

REVIEW

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Prevalence and molecular characteristics of *Staphylococcus aureus* in raw milk and milk products in Ethiopia: a systematic review and meta-analysis

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Abstract

Background: *Staphylococcus aureus* is an important opportunistic pathogen of raw milk and milk products, and the enterotoxins cause food poisoning. Milk and milk products are important reservoirs of enterotoxin-producing *S. aureus*. The aims of this systematic review were to estimate the pooled prevalence of *S. aureus*, including methicillin-resistant *Staphylococcus aureus* (MRSA), and to summarize their molecular characteristics, assess the potential sources of *S. aureus* contamination in bulk milk and analyse the antimicrobial resistance patterns of the isolates.

Methods: Following Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, we searched publicly available scientific online databases and search engines: PubMed, Research for Life, African Journal Online (AJOL), and Google Scholar. In addition, the reference lists of the identified studies were manually checked for relevant literature. A random effects model using the DerSimonian method was used to compute pooled prevalence estimates, and the data were transformed using variance stabilizing Freeman-Tukey double arcsine transformation.

Results: A total of 38 studies were included in this systematic review. The pooled prevalence of *S. aureus* was highest in raw cow milk (30.7%), followed by camel milk (19.3%), goat milk (13.6%) and pasteurized milk (3.8%). The pooled prevalence of *S. aureus* in locally produced soft cheese (*ayib*) and traditional fermented milk (*ergo*) was 18.6% and 14.9%, respectively. The pooled prevalence of MRSA in milk and milk products was 0.73%. In this study, 58.9% of *S. aureus* isolates recovered from milk and milk products harbored at least one type of enterotoxin gene. Raw milk of the three species (cow, goat and camel) showed the highest *S. aureus* pooled prevalence rate at processing plants (50.3%), followed by milk collection centers (MCCs) (47.1%), selling points (34.5%), farm bulk milk (25.8%), milking buckets (24.8%) and udder milk (20.3%). Water for washing milking utensils (39.3%) was more contaminated than swab samples from farm workers' nares (31.5%), milkers' hands (25.9%), MCCs containers (23.8%), bulk tanks (20.4%), udders (15.6%), milking buckets (14.2%) and towels (10%). *S. aureus* isolates were highly resistant to penicillin G (92%), followed by ampicillin (82%) and amoxicillin (62.6%). The pooled multidrug resistance (MDR) was high (62.1%).

Conclusion: This systematic review revealed a high and increasing level of *S. aureus* contamination of raw milk from udder to MCCs or processing plants. Enterotoxin genes and MRSA were reported in milk, milk products and samples from farm workers. *S. aureus* showed resistance to different antimicrobial agents, with β -lactams showing the highest

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pooled antimicrobial resistance and the level of MDR was high. The results of this study indicated that the consumption of raw milk and milk products may predispose consumers to staphylococcal food poisoning. Application of good hygiene and handling practices across the dairy value chain starting from farm, udder health, milk cooling, heat treatment of milk before drinking and rational use of antibiotics in veterinary medicine can reduce the potential health risks from *S. aureus* and MRSA contamination of milk and milk products.

Keywords: Antimicrobial resistance, Enterotoxin genes, Ethiopia, Milk, Milk products, MRSA, *Staphylococcus aureus*, Systematic review and meta-analysis

Introduction

Milk is considered to be the most balanced food containing most of the nutrients. Being nutritious in nature, milk provides an ideal growth environment for many microorganisms, including pathogenic bacteria (FAO 2013). Milk is considered to be free of bacteria in healthy mammary glands. However, once it is secreted from the udder, milk can easily be contaminated by spoilage bacteria and pathogens from various sources, including feed, water, dairy farm environments, udder and teat surfaces, equipment, raw milk tanks and personnel. Milk can be contaminated before it is secreted from the udder by mastitis organisms, and this is the main source of milk contamination with pathogenic bacteria (Oliver et al. 2005; Owusu-kwarteng et al. 2020). The high temperature in sub-Saharan Africa, including Ethiopia, may also exacerbate the conditions by accelerating the growth and multiplication of microorganisms (Owusu-kwarteng et al. 2020).

Dairy marketing and trade in most African countries, including Ethiopia, is dominated by informal and small-scale channels selling unpasteurized milk. The informal market is generally characterized by the predominance of traditional processing, products and retail practices and limited access to infrastructures including water, electricity, sanitation, cold chain and refrigeration. There are few or no health and safety regulations (Blackmore et al. 2020; Roesel and Grace 2014). The informal dairy sector is associated with low-quality milk and food safety risks (Nacul and Giha 2022). Despite the fact that numerous studies in various parts of Ethiopia isolated food-borne pathogens of public health significance from raw milk, people still consume raw milk claiming flavor, availability, price and perceived higher nutritional value benefits (Amenu et al. 2019; Ayele et al. 2017; Keba et al. 2020).

Staphylococcus aureus is part of normal microflora of skin and mucous membranes of animals and humans (Rajkovic et al. 2019; Hennekinne et al. 2012). *S. aureus* is a major cause of mastitis in dairy cows and incurs a significant economic loss to the dairy industry worldwide (Haag et al. 2019; Peton and Le 2014). *S. aureus* can be shed into milk from udders infected with mastitis and poses a significant public health hazard (Haag et al. 2019). *S. aureus* is the most significant human

pathogenic staphylococcal species and causes a variety of infections ranging from mild superficial skin lesions to life-threatening diseases (Tong et al. 2015). Animals can act as reservoirs for staphylococcal transmission to humans (Haag et al. 2019).

Staphylococcus aureus is an important opportunistic pathogen of raw milk, and enterotoxins cause food poisoning. Milk and milk products are important reservoirs of enterotoxin-producing *S. aureus*. Staphylococcal food poisoning (SFP) is among the most prevalent causes of gastroenteritis in the world. SFP results from the consumption of preformed *S. aureus* enterotoxins in food (Rajkovic et al. 2019; Park et al. 2019). A small dose of staphylococcal enterotoxins (SEs) can cause illness. The toxin dose to cause symptoms of SFP is 100–200 ng of enterotoxin, which is produced when the *S. aureus* count in food exceeds 10^5 cfu/gm. Improper holding times and temperatures are the most important factors associated with SFP outbreaks (Park et al. 2019; Rajkovic 2012). SEs are heat-stable and are able to survive pasteurization (Park et al. 2019).

To date, 24 different SEs have been determined: classical enterotoxins (SEA, SEB, SEC, SED, SEE) and non-classical or new enterotoxins (SEG, SEH, SEI, SER, SES, SET) and SEIs (SEJ, SEK, SEL, SEM, SEN, SEO, SEI, SEQ, SEU, SEW, SEV, SEY and SEZ) (Rajkovic 2012). The five classical SEs are encoded by specific enterotoxin genes, such as *sea*, *seb*, *sec*, *sed*, and *see*. Classical enterotoxins are implicated in 95% of food related enterotoxin outbreaks in the United States (Kou et al. 2021). SFP is characterized by a sudden onset of symptoms, with vomiting, abdominal pain and nausea being the most common. Certain foods, such as raw milk and milk products, meat and meat products, poultry and egg products and salad, are regarded as potential vehicles for the transmission of *S. aureus* to humans (Rajkovic et al. 2019). Several SFP outbreaks associated with the consumption of contaminated milk and dairy products have been reported (Hennekinne et al. 2012; Johler et al. 2015; Fetsch et al. 2014; Motarjemi et al. 2014; Verraes et al. 2014).

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major drug-resistant pathogen responsible for a wide variety of infections worldwide, ranging from

mild skin infections to life-threatening invasive diseases (Aires-de-Sousa 2017). MRSA causes mastitis in cattle. *S. aureus* becomes methicillin resistant by acquisition of the *mecA* gene. The gene is responsible for the production of an altered penicillin-binding protein 2a (PBP2a). PBP2a has a lower affinity for β -lactams than normal PBP. Thus, β -lactams are not effective for treating mastitis caused by MRSA, and culling might be the only chance to remove MRSA from dairy herds. MRSA in dairy herds represents a possible health hazard for both humans and cattle (Schnitt and Tenhagen 2019).

Several molecular typing methods, such as multilocus sequence typing (MLST), staphylococcal protein A (*spa*) typing, staphylococcal cassette chromosome (*SCC_{mec}*) typing and pulse-field gel electrophoresis (PFGE), are widely used to determine the genetic relatedness of *S. aureus* isolates. Whole genome sequencing has become a powerful tool in food safety and may play an extremely important role in food safety surveillance and risk assessment (Rajkovic et al. 2019).

Antibiotic therapy is an important measure to treat infections in animals and humans. However, heavy use and misuse of antibiotics have led to the emergence of resistant strains, leading to reduced effectiveness in treating diseases in humans and animals (Chambers and Deleo 2010; Economou and Gousia 2015). In developing countries, including Ethiopia, antibiotics are misused and abused due to their availability over the counter, lack of access to appropriate antimicrobial therapy, unregulated supply chains, purchase without prescriptions and engaging unskilled people to treat animals (Manyi-Loh et al. 2018; Kimera et al. 2020). Antibiotic-resistant bacteria associated with animals can be transmitted to humans through the food chain (Guo et al. 2021). *S. aureus* has an extraordinary ability to develop resistance to antibiotic drugs used for therapeutics, narrowing treatment options in animal and human medicines (Fuda et al. 2005). Widespread nonadherence to rational drug use, which favors the selection of drug-resistant pathogens, was reported in Ethiopia (Kifle and Tadesse 2014).

