 17.5–35.0%), respectively. The contamination prevalence rate of raw cow's milk was double that of pasteurized and boiled cow's milk. On one hand, these results indicate that pasteurization (71.6°C for 15 s and 62.7°C for 30 min) and boiling treatment (when the milk temperature was approximately 95°C) can be very effective in lowering *S. aureus* contamination of milk. On the other hand, *S. aureus* contamination was still observed in milk that had been pasteurized and heated. If these findings are true, they may be attributable to insufficient heat treatment during pasteurization or post-pasteurization contamination after a heat process, such as

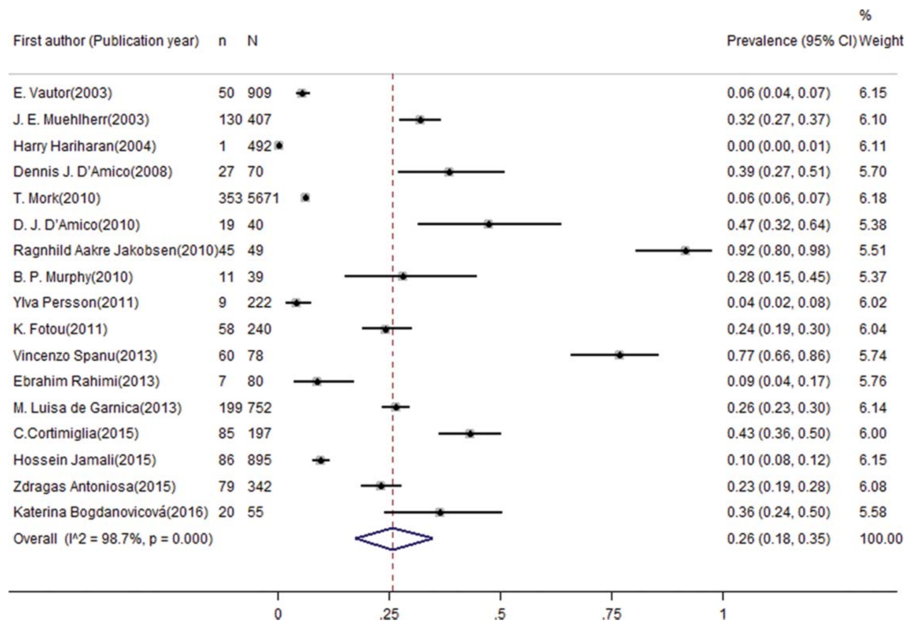


**Figure 4.** Forest plot of the prevalence of *Staphylococcus aureus* (*S. aureus*) and its 95% confidence interval (CI) in raw Caprinae milk. **Label**: The combined prevalence was calculated using a random-effects model; [n]*S. aureus*-positive sample size; [N]total sample size.

contamination by food handlers. Kamana et al. (2014) also noted that increasing bacterial numbers occurred along the retail chain and could be attributed to insufficient temperature control during storage. Milk handling after pasteurization was recognized as an important problem on the farms studied (Elizondo-Salazar et al., 2010). The hygienic conditions and pasteurization process along with the cold chain of milk from suppliers to consumers need to be improved. Agarwal et al. (2012) suggested that milk should be boiled before consumption and refrigerated for storage to improve its keeping quality and shelf life. However, the pooled prevalence rates of MRSA in pasteurized and boiled cow's milk, raw cow's milk and raw Caprinae milk were 4.9% (95% CI, 0.0–15.7%), 2.3% (95% CI, 1.3–3.6%) and 1.1% (95% CI, 0.5–1.8%), respectively, and it was highest in pasteurized and boiled cow's milk. However, the limited number of studies focused on MRSA contamination in pasteurized and boiled cow's milk, raw cow's milk and raw Caprinae milk should be noticed.

Subgroup analyses demonstrated that some factors with available information, such as sample size, may partly lead to the heterogeneity of *S. aureus* contamination in raw cow's milk and raw Caprinae milk. Subgroup analysis indicated an increasing trend in the pooled prevalence rates of *S. aureus* contamination in raw cow's milk over time. The prevalence rate of 2007–2016 was 1.65 times that of 1998–2006. Raw milk contamination is the leading source of contamination in final milk products (Tondo et al., 2000), such as cheese, milk powder, fermented milk, etc. To improve the quality of milk and consequently the dairy products, correct handling of milk and dairy products, sanitization of milk containers, effective cleaning and use of HACCP programs must be put into practice (Sospedra et al., 2009).