Many studies have reported contamination of milk and milk products by *S. aureus* in Ethiopia. However, the data are fragmented, and the national contamination burden remains unknown. Quantitative synthesis of data from previous study reports enables understanding the burden of the problem and helps in designing intervention measures to reduce the risk to consumers and livestock production. Therefore, the objectives of this systematic review were to estimate the pooled prevalence of *S. aureus*, including MRSA, summarize their molecular characteristics, assess the potential sources of *S. aureus*

contamination in bulk milk and analyse the antimicrobial resistance patterns of the isolates.

Methods

Systematic review protocol

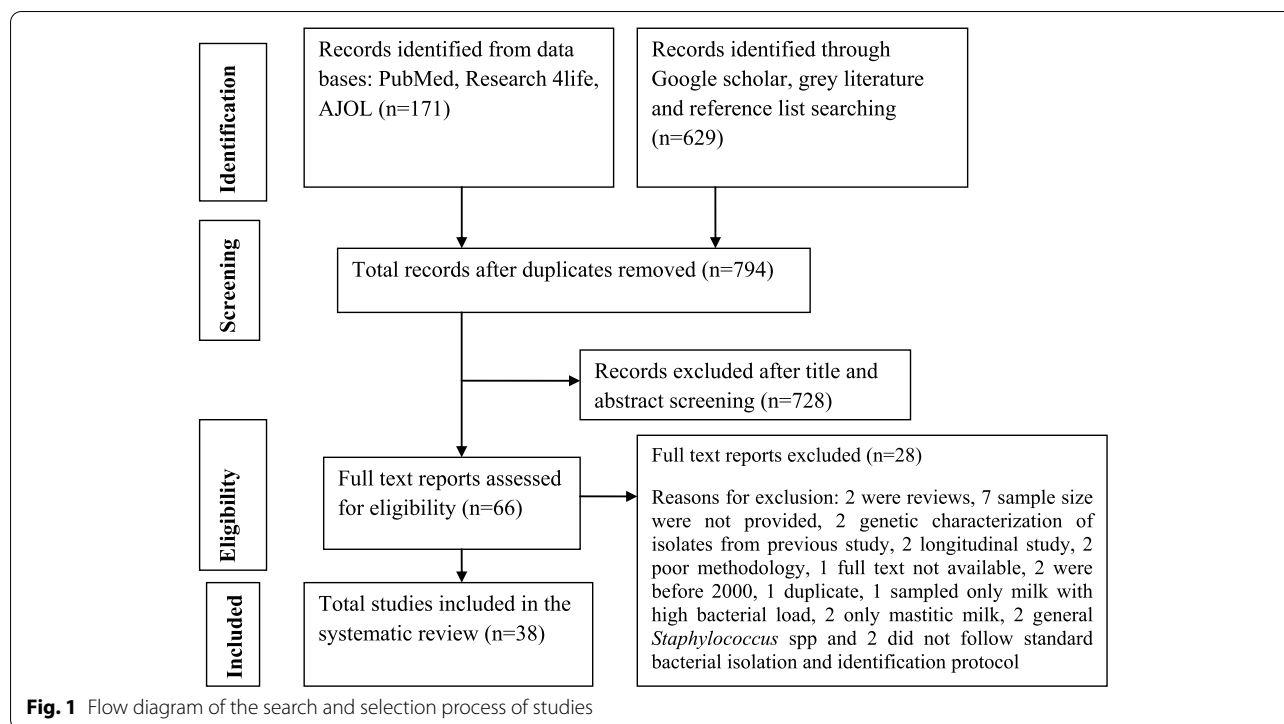
This systematic review and meta-analysis was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines. The PRISMA 2020 item checklist (Additional file 1) was used to ensure the inclusion of relevant information in the analysis (Moher et al. 2009; Page et al. 2020).

Search strategy

The search and selection strategy of the studies is presented in Fig. 1. Study reports were searched in publicly available online databases including PubMed, Research for Life, African Journal Online (AJOL) and the Web search engine of Google Scholar. In addition, the reference lists of the identified studies were screened to capture any relevant report that might have been missed. Studies published since 2000 were included. The search terms used were “milk”, “*Staphylococcus aureus/S. aureus/methicillin resistant Staphylococcus aureus/MRSA*”, “prevalence/Occurrence” and “Ethiopia”. Additional searches were performed by combining these search terms with local cheese, yoghurt, fermented milk, cow milk, camel milk or goat milk. The last search was performed on July 25, 2021.

Eligibility criteria

The studies were selected based on predefined inclusion and exclusion criteria. The studies were selected by screening title and abstract and then followed by full text reading. To qualify for inclusion in the systematic review, studies had to meet the following criteria: (i) they were published in English; (ii) the study design was cross-sectional; (iii) the study was published between 2000 and July 25, 2021; (iv) the samples were milk or milk products; for udder milk, it was randomly sampled without considering mastitis status; (v) standard bacteriological methods were used for isolation and identification of *S. aureus*: growth and colony morphology on blood agar or selective media, Gram staining, coagulase test and biochemical tests; (vi) for MRSA, studies that used molecular methods to detect the *mecA* gene were selected and (vii) relevant data to compute prevalence were provided. Reviews, longitudinal studies, studies published before 2000, studies with full text irretrievable, studies that did not follow standard bacteriological procedures and studies based on samples that selectively taken from diseased or mastitic cows were excluded.



Data extraction

After downloading the full papers, the relevant data were extracted from each article and recorded in a Microsoft Excel template. From each eligible study, the author, year of study, study region and area, livestock species, sampling point along the milk value chain, sample size and the numbers of *S. aureus*- or MRSA-positive samples were extracted. Data on the proportion of *S. aureus* isolates that carried enterotoxin genes and the likely risk of illness from *S. aureus* due to consumption of raw milk and milk products were also extracted. For the study on antimicrobial resistance, the type of antimicrobial drug, the number of isolates tested and the number of resistant isolates were extracted in addition to the variables mentioned above. Milk sampling points along the dairy chain were grouped into udder, milking bucket, farm bulk milk, milk collection center, selling point and processing plant levels. Milk samples collected from any milk selling establishment (wholesale, cafeteria, restaurant, milk shop, open market) were grouped into selling point milk. Processing plant milk is milk sampled from transport containers upon reaching the milk processing plant. Study selection and data extraction were performed by AD and KA and independently checked by GM and SL. Disagreements among reviewers were resolved by discussion.

Data analysis

We used variance stabilizing Freeman-Tukey double arcsine transformation to compute pooled prevalence estimates so that studies with a prevalence of 0 or 100% would not be excluded from the analysis. The study-level prevalence was transformed by the formula $t = \sin^{-1}(\sqrt{n/(N+1)} + \sin^{-1}(\sqrt{(n+1)/(N+1)})$, and the standard error of the estimate was calculated by formula $se(t) = \sqrt{1/(N+0.5)}$ (Barendregt et al. 2013). Where, t is the transformed value, N is the sample size and n is the number of *S. aureus*-positive samples. Pooled estimates were back transformed to prevalence by the formula $p = 0.5\{1 - \text{sgn}(\cos t) [1 - (\sin t + (\sin t - 1/\sin t)/N)^2]^{1/2}\}$, where $\text{sgn}(\cos t) = 1$ if $\cos t > 0$ and -1 if $\cos t < 0$ (Miller 1978). A random effects model using the DerSimonian and Laird (DerSimonian and Laird 1986) method was used to pool double arcsine estimates. Sensitivity analysis was conducted to assess the robustness of the synthesized results. Data analysis was performed using STATA 16 (StataCorp, Lakeway Drive, College Station, Texas, USA). A p -value of 0.05 or lower was considered to be statistically significant.

Assessment of risk of bias

The within study bias was assessed by the quality of the study design and the microbiological methods used. A funnel plot was used to visually examine the study bias (small study effects), where an asymmetrical distribution of studies was suggestive of bias (Sterne and Harbord

2004). Egger's regression test was used to check the statistical significance of bias, with $p < 0.05$ indicating significant publication bias (Egger et al. 1997).

Heterogeneity analysis

The between-study heterogeneity was quantified using inverse variance index (I^2) statistics, which estimate the percentage of variation between studies that is attributable to heterogeneity. I^2 values were interpreted as low, moderate and high referring to 25%, 50% and 75% degrees of heterogeneity, respectively. The significance of the heterogeneities was assessed by Cochran's Q test, and a nonsignificant heterogeneity was accepted if the ratio of Q and the degree of freedom (Q/df) was less than one (Higgins et al. 2003).

Results

Included studies

We retrieved a total of 794 (after duplicates were removed) potentially relevant studies, of which 728 were excluded following screening of titles and abstracts. Full texts of 66 studies were evaluated for eligibility, out of which 28 were excluded for various reasons. Finally, 38 studies that met the eligibility criteria were included in the systematic review. Of these, 35 were published research articles, while 3 were unpublished MSC dissertation papers. The flow diagram of the eligible study search and selection process is presented in Fig. 1.

Characteristics of eligible studies

The characteristics of the eligible studies are summarized in Tables 1, 2 and 3. The studies were conducted between 2003 and 2020. A total of 5718 milk samples (4856 cow, 560 camel, and 157 goats, 145 pasteurized), 637 local soft cheese (*ayib*) and 437 traditional fermented milk (*ergo*) collected from various sampling points along the dairy value chain were examined for *S. aureus* contamination. A total of 422 and 269 *S. aureus* isolates were tested for the presence of MRSA and virulence genes, respectively. For the production of *ergo*, raw milk is stored in a warm place to induce spontaneous fermentation. After approximately 24 h, the product is similar to sour milk (yogurt). To produce *ayib* (local soft cheese), *ergo* is further processed by churning, and the fat is removed. The residue (butter milk) is heated to 40–50°C, until the curd and whey separate. After cooling, the whey is strained off with fiber from false banana or muslin cloth, and then the cheese curd (*ayib*) is kept in a clean bowl or pot (Roesel and Grace 2014; Yilma 2003). Out of the 38 studies selected for analysis, 14 studies reported on antimicrobial resistance profiles of the *S. aureus* isolates. A total of 7133 *S. aureus* isolates were tested for their antimicrobial resistance profiles.

Pooled prevalence estimates

Raw and pasteurized milk

The pooled prevalence rates of *S. aureus* in milk of different species at different sampling points are summarized in Table 4. The overall pooled prevalence of *S. aureus* in raw milk (cow, camel, and goat) sampled from different points along the dairy chain was 28.8% (95% CI: 25, 32.8). The pooled prevalence of *S. aureus* was highest in raw cow milk (30.7%, 95% CI: 26.6, 35), followed by camel (19.3%, 95% CI: 9.6, 31.5), and goat milk (13.6%, 95% CI: 8.7, 19.5), with statistically significant difference in contamination rates among them. A forest plot of *S. aureus* contamination of raw milk in different species is indicated in Additional files 2 and 3. All single studies omitted pooled estimates lie within 95% confidence interval of the respective overall pooled estimates. This indicates that none of the single studies influenced the overall pooled prevalence of *S. aureus*.

Analysis by sampling points showed that significantly higher *S. aureus* contamination of raw milk (of the three species) was recorded in milk sampled from processing plants (50.3%, CI: 25.8, 74.7), followed by MCCs (47.1%, CI: 27.9, 66.7), selling points (34.5%, CI: 24.9, 44.9), farm bulk milk (25.8%, CI: 19, 33.1), milking buckets (24.8%, CI: 19.5, 30.5) and udders (20.3%, CI: 15.9, 25.1). The pooled prevalence of *S. aureus* in pasteurized milk was 3.8% (CI: 1.1, 16.7). The forest plots of *S. aureus* prevalence in milk at the levels of udder, milking bucket, farm bulk milk, selling point, MCCs and processing plant are indicated in Figs. 2, 3, and 4.

The pooled prevalence of *S. aureus* contamination in raw cow milk was significantly ($P < 0.05$) higher at MCCs (52.1%, CI: 31.2, 72.7), followed by processing plants (50.3%, CI: 25.8, 74.7), selling points (35%, CI: 24.5, 46.2), farm bulk tanks (29.7%, CI: 22.8, 37.1), milking buckets (24.8%, CI: 19.5, 30.5) and udder milk (20.3%, CI: 15.8, 25.3). There are limited numbers of studies reported on *S. aureus* contamination of camel and goat milk.

Local cheese and traditional fermented milk

The pooled prevalence rates of *S. aureus* in locally produced soft cheese (*ayib*) and traditional fermented milk (*ergo*) were 18.6% (95% CI: 6.8, 34.4) and 14.9% (95% CI: 4.3, 30.2), respectively. The forest plot of *S. aureus* prevalence estimates in local cheese and traditional fermented milk is presented in Additional file 3.

Contamination of water, farm workers and different swab samples

The pooled prevalence estimate of *S. aureus* was highest in water for washing milking utensils (39.3%, CI: 28.7, 50.3), followed by farm worker nasal swab samples (31.5%, 95% CI: 21.7, 42), milkers' hand swabs (25.9%, 95% CI: 19.3, 33.1), milk collection center container swabs (23.8%, 95% CI: 7.6, 44.7), bulk tank swabs (20.4%,

Table 1 Characteristics of eligible studies reported on milk of different species

Study	Study year	Region	Study area (as stated in the paper)	Livestock species	Sampling point along milk value chain	N	n (%)
(Wodaje and Beyene 2021)	2015/16	Oromia	Mojo	Cow	Udder milk	152	22(14.5)
(Wodaje and Beyene 2021)	2015/16	Oromia	Mojo	Cow	Farm bulk milk	15	4(26.7)
(Lemma et al. 2021)	2019/20	A/Ababa	Addis Ababa	Cow	Farm bulk milk	175	43(24.6)
(Wedeselassie et al. 2020)	2014/15	Tigray	Mekelle	Cow	Udder milk	100	17(17)
(Wedeselassie et al. 2020)	2014/15	Tigray	Mekelle	Cow	Selling point	45	21(46.7)
(Kalayu et al. 2020)	2016/17	Tigray	Mekelle	Cow	Udder milk	385	48(12.5)
(Amenu et al. 2019)	2015	Oromia	Borena	Cow	Udder milk	33	7(21.2)
(Amenu et al. 2019)	2015	Oromia	Borena	Cow	Farm bulk milk	42	1(2.3)
(Amenu et al. 2019)	2015	Oromia	Borena	Cow	Selling point	51	6(11.8)
(Amenu et al. 2019)	2015	Oromia	Borena	Camel	Udder milk	4	0(0)
(Amenu et al. 2019)	2015	Oromia	Borena	Camel	Farm bulk milk	25	1(4)
(Amenu et al. 2019)	2015	Oromia	Borena	Camel	Selling point	15	4(26.7)
(Amenu et al. 2019)	2015	Oromia	Borena	Goat	Udder milk	3	1(33.3)
(Amenu et al. 2019)	2015	Oromia	Borena	Goat	Farm bulk milk	12	1(8.3)
(Regasa et al. 2019)	2017/18	Oromia	Mukatari & Sululta	Cow	Udder milk	183	28(15.3)
(Weldeabezgi et al. 2019)	2017	Tigray	Mekelle, Maichew, H/selam	Cow	Farm bulk milk	53	15(28.3)
(Weldeabezgi et al. 2019)	2017	Tigray	Mekelle, Maichew, H/selam	Cow	Selling point	93	51(54.8)
(Abraha et al. 2018)	2016/17	Tigray	Mekelle	Cow	Farm bulk milk	86	23(26.7)
(Abraha et al. 2018)	2016/17	Tigray	Mekelle	Cow	Selling point	86	20(23.3)
(Serda et al. 2018)	2014/15	Somali	Jigjiga	Camel	Farm bulk milk	128	9(7)
(Serda et al. 2018)	2014/15	Somali	Jigjiga	Camel	MCC	128	15(11.71)
(Serda et al. 2018)	2014/15	Somali	Jigjiga	Camel	Selling point	128	20(15.62)
(Tsige 2018)	2017/18	Oromia	Arsi Negelle	Cow	Udder milk	224	40(17.9)
(Tsige 2018)	2017/18	Oromia	Arsi Negelle	Cow	MCC	38	24(63.2)
(Ayele et al. 2017)	2014/15	Oromia	Sebeta	Cow	Udder milk	209	41(19.6)
(Ayele et al. 2017)	2014/15	Oromia	Sebeta	Cow	MCC	20	16(80)
(Ayele et al. 2017)	2014/15	Oromia	Sebeta	Cow	Pasteurized milk	10	0(0)
(Abunna et al. 2017)	2014/15	Oromia	Sebeta	Cow	Udder milk	60	14(23.3)
(Abunna et al. 2017)	2014/15	Oromia	Sebeta	Cow	Farm bulk milk	10	4(40)
(Beyene et al. 2017)	2013/14	A/Ababa	Addis Ababa	Cow	Udder milk	40	8(20)
(Beyene et al. 2017)	2013/14	A/Ababa	Addis Ababa	Cow	Farm bulk milk	8	2(25)
(Tegegne and Tesfaye 2017)	2013/14	Amhara	Gondar	Cow	Udder milk	60	11(18.3)
(Tegegne and Tesfaye 2017)	2013/14	Amhara	Gondar	Cow	Milking bucket	12	3(25)
(Tessema and Tsegaye 2017)	2011/12	Oromia	Alage	Cow	Milking bucket	85	18(21.2)
(Tessema and Tsegaye 2017)	2011/12	Oromia	Alage	Cow	Selling point	85	30(35.3)
(Ebissa and Aki 2017)	2011/12	B/Gumuz	Asossa	Cow	Farm bulk milk	68	34(50)
(Ebissa and Aki 2017)	2015/16	B/Gumuz	Asossa	Cow	Selling point	32	4(12.5)
(Abunna et al. 2017)	2014	Oromia	Asella	Cow	Udder milk	42	5(11.9)
(Abunna et al. 2017)	2014	Oromia	Asella	Cow	Farm bulk milk	9	1(11.1)
(Reta et al. 2016)	2013/14	Somali	Jigjiga	Cow	Udder milk	30	6(20)
(Reta et al. 2016)	2013/14	Somali	Jigjiga	Cow	Milking bucket	30	7(23.3)
(Reta et al. 2016)	2013/14	Somali	Jigjiga	Cow	MCC	30	6(20)
(Reta et al. 2016)	2013/14	Somali	Jigjiga	Cow	Selling point	30	10(33.3)
(Kiros et al. 2016)	2013/14	SNNPR	Wolaita Sodo	Cow	Selling point	56	41(73.2)
(Teshome et al. 2016)	2013/14	Somali	Pastoral districts	Camel	Udder milk	62	4(6.5)
(Teshome et al. 2016)	2013/14	Somali	Pastoral districts	Goat	Udder milk	142	19(13.4)
(Tolosa et al. 2016)	2012/13	Oromia	Jimma	Cow	Farm bulk milk	32	12(37.5)
(Tolosa et al. 2016)	2012/13	Oromia	Jimma	Cow	MCC	3	2(66.7)