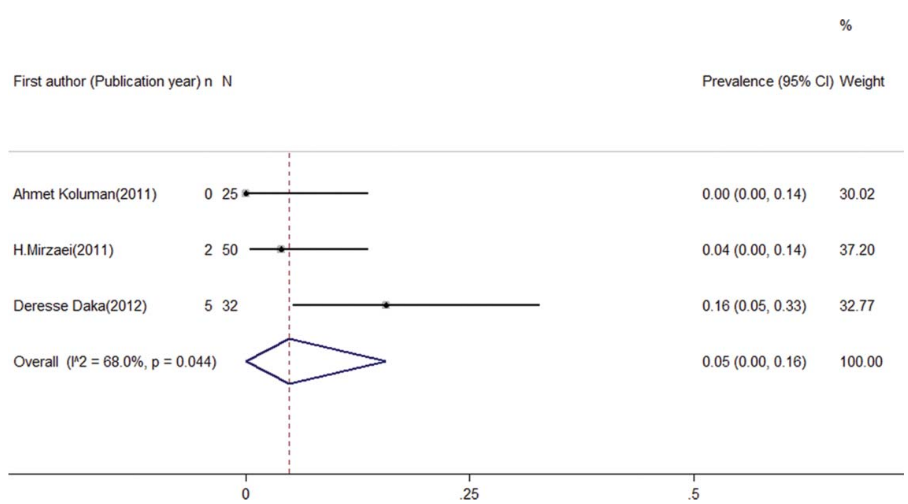


**Figure 5.** Forest plot for the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) and its 95% confidence interval (CI) in pasteurized and boiled cow's milk. **Label**: The combined prevalence was calculated using a random-effects model; [n]MRSA-positive sample size; [N]total sample size.

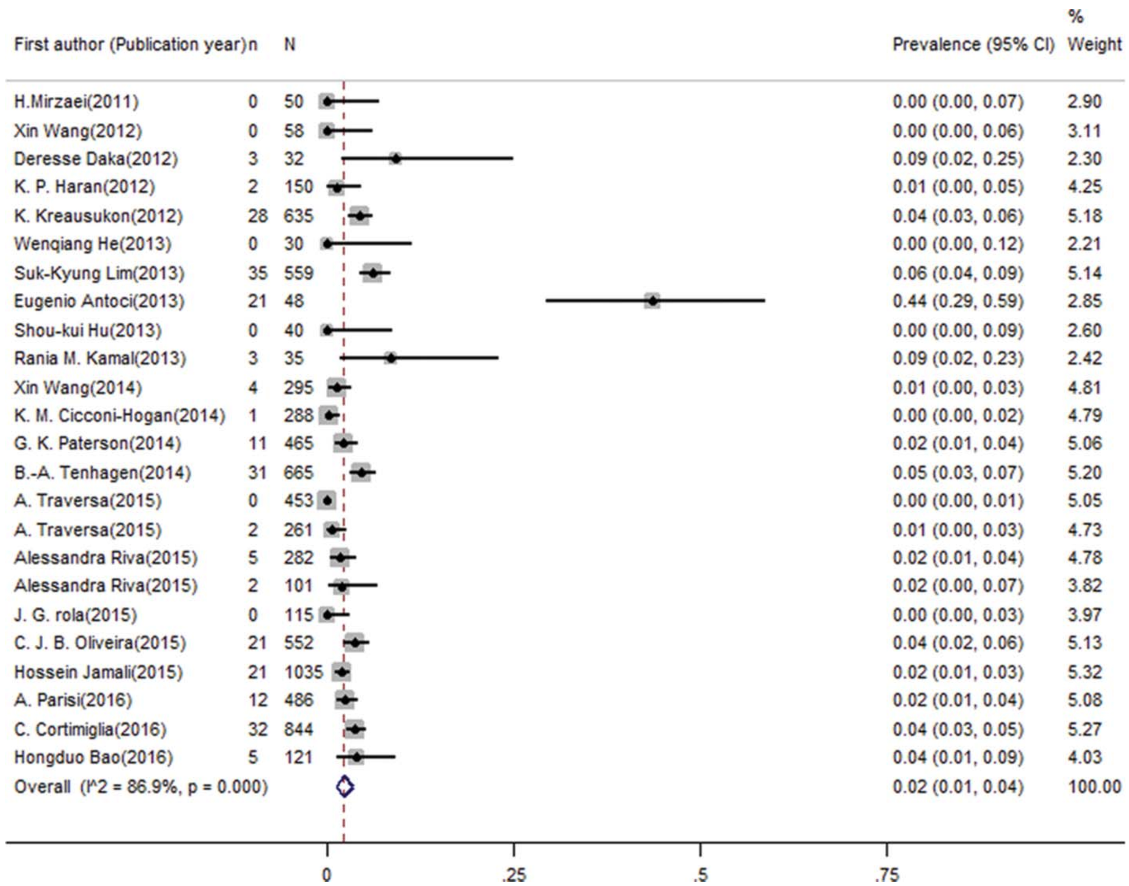


**Figure 6.** Forest plot for the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) and its 95% confidence interval (CI) in raw cow's milk. **Label**: The combined prevalence was calculated using a random-effects model; $^{n}$MRSA-positive sample size; $^{N}$total sample size; repeated first authors' names and publication years represent the same study but different sampling locations.