Table 1 (continued)

Study	Study year	Region	Study area (as stated in the paper)	Livestock species	Sampling point along milk value chain	N	n (%)
(Tolosa et al. 2016)	2012/13	Oromia	Jimma	Cow	Selling point	40	13(33)
(Shiferaw and Ahmad 2016)	2012/13	Amhara	Bahira Dar	Cow	Udder milk	218	98(45)
(Tigabu et al. 2015)	2011/12	Central Ethiopia	Asella, Bishoftu, Selalle, A.A	Cow	Farm bulk milk	433	103(23.8)
(Tigabu et al. 2015)	2011/12	Central Ethiopia	Asella, Bishoftu, Selalle, A.A	Cow	MCC	44	17(38.6)
(Tarekgne et al. 2015)	2012/13	Tigray	Mekelle & major cities	Cow	Farm bulk milk	195	67(34.4)
(Tarekgne et al. 2015)	2012/13	Tigray	Mekelle & other cities	Cow	Selling point	115	53(46.1)
(Garedew et al. 2015)	2010/11	Amhara	Gondar	Cow	Udder milk	37	10(27)
(Garedew et al. 2015)	2010/11	Amhara	Gondar	Cow	Milking bucket	33	10(30.3)
(Garedew et al. 2015)	2010/11	Amhara	Gondar	Cow	Processing plant	13	3(23.1)
(Garedew et al. 2015)	2010/11	Amhara	Gondar	Cow	Pasteurized milk	15	4(26.7)
(Megersa 2015)	2014/15	Oromia	Ambo and Guder	Cow	Udder milk	135	17(12.6)
(Wasie et al. 2015)	2014/15	Afar	Dubti	Camel	Udder milk	35	19(54.3)
(Wasie et al. 2015)	2014/15	Afar	Dubti	Camel	Selling point	35	21(60)
(Shunda et al. 2013)	2011/12	Tigray	Mekelle	Cow	Farm bulk milk	60	24(40)
(Shunda et al. 2013)	2011/12	Tigray	Mekelle	Cow	Selling point	120	24(20)
(Desissa et al. 2012)	2009/10	Oromia	Bishoftu	Cow	Farm bulk milk	170	74(43.5)
(Desissa et al. 2012)	2009/10	Oromia	Bishoftu	Cow	MCC	25	18(72)
(Desissa et al. 2012)	2009/10	Oromia	Bishoftu	Cow	Pasteurized milk	20	0(0)
(Daka et al. 2012)	2011/12	SNNPR	Hawassa	Cow	Udder milk	32	14(43.8)
(Daka et al. 2012)	2011/12	SNNPR	Hawassa	Cow	Farm bulk milk	32	20(62.5)
(Daka et al. 2012)	2011/12	SNNPR	Hawassa	Cow	MCC	32	21(65.6)
(Daka et al. 2012)	2011/12	SNNPR	Hawassa	Cow	Processing plant	32	17(53.1)
(Addis et al. 2011)	2008/9	Oromia	Bishoftu	Cow	Farm bulk milk	100	8(8)
(Addis et al. 2011)	2008/9	Oromia	Bishoftu	Cow	MCC	100	10(10)
(Wubete 2004)	2003/4	Oromia	Bishoftu	Cow	Udder milk	77	12(15.6)
(Wubete 2004)	2003/4	Oromia	Bishoftu	Cow	Milking bucket	77	20(26)
(Wubete 2004)	2003/4	Oromia	Bishoftu	Cow	MCC	12	9(75)
(Wubete 2004)	2003/4	Oromia	Bishoftu	Cow	Processing plant	12	9(75)
(Wubete 2004)	2003/4	Oromia	Bishoftu	Cow	Pasteurized milk	100	0(0)

N sample size, n number of positive, SNNPR South Nations Nationalities and Peoples Region, A.A Addis Ababa, MCC Milk collection center

95% CI: 9.6, 33.9), udder swabs (15.6%, 95% CI: 9.9, 22.2), milking bucket swabs (14.2%, 95% CI: 8.8, 20.6) and towel swabs (10%, 95% CI: 8.3, 38.1). The difference was statistically significant ($P < 0.01$).

Molecular characterization of *Staphylococcus aureus* isolated from milk, milk products and farm workers in Ethiopia

Studies that used polymerase chain reaction (PCR) to detect the enterotoxin gene and MRSA were included in this review. According to Seyoum et al. (2016), of the 109 *S. aureus* isolates recovered from bulk milk in central Ethiopia, 73 (66.9%) harbored at least one type of *S. aureus* enterotoxin gene. The most frequently encountered gene was *sea* (36.7%), followed by *seb* (17.4%), *see* (16.5%), *tst* (14.7%), *sec-1* (11.01%), and *sed* (6.4%). Of these *S. aureus* isolates that harbored at least one of the

enterotoxin genes, 35.6% harbored more than one enterotoxin gene.

Out of 160 *S. aureus* isolates recovered from bulk milk and milk products in the Tigray region, 82 (51%) were reported to carry staphylococcal enterotoxin genes by modified multiplex PCR. Nine SE genes were identified in this study: *sea*, *seb*, *sec*, *sed*, *seg*, *seh*, *sei*, *sej* and *tsst-1*. The gene identified most frequently was *seg*, followed by *sei* and *tsst-1*. Of the 82 enterotoxigenic isolates, 41.5 and 12.4% harbored two or more SE genes, respectively. The pooled prevalence of *S. aureus* that harbored at least one type of enterotoxin gene in this systematic review was 58.9% (CI: 43.4, 73.7).

The pooled prevalence of MRSA in mastitic milk was 15.9% (CI: 6.4, 28.4.7, $p < 0.001$) which was higher than the pooled rate in raw milk (without considering mastitis status) and milk products which was calculated

Table 2 Characteristics of eligible studies reported on local cheese and fermented milk

Study	Study year	Study region	Study area	Product type	Sampling point	N	n (%)
(Lemma et al. 2021)	2019/20	Addis Ababa	Addis Ababa	Cheese	Market	40	2(5)
(Lemma et al. 2021)	2019/20	Addis Ababa	Addis Ababa	Fermented milk	Market	40	7(17.5)
(Wedeselassie et al. 2020)	2014/15	Tigray	Mekelle	Fermented milk	Dairy farm, cafeteria	48	22(45.8)
(Amenu et al. 2019)	2015	Oromia	Borena	Fermented milk	Open market	18	1(5.6)
(Weldeabezgi et al. 2019)	2017	Tigray	Mekelle, Maichew, H/selam	Fermented milk	Market	80	3(3.75)
(Argaw et al. 2018)	2015/16	Oromia	Jimma	Cheese	Retailer, restaurant, household	200	14(7)
(Argaw et al. 2018)	2015/16	Oromia	Jimma	Fermented milk	Retailer, restaurant, household	200	6(3)
(Kiros et al. 2016)	2013/14	SNNPR	Wolaita Sodo	Cheese	Market, Cafeteria/restaurant	40	28(70)
(Tarekgne et al. 2015)	2012/13	Tigray	Major cities	Fermented milk	Milk and product shop, cafeteria	51	13(25.4)
(Tarekgne et al. 2015)	2012/13	Tigray	Major cities	Cheese	Market	7	2(28.6)
(Birhanu et al. 2013)	2010	Oromia	Jimma	Cheese	Open market	150	27(18)
(Addis et al. 2011)	2008/9	Oromia	Bishoftu	Cheese	Open market	200	10(5)

N sample size, n number of positive, SNNPR South Nations Nationalities and Peoples Region

Table 3 Characteristics of eligible studies reported on sources of milk contamination

Study	Study year	Region	Study area	Sample type	N	n (%)
(Wodaje and Beyene 2021)	2015/2016	Oromia	Mojo	Milker hand swab	15	3(20)
(Wodaje and Beyene 2021)	2015/2016	Oromia	Mojo	Milking bucket swab	15	1(6.7)
(Wodaje and Beyene 2021)	2015/2016	Oromia	Mojo	Bulk tank swab	15	2(13.3)
(Kalayu et al. 2020)	2014/15	Tigray	Mekelle	Nasal swab	71	22(31)
(Regasa et al. 2019)	2017/18	Oromia	Mukatari & Sululta	Milking bucket swab	30	6(20)
(Regasa et al. 2019)	2017/18	Oromia	Mukatari & Sululta	Milker hand swab	24	6(25)
(Regasa et al. 2019)	2017/18	Oromia	Mukatari & Sululta	Towel swab	10	1(10)
(Tsige 2018)	2017/18	Oromia	Arsi Negelle	Milker hand swab	35	12(34.3)
(Tsige 2018)	2017/18	Oromia	Arsi Negelle	MCC Container swab	21	5(23.8)
(Ayele et al. 2017)	2014/15	Oromia	Sebeta	Milking bucket swab	27	3(11.1)
(Ayele et al. 2017)	2014/15	Oromia	Sebeta	Milker hand swab	25	8(32)
(Abunna et al. 2017)	2014/15	Oromia	Sebeta	Bulk tank swab	10	2(20)
(Abunna et al. 2017)	2014/15	Oromia	Sebeta	Milking bucket swab	10	2(20)
(Abunna et al. 2017)	2014/15	Oromia	Sebeta	Milker hand swab	10	1(10)
(Beyene et al. 2017)	2013/14	Addis Ababa	Addis Ababa	Milking bucket swab	8	2(25)
(Beyene et al. 2017)	2013/14	Addis Ababa	Addis Ababa	Milker hand swab	8	2(25)
(Beyene et al. 2017)	2013/14	Addis Ababa	Addis Ababa	Bulk tank swab	8	1(12.5)
(Amenu et al. 2016)	2015	SNNPR	Hawassa	Water for cleaning	79	31(39.2)
(Abunna et al. 2017)	2014	Oromia	Asella	Bulk tank swab	9	3(33.3)
(Abunna et al. 2017)	2014	Oromia	Asella	Milker hand swab	9	1(11.1)
(Abunna et al. 2017)	2014	Oromia	Asella	Nasal swab	9	3(33.3)
(Megersa 2015)	2014/15	Oromia	Ambo and Guder	Milking bucket swab	44	4(9)
(Megersa 2015)	2014/15	Oromia	Ambo and Guder	Milker hand swab	25	5(20)
(Megersa 2015)	2014/15	Oromia	Ambo and Guder	Udder swab	135	21(15.6)

N sample size, n number of positive, p prevalence, SNNPR South Nations Nationalities and Peoples Region, MCC Milk collection center

to be 0.73% (CI: 0.07, 2.7, $p=0.008$). Molecular characterization of 70 *S. aureus* isolates recovered from cow udder quarters and farmers' nares in the Tigray region detected the *mecA* gene in only one sample

from the nares of farm workers (Kalayu et al. 2020). In another study, of the 52 *S. aureus* strains isolated from milk and milk product samples collected from farms and retail markets in Addis Ababa, only one isolate was found

Table 4 Pooled prevalence estimates of *S. aureus* in milk at different sampling points

Livestock Spp	Sampling points along dairy chain	Pooled estimate p(95%CI)	Heterogeneity			
			df	Q	Q/df	I ²
All(cow, camel, goat)	Overall	28.8 (25, 32.8)	72	688.74	9.6	89.55
	Udder milk	20.3 (15.9, 25.1)	21	137.43	6.6	84.72
	Milking bucket	24.8 (19.5, 30.5)	4	1.26	0.3	0
	Farm bulk milk	25.8 (19, 33.1)	18	166.18	9.2	89.17
	Selling points	34.5 (24.9, 44.9)	13	134.21	10.3	90.31
	MCC	47.1 (27.9, 66.7)	9	139.94	15.5	93.57
	Processing plant	50.3 (25.8, 74.7)	2	6.74	3.4	70.31
Cow	Overall	30.7 (26.6, 35)	60	560.3	9.3	89.29
	Udder milk	20.3 (15.8, 25.3)	16	104.56	6.5	84.7
	Milking bucket	24.8 (19.5, 30.5)	4	1.26	0.3	0
	Farm bulk milk	29.7 (22.8, 37.1)	15	115.55	7.7	87.02
	Selling points	35 (24.5, 46.2)	10	100.32	10	90.03
	MCC	52.1 (31.2, 72.7)	8	103.32	12.9	92.26
	Processing plant	50.3 (25.8, 74.7)	2	6.74	3.4	70.31
Camel	Overall	19.3 (9.6, 31.5)	8	77.19	9.6	89.64
	Udder milk	19.5 (0.6, 61.4)	2	29.09	14.5	93.12
	Farm bulk milk	6.8 (3.3, 11.4)	1	0.11	0.11	0
	Selling points	33.1 (8.1, 64.9)	2	25.04	12.5	92.01
	Primary collection center	11.7 (6.7, 17.9)	0	0	-	-
Goat	Overall	13.6 (8.7, 19.5)	2	1.13	0.6	0
	Udder milk	14.2 (7.3, 22.9)	1	1.04	1.04	4.14
	Farm bulk milk	8.3 (6.8, 32.4)	0	0	-	-
Pasteurized milk		3.8 (1.1, 16.7)	3	13.95	4.7	78.49

P prevalence, Q Cochran's Q, df degrees of freedom, I² Inverse variance index, MCC Milk collection center, CI Confidence interval

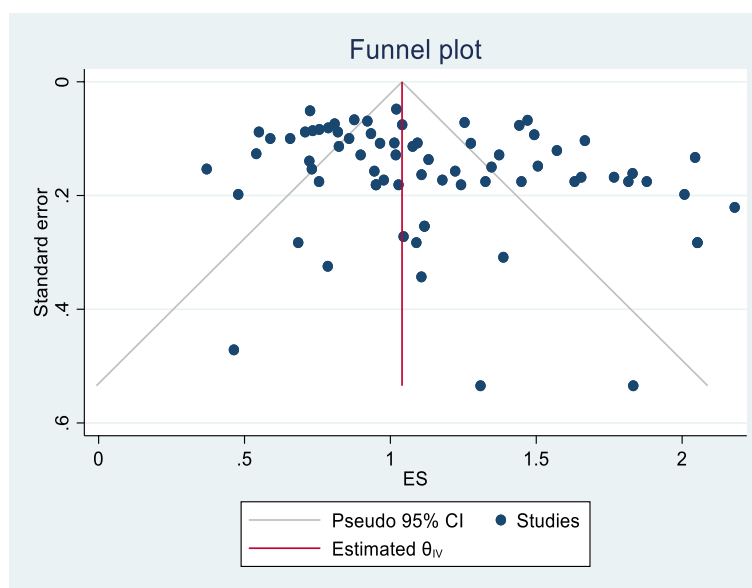
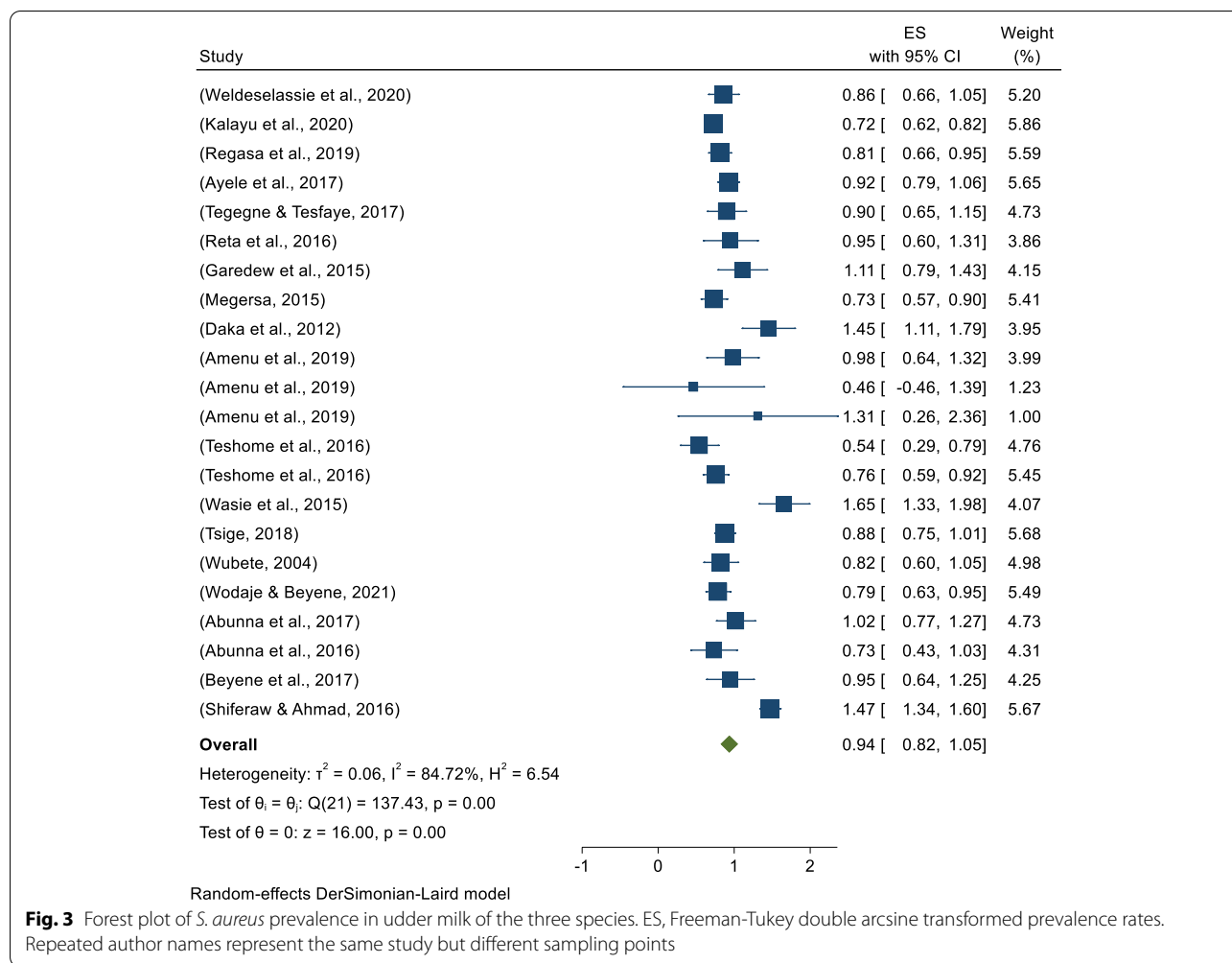