The results showed the high quality of European and Asian milk, with low prevalence rates of raw cow's milk and raw Caprinae milk contaminated with *S. aureus*. The high quality of raw cow's milk in Europe is mainly attributable to high standards of breeding and regulation. Humid climates, natural farms and grazing raising patterns in many European countries also reduce the incidence of metabolic diseases in dairy cows, goats and sheep. Moreover, there are complete systems of food security and rigorous punishment, especially for milk regulation. The institution of the European Food Safety Agency by the European Union (EU) mandated a high level of consumer protection in regard to food security. According to European legislation, the producer is legally responsible for the safety of primary foods. To ensure food security, the official supervision

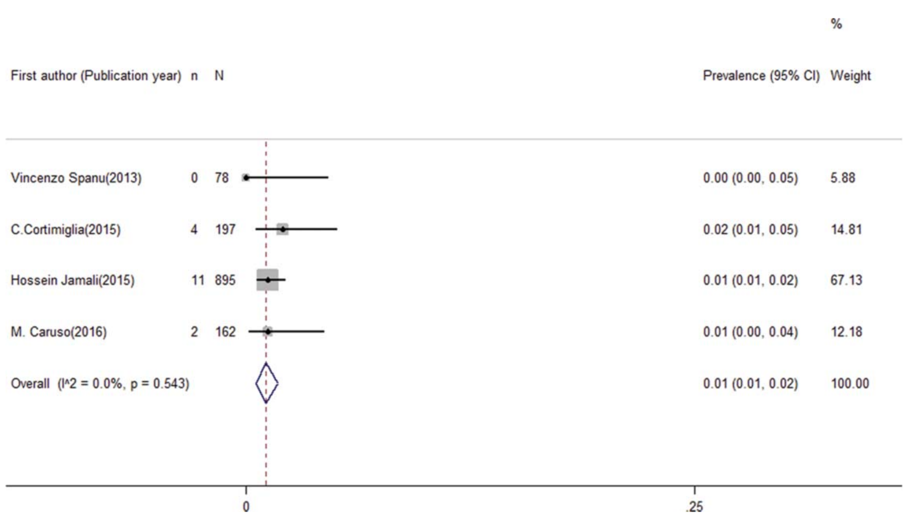


**Figure 7.** Forest plot of the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) and its 95% confidence interval (CI) in raw Caprinae milk. **Label**: The combined prevalence was calculated using a fixed-effects model; $^{n}$MRSA-positive sample size; $^{N}$total sample size.

**Table 2.** Prevalence and 95% confidence interval (CI) of *S. aureus* and methicillin-resistant *S. aureus* (MRSA) by sample size, study year, continent, economic condition, and sampling location.