Fig. 2 Funnel plot of prevalence estimate of *S. aureus* in raw milk of the three species



to carry the *mecA* gene (Lemma et al. 2021). The prevalence of MRSA in milk and milk products is presented in Table 5.

Antimicrobial resistance patterns of *S. aureus* isolates

The pooled prevalence of *S. aureus* resistance to different antimicrobials is indicated in Table 6. We estimated the pooled prevalence of *S. aureus* resistance to 17 antimicrobials used in human and veterinary medicine. Analysis of AMR patterns of *S. aureus* revealed that penicillin recorded the highest pooled resistance rate (92%), followed by ampicillin (82%), amoxicillin (62.6%), cefoxitin (48.2%), nalidixic acid (45.5%), amoxicillin-clavulanic acid (45.1%), tetracycline (43.1%), streptomycin (41.1%), oxacillin (33.4%), erythromycin (23.7%), cephalothin (20.6%), vancomycin (19.8%), kanamycin (18.2%), trimethoprim-sulfamethoxazole (15.9%), gentamycin (15.7%), chloramphenicol (15.3%) and ciprofloxacin (3.8%). The pooled prevalence of multidrug resistant *S. aureus* was 62.1%. Multidrug resistance was defined

as resistance to three or more antimicrobials. Raw data for the antimicrobial resistance patterns of the isolates is indicated in Additional file 5.

Risk assessment of *Staphylococcus aureus* associated with the consumption of milk and milk products in Ethiopia

Only two studies have been conducted on the risk assessment of *S. aureus*-associated illness due to the consumption of milk and milk products in Ethiopia. Using a stochastic model, (Makita et al. 2012) estimated the annual incidence rate of SFP to be 20 per 1000 people in milk and 5.4 cases per 10,000 people in traditional fermented milk. Another quantitative risk assessment model reported 11 and 24 illnesses per 100 consumers from a single exposure to *S. aureus* for raw milk purchased from farm gates and MCCs, respectively. However, a single serving of traditionally fermented milk was not linked to likely risk of illness in this study. In this study, the annual likely risk of illness from *S. aureus* due to consumption of raw milk and milk products was 24.2 and

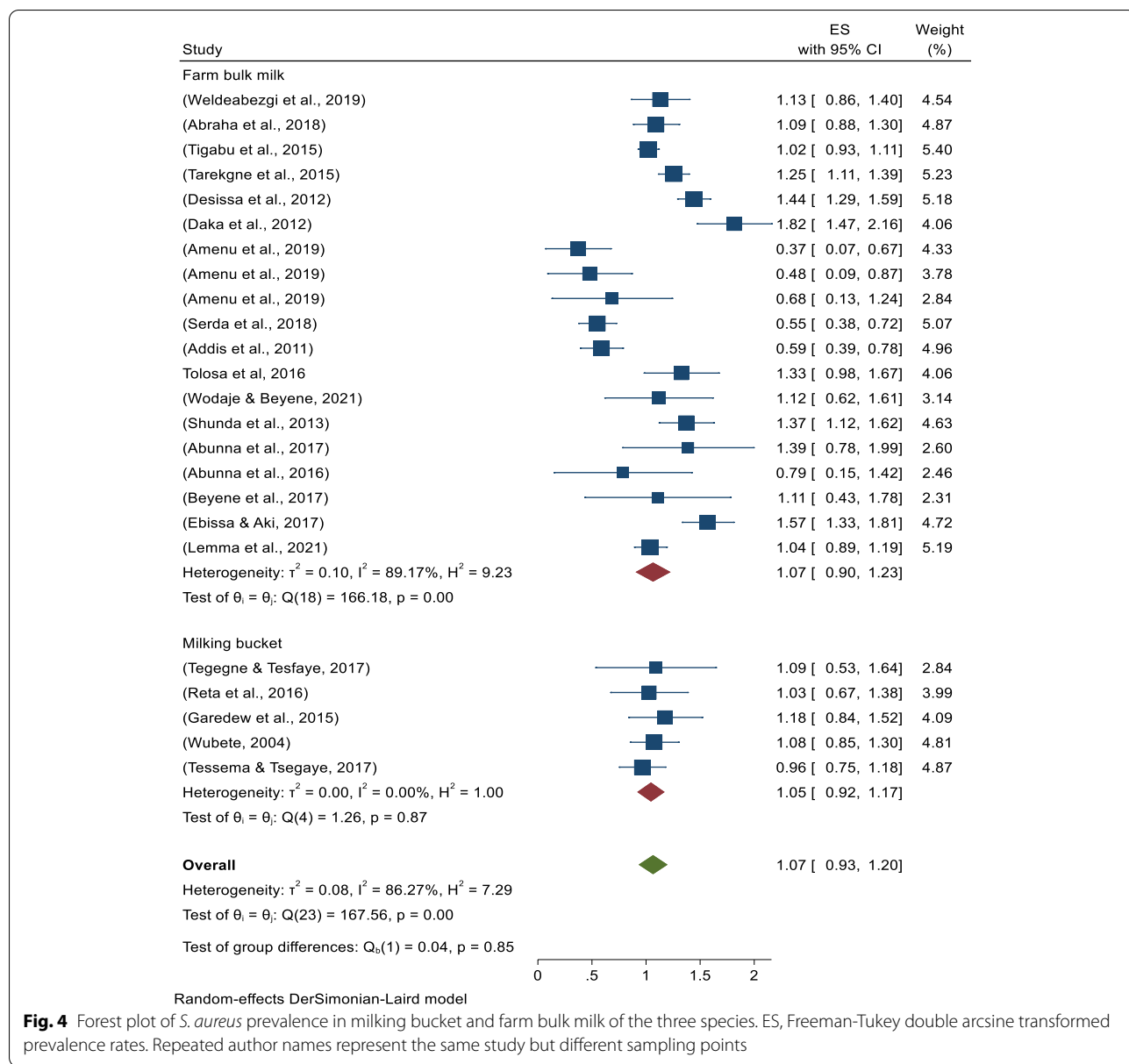


Fig. 4 Forest plot of *S. aureus* prevalence in milking bucket and farm bulk milk of the three species. ES, Freeman-Tukey double arcsine transformed prevalence rates. Repeated author names represent the same study but different sampling points

Table 5 Prevalence of MRSA in milk and milk products and farm workers in Ethiopia

Study	Study year	Region	Study area	Sample type	Total sample size	N (%)	n	n/N (%)
(Kalayu et al. 2020)	2016/17	Tigray	Mekelle	Udder milk	385	48 (12.5)	0	0
(Kalayu et al. 2020)	2016/17	Tigray	Mekelle	Nares of farmers	71	22 (31)	1	4.55
(Lemma et al. 2021)	2019/20	Addis Ababa	Addis Ababa	Raw farm milk	174	43 (24.6)	1	2.33
(Lemma et al. 2021)	2019/20	Addis Ababa	Addis Ababa	Yogurt	40	7 (17.5)	0	0
(Lemma et al. 2021)	2019/20	Addis Ababa	Addis Ababa	Cheese	40	2 (5)	0	0
(Mekonnen et al. 2018)	2014–16	Amhara	Gondar/Bahirdar	Udder milk	107	79 (73.8)	0	0
(Girmay et al. 2020)	2014/15	Tigray	Shire	Mastitic milk	64	21 (32.81)	5	23.8
(Tegegne et al., 2021)	2018/19	Central Ethiopia	West and East Shoa	Mastitic milk	130	100 (76.9)	12	12

N total number of *S. aureus* isolates, *n* number of MRSA isolates (*mecA* gene), Addis Ababa

Table 6 Pooled prevalence of *S. aureus* resistance to different antimicrobials

Antimicrobial type	Number of reports	Pooled prevalence of AMR (%)	95% CI
Penicillin G	12	92	85–97
Amoxicillin	7	62.6	33.5–87.5
Ampicillin	5	82	44.3–99.9
Nalidixic acid	5	45.5	26.5–65.3
Gentamycin	8	15.7	9.1–23.8
Kanamycin	3	18.2	5.6–35.4
Vancomycin	11	19.8	8.1–35.2
Tetracycline	11	43.1	34.1–52.4
Ciprofloxacin	9	3.8	1.3–7.5
Cefoxitin	6	48.2	11.9–85.7
Trimetoprim-Sulfametoxazole	11	15.9	9.2–24
Erythromycin	12	23.7	13.5–35.6
Chloramphenicol	5	15.3	7.6–25
Streptomycin	5	41.1	11–75.6
Amoxicillin-Clavulanic Acid	5	45.1	14.6–77.8
Oxacillin	4	33.4	16.2–53.3
Cephalothin	3	20.6	11.7–31.2
MDR	9	62.1	40.1–81.7

48.3% in milk collected from dairy farms and MCCs, respectively. This means that nearly a quarter of farm gate and half of MCC milk consumers might experience illnesses annually. Traditionally fermented milk was reported to be less risky, with 2.5% (2540 illnesses out of 100,000 consumers yearly) estimated illness per serving-per year (Weldeabezgi et al. 2019).

Analysis of publication bias

The funnel plot of prevalence estimates of *S. aureus* in raw milk (cow, camel and goat) is shown in Fig. 5. Egger’s regression asymmetry test did not suggest the presence of statistically significant publication bias ($p > 0.05$) in all study types: among raw milk of the three species, naturally fermented milk and local cheese, water and swab samples and antimicrobial resistance. In all studies, samples were collected randomly from different farms, selling points, MCCs and study areas, which makes bias unlikely. Moreover, *S. aureus* was isolated and identified according to standard microbiological techniques. We conclude that there is no within- and across-study bias and that the asymmetry of the funnel plot was not due to small study effects.

Heterogeneity analysis

Heterogeneities and Cochran’s Q statistics (Q/df) at each sampling point are indicated in Table 4. The study-level prevalence estimates of *S. aureus* were heterogeneous

($I^2 = 89.55\%$, $p < 0.001$) among milk of the three species. Study-level prevalence estimates (of the three species) were heterogeneous, with I^2 values of 93.57%, 90.31%, 89.17%, 84.72%, 78.49 and 70.31% at the MCC, selling point, farm bulk milk, udder, pasteurized milk and processing plant levels, respectively ($p < 0.05$). However, studies that reported on the prevalence of *S. aureus* in milk collected from milking buckets were not heterogeneous. Significant heterogeneity in the prevalence of *S. aureus* was observed across studies that reported on raw cow milk ($I^2 = 89.29\%$), camel milk ($I^2 = 89.64\%$) and pasteurized milk ($I^2 = 78.49\%$), but studies reported on raw goat milk were not heterogeneous ($I^2 = 0$). There was significant heterogeneity among individual studies that reported on local cheese ($I^2 = 94.46\%$, $p < 0.0001$) and fermented milk ($I^2 = 92.48\%$, $p < 0.0001$). Studies that reported on sources of milk contamination (water and swab samples) were also moderately heterogeneous ($I^2 = 34.72\%$, $p < 0.05$). We calculated heterogeneity among studies in all antimicrobial types separately, and all were found to be significantly heterogeneous. The high heterogeneity observed in this systematic review could be because of the small sample size and outliers in some studies.

Discussion

This systematic review of previous studies revealed a significantly higher pooled prevalence of *S. aureus* contamination in raw cow milk (30.7%), followed by camel milk (19.3%) and goat milk (13.6%) in Ethiopia. A meta-analysis study of the global prevalence rate of *S. aureus* contamination recorded a comparable pooled prevalence of 33.5% in raw cow milk, but a higher prevalence rate in raw goat (25.8%) and pasteurized and boiled cow milk (15.4%) compared to this systematic review (Ou et al. 2017). In agreement with this systematic review, the isolation rate of *S. aureus* was higher in raw cow milk (61.7%) than in camel milk (35.9%) in China (Kou et al. 2021). In contrast, bulk goat milk was reported to be more contaminated than cow milk in northwestern Greece (Papadopoulos et al. 2018) and Norway (Jørgensen et al. 2005). A study in Iran reported *S. aureus* prevalence rates of 16%, 7.5% and 3.4% in cow, goat and camel bulk milk samples, respectively (Rahimi and Alian 2013).

In this systematic review, raw milk (of the three species) showed a statistically significant highest pooled *S. aureus* prevalence at processing plants (50.3%), followed by MCCs (47.1%), selling points (34.5%), farm bulk milk (25.8%), milking buckets (24.8%), and udder milk (20.3%). In line with the results of this systematic review, the rate of contamination is indicated to increase along the production chain (Titouche et al. 2019). This could be due

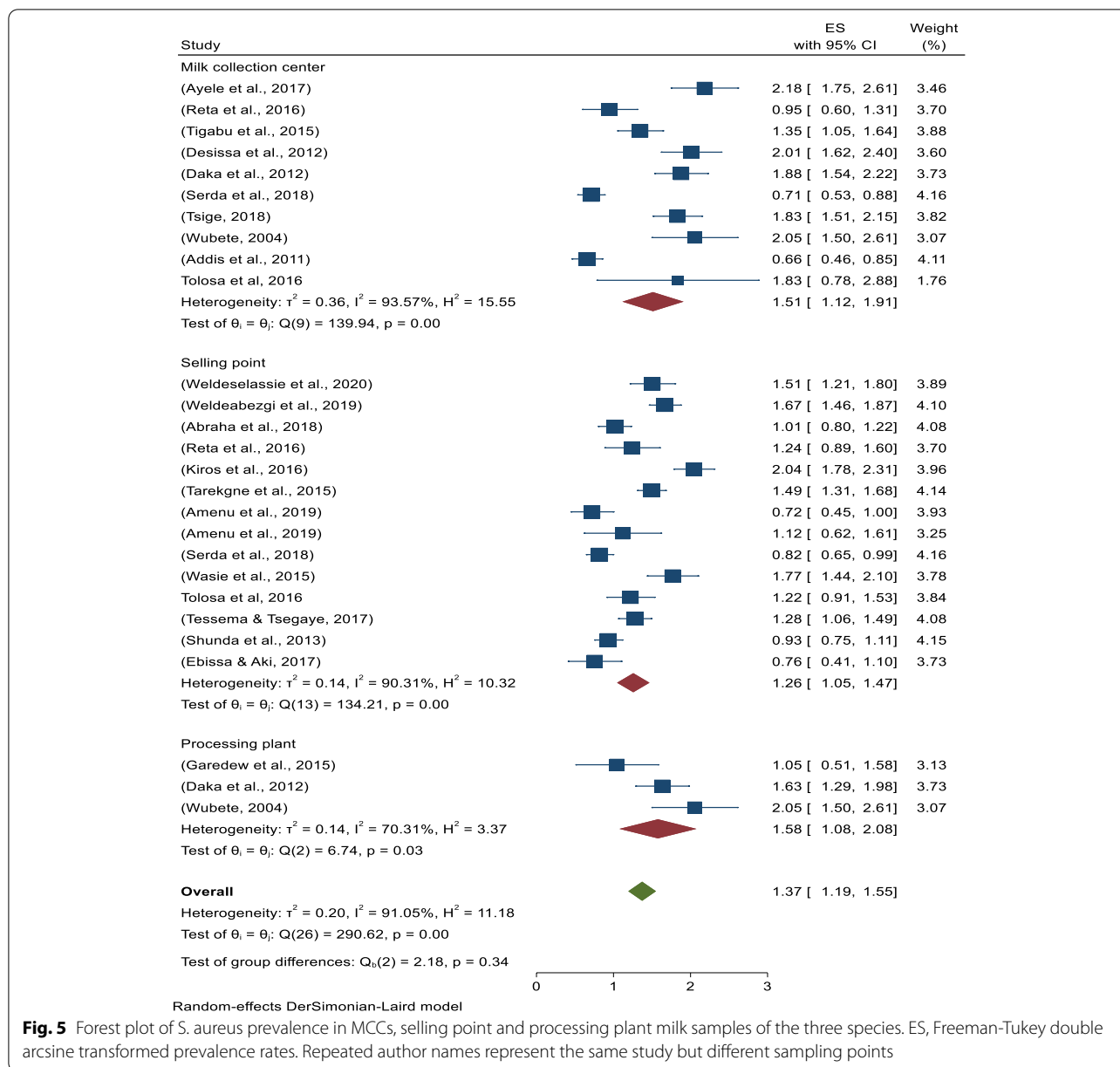


Fig. 5 Forest plot of *S. aureus* prevalence in MCCs, selling point and processing plant milk samples of the three species. ES, Freeman-Tukey double arcsine transformed prevalence rates. Repeated author names represent the same study but different sampling points

to exposure to additional sources of contamination and bacterial multiplication in inappropriate storage conditions. In Ethiopia, poor sanitation of milking utensils, suboptimal hygiene of milking personnel, poor udder preparation, poor hygiene of farms, milk transportation without cold chains and an absence of awareness of food-borne diseases were frequently reported (Ayele et al. 2017; Regasa et al. 2019; Tigabu et al. 2015; Abebe et al. 2016). These practices might have contributed to the high pooled prevalence estimate at each sampling location and an increase in *S. aureus* contamination along the value chain. Mastitis, which was reported to be highly

prevalent in the country, might be another contributing factor for the high pooled prevalence of *S. aureus* along the chain (Abebe et al. 2016; Balemi et al. 2021; Fulasa and Deressa 2021). *S. aureus* may be directly excreted from the mastitic udder into milk and subsequently contaminate bulk milk and raw milk products (Kadariya and Smith 2014). Mixing of milk from different farms including mastitic milk, may also contribute to higher contamination at MCC and processing plant levels (Ayele et al. 2017; Abebe et al. 2016). Plastic containers are commonly used for milking, storage and transporting milk in Ethiopia. They easily scratch and create favorable conditions

for bacterial growth (Ayele et al. 2017; Mpatwenumugabo et al. 2019; Wafula et al. 2016). Inadequate cleaning of milk utensils leads to milk residue that provides nutrients for the growth of bacteria (Kou et al. 2021). The use of poor quality water for cleaning, absence of discarding the first squirts of milk during milking and absence of teat dipping into antiseptic solution were mentioned as practices that could account for bacterial contamination of raw milk in Ethiopia (Roesel and Grace 2014).

The current systematic review revealed that raw cow milk was more contaminated by *S. aureus* at MCCs (52.1%) than processing plants (50.3%), selling points (35%), farm bulk tanks (29.7%), milking buckets (24.8%), and udder milk (20.3%). A worldwide meta-analysis study reported a higher pooled prevalence of *S. aureus* in raw cow milk obtained from processing companies (54%), followed by farms (32.3%) and retailers (27%) (Ou et al. 2017). Comparable to this systematic review result, prevalence rates of 17.9% in Iran (Alian et al. 2012), 20.8% in Tunisia (Said et al. 2016) and 25.3% in Sudan (Ibrahim et al. 2018) were reported in udder milk. In contrast, two studies conducted in Brazil reported lower prevalence rates of 12.8 and 5.5% (Girardini et al. 2016; Lee et al. 2012). Consistent with the pooled prevalence estimated in this systematic review, prevalence rates of 27.7% (Liu et al. 2017) and 28.9% (Zhao et al. 2020) were reported in China, while slightly lower rates of 20.3% and 21.7% were recorded in Uganda (Asiimwe et al. 2017) and Brazil (Lee et al. 2012) respectively, in bulk tank milk. Higher *S. aureus* prevalence rates of 80% in Egypt (Kandil et al. 2018), 40% in Greece (Papadopoulos et al. 2018), 50% in Tunisia (Said et al. 2016), 65.9% in Jordan (Obaidat et al. 2018) and 75% in Norway (Jørgensen et al. 2005) were reported in bulk tank milk samples. At selling points, compared to the 35% calculated in this review, higher *S. aureus* prevalence rates of 75% and 68% were reported in Turkey (Gucukoglu et al. 2012) and Brazil (Oliveira et al. 2011) respectively, while a comparable prevalence of 39.1% was recorded in India (Sudhantiramani et al. 2015). In contrast, lower prevalence rates of 15.7% (Jamali et al. 2015) and 16.3% (Chao et al. 2007) were reported in Iran and China, respectively.

The pooled prevalence of *S. aureus* in this systematic review in pasteurized milk was 3.8%. Similarly, a low *S. aureus* prevalence rate of 3.9% was reported in pasteurized milk in China (Dai et al. 2019). Pasteurization is regarded as an effective method for eliminating food-borne pathogens and other bacteria from milk. Proper pasteurization followed by refrigeration can minimize the chance of milk contamination by *S. aureus*. However, an increasing number of reports have detected food-borne pathogens in pasteurized milk (Garedew et al. 2015; Dai et al. 2019; Rall et al. 2008; Gundogan

et al. 2006). Raw milk contaminated with food-borne pathogens can introduce the organism into processing plants, which could be a source of postpasteurization contamination (Oliver et al. 2005).

The pooled prevalence rates of *S. aureus* contamination in camel milk in this systematic review was 33.1%, 19.5%, 11.7% and 6.8% at the selling point, udder, primary collection center and farm bulk tank levels, respectively. Compared to the result in this study, a higher camel udder milk contamination (36.8%) was reported in the United Arab Emirates (Omer and Eltinay 2008). A higher prevalence rate of 70% was reported in Saudi Arabia from milk samples collected from milking bowls (El-Ziney and Al-Turki 2007). Nearly in agreement with our result, a prevalence rate of 3.4% was reported in Iran in bulk tank milk (Rahimi and Alian 2013).

The pooled prevalence of *S. aureus* in raw goat milk at the udder and farm bulk tank levels was 14.2% and 8.3%, respectively. Lower prevalence rates of 6.3% in Iran (Alian et al. 2012), 6.2% in Norway (Mørk et al. 2010) and 1.4% in the USA (Anderson et al. 2018) were reported in udder milk samples. Higher prevalence rates of *S. aureus* were recorded in bulk tank milk samples of dairy goats: 96.2% in Norway (Jørgensen et al. 2005), 80% in Greece (Papadopoulos et al. 2018), 76.9% in Jordan (Obaidat et al. 2018) and 43.1% in Italy (Cortimiglia et al. 2015), 40% in Switzerland (Merz et al. 2016), 31.4% in Greece (Pexara et al. 2015), 31.7% in Switzerland (Muehlherr et al. 2003) and 17.6% in China (Qian et al. 2019). The presence of *S. aureus* in camel and goat milk is of public health importance because milk from these species is often consumed in raw form in pastoral areas of Ethiopia (Amenu et al. 2019).

The pooled prevalence of *S. aureus* in local cheese and traditional fermented milk in this review was 18.6% and 14.9%, respectively. A comparable prevalence rate of 12% was reported in sour (fermented) milk in Uganda (Asiimwe et al. 2017). A meta-analysis study on African indigenous fermented milk reported a higher *S. aureus* prevalence estimate of 37% (Wambui et al. 2019). *S. aureus* was detected in 18% of Egyptian Kareish soft cheese samples (Zeinhom and Abed 2021). Comparable with our result, a prevalence rate of 21.96% was reported in traditional cheese in Turkey (Kayili and Sanlibaba 2020), while higher prevalence rates of 37.5 and 30% were reported in white cheese and Kashar cheese, respectively, in other region of the same country (Gucukoglu et al. 2012).

The pooled prevalence of *S. aureus* in this systematic review was highest in water intended for washing milk containers (39.3%), followed by swab samples from human nares (31.5%), milkers' hands (25.9%), MCC containers (23.8%), bulk tanks (20.4%, 95%), udders

(15.6%), milking buckets (14.2%) and towels (10%). Similarly, a study conducted in Algeria reported the highest *S. aureus* contamination rate in water used for cleaning (50.9%), followed by hand swabs (39.6%), udder swabs (28.9%) and swab samples from utensils (5.7%) (Hamiroune et al. 2016). Comparatively, lower occurrences of *S. aureus* were reported in swab samples from milk equipment (3.6%) and milkers' hand swabs (3.3%) (Lee et al. 2012) in Brazil. The prevalence of *S. aureus* on teat skin swabs in dairy goats in Norway was 9.9% (Mørk et al. 2010). The variation might be attributed to differences in hygiene practices. Several studies conducted in Ethiopia revealed the absence of cleaning of hands, milk utensils, udder and teats which could explain the reason for the high *S. aureus* contamination rate in this review (Amenu et al. 2019; Ayele et al. 2017; Regasa et al. 2019). Human handlers, milking equipment, the environment and udder and teat skin of dairy animals are potential sources of bulk milk contamination (Lee et al. 2012). In most small-scale milk production farms in Africa, there are no strict implementations of procedures for cleaning and disinfection of materials used during production processes, from milking to the sale of final products. Adequate milking hygiene potentially reduces the contaminating microorganisms and prevents them from inhabiting the immediate environment or skin of the animals, hands of personnel and milking equipment, thereby minimizing their spread during milking (Owusu-kwarteng et al. 2020).

The pooled prevalence of *S. aureus* that harbored at least one type of enterotoxin gene in this systematic review was 58.9% (CI: 43.4, 73.7). Comparatively, a higher *S. aureus* enterotoxin gene carriage was reported in Kenya (74.11%) (Omwenga et al. 2019) and Uganda (90.2%) (Asiimwe et al. 2017) in milk and milk products. In China, 12.9% of *S. aureus* isolates from retail milk of different livestock species carried at least one classical virulence gene (Kou