| | No. of records | | | | | | Prevalence % (95% CI) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *S. aureus* | | | MRSA | | | *S. aureus* | | | MRSA | | |
| Subgroups | a | b | c | a | b | c | a | b | c | a | b | c |
| Sample size | | | | | | | | | | | | |
| ≤110 | 11 | 33 | 7 | 3 | 8 | 1 | 15.4(6.1–27.5) | *44.0(31.3–57.1) | *46.9(23.2–71.4) | 4.9(0.0–15.7) | 4.1(0.0–13.3) | 0.0(0.0–2.2) |
| >110 | 0 | 44 | 10 | 0 | 16 | 3 | — | 28.1(23.6–32.8) | 14.6(8.0–22.7) | — | 2.1(1.3–3.2) | 1.3(0.7–2.0) |
| Study year | | | | | | | | | | | | |
| 1998–2006 | 0 | 19 | 5 | 0 | 0 | 0 | — | *23.0(19.1–27.2) | 19.1(9.1–31.7) | — | — | — |
| 2007–2016 | 10 | 44 | 8 | 2 | 22 | 2 | 16.2(6.1–29.4) | 38.1(29.3–47.3) | 22.3(13.0–33.2) | 8.6(0.5–23.0) | 2.2(1.1–3.5) | 1.3(0.7–2.1) |
| Continent | | | | | | | | | | | | |
| Asia | 5 | 20 | 2 | 2 | 8 | 1 | 9.0(2.2–18.9) | *27.8(18.5–38.1) | *9.3(7.6–11.3) | *2.0(0.0–7.3) | 1.6(0.3–3.4) | 1.2(0.6–2.1) |
| Africa | 6 | 15 | 0 | 1 | 2 | 0 | 21.8(5.7–43.5) | 50.4(34.7–66.0) | — | 15.6(4.8–30.6) | 8.9(2.9–17.4) | — |
| Americas | 0 | 15 | 2 | 0 | 3 | 0 | — | 38.0(21.8–55.6) | 41.8(32.6–51.2) | — | 1.6(0.1–4.5) | — |
| Oceania | 0 | 4 | 0 | 0 | 0 | 0 | — | 62.3(17.1–97.3) | — | — | — | — |
| Europe | 0 | 23 | 13 | 0 | 11 | 3 | — | 22.8(19.0–27.0) | 26.6(16.5–38.1) | — | 2.9(1.3–5.2) | 1.2(0.3–2.5) |
| Economic condition | | | | | | | | | | | | |
| developed | 0 | 34 | 15 | 0 | 13 | 3 | — | 29.1(24.6–33.7) | *28.6(18.7–39.6) | — | 3.1(1.5–5.0) | 1.2(0.3–2.5) |
| developing | 11 | 43 | 2 | 3 | 11 | 1 | 15.4(6.1–27.5) | 37.9(29.4–46.8) | 9.3(7.6–11.3) | 4.9(0.0–15.7) | 1.5(0.5–2.8) | 1.2(0.6–2.1) |
| Sampling location | | | | | | | | | | | | |
| retailers | 6 | 12 | 1 | 2 | 4 | 1 | 13.1(1.3–32.8) | *27.0(18.1–36.9) | *9.6(7.8–11.6) | *2.0(0.0–7.3) | 0.6(0.0–2.8) | 1.2(0.6–2.1) |
| farms | 1 | 54 | 15 | 1 | 18 | 3 | 18.7(6.8–34.4) | 32.3(27.7–36.9) | 23.1(14.7–32.8) | 15.6(4.8–30.6) | 2.9(1.7–4.3) | 1.2(0.3–2.5) |
| processing companies | 1 | 5 | 1 | 0 | 0 | 0 | 18.5(5.7–35.7) | 54.0(33.4–74.0) | 91.8(82.2–98.2) | — | — | — |

[a]Pasteurized and boiled cow's milk;
[b]raw cow's milk;
[c]raw Caprinae milk; - no available data; *significant difference, $P < 0.05$.

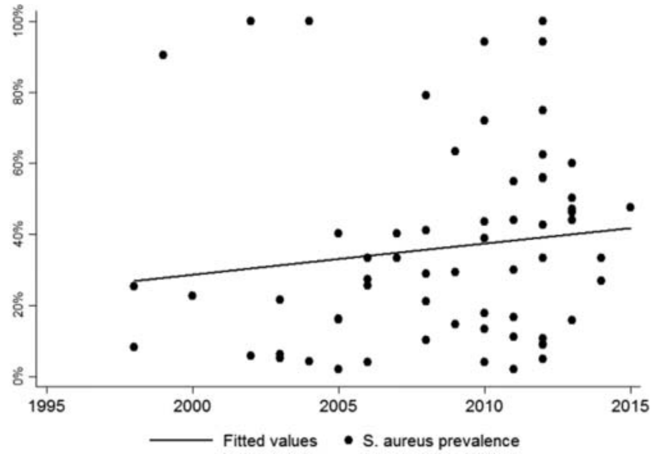


**Figure 8.** Trends in *Staphylococcus aureus* (*S. aureus*) prevalence and its 95% confidence interval (CI) in sampled raw cow's milk based on the year during which the study was conducted.

must be applied along with a self-regulation regimen by farmers (Cavirani, 2008). We also identified the lowest *S. aureus* prevalence rate in raw Caprinae milk and the lowest MRSA prevalence rate in pasteurized and boiled cow's milk in Asia. However, there were no available studies from other continents, and perhaps the number of included studies was too small to yield a reliable result. The causal relationship between raw Caprinae milk and pasteurized and boiled cow's milk contamination proportions and different continents still needs to be further clarified.

There were no significant differences of *S. aureus* or MRSA contamination in raw cow's milk between developed and developing nations. However, we found that the pooled prevalence rate of *S. aureus* contamination in raw Caprinae milk in studies of developed countries was higher than that of developing countries. Explanations for these results, if true, are unclear. These findings underscore the need for further study to clarify the matter. Perhaps the limited number of articles included in this subgroup analysis should be taken into account.

We reported that the pooled prevalence rates of *S. aureus* contamination in raw cow's milk and raw Caprinae milk sold by farms and processing companies were significantly higher than in similar products sold in retail contexts, such as markets and grocery stores. The high prevalence rates of *S. aureus* contamination in raw and processed milk at farms and processing companies generally suggested low levels of milking hygienic practices. The high *S. aureus* counts of both raw and processed milk may represent a public health risk (Mhone et al., 2011). Some studies indicated that *S. aureus* was found throughout the farm and spread with the milk to the equipment, environment, and dairy products (Jørgensen et al., 2005). Moreover, Lim et al. (2013) also noted the evidence of transmission of MRSA among cattle, farmers, and the environment as well as the long-term existence of MRSA in animals, emphasizing the need to improve sanitary conditions in the milking environment. Since food handlers could carry *S. aureus*, good hygiene behavior should be emphasized. Installing effective cooling facilities on farms, educating the farmers on general hygienic practices, and shortening the delivery time of milk to collection centers will improve the safety and microbiological quality of milk (Mhone et al., 2011).

Raw cow's milk contaminated by *S. aureus* probably causes human alimentary tract diseases and poses a severe public health risk to consumers. Milk contamination could occur directly from infected cows, goats and sheep, or it results from poor cleaning of the pasteurizer or lack of refrigerated storage. Normanno et al. (2007) noted that the presence of enterotoxigenic and antimicrobial resistant *S. aureus* (ARSA) strains was widespread in meat and milk. Therefore, prudent use of antibiotics in veterinary medicine and correct pasteurization are significant to ensure the safety of milk. Strategies to reduce the presence of *S. aureus* in raw milk are particularly important because raw milk is used for raw milk products (Jørgensen et al., 2005). Krishnamurthy et al. (2008) also suggested that the infrared (IR) heating can potentially be a selective heating method for *S. aureus* in milk. The risk of human diseases from consumption of raw milk could be eliminated by raising awareness of heat treatment and good hygiene practices throughout the dairy chain (Kouamé-Sina et al., 2012). Heat treatment used to control *S. aureus* may be an appropriate method to improve the safety of milk and dairy products. What is worse, contact with or consumption of milk and dairy products without heat treatment may result in the spread of antimicrobial resistance genes in *Staphylococcus* spp. to humans (McKay, 2008). It has been demonstrated that proper management, including better awareness for efficient milking and hygienic handling, has controlled the transmission of gram-positive bacteria and significantly decreased subclinical mastitis and the proportion of *S. aureus* isolates (Burvenich et al., 2003).

There are several limitations to this meta-analysis. First, some information may be lost, because we only included studies that reported sample size. Studies that did not report the study year or sampling location also restricted the results of the subgroup analyses. Second, among the 93 included studies, the sample size, sampling method, study year, studied country and sampling location varied. Differences observed across the included studies resulted in high heterogeneity in this meta-analysis. However, some predefined variables can explain the variance in pooled prevalence rates of *S. aureus* and MRSA contamination in subgroup analyses. Despite the limitations of this meta-analysis, it is the first meta-analysis about the pooled prevalence rates of *S. aureus* and MRSA contamination in cow's milk and Caprinae milk all over the world. Moreover, we systematically analyzed *S. aureus* and MRSA contamination in cow's milk and Caprinae milk across different periods, continents, economic conditions and sampling locations in the subgroup analyses. Furthermore, a large number of studies were included. Publication bias and sensitivity analysis indicated that the results were reliable and stable.

## Conclusion

In conclusion, our meta-analysis demonstrates that the pooled prevalence rates of *S. aureus* contamination in pasteurized and boiled cow's milk, raw cow's milk and raw Caprinae milk were 15.4% (95% CI, 6.1–27.5%), 33.5% (95% CI, 29.5–37.7%) and 25.8% (95% CI, 17.5–35.0%), respectively. The pooled prevalence rates of MRSA contamination were 4.9% (95% CI, 0.0–15.7%), 2.3% (95% CI, 1.3–3.6%), and 1.1% (95% CI, 0.5–1.8%), respectively. We also found that *S. aureus* contamination in raw cow's milk has significantly increased over time. The prevalence rate of *S. aureus* contamination in raw cow's milk was lowest in Europe. These findings give an indication of the consequences of better milk regulation in Europe. The high prevalence rates of *S. aureus* contamination in raw milk sampled from farms and processing companies pose a potential threat to consumers and justify the need for better control of sources of contamination.

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## Conflict of interest

The authors have no conflicts of interest.

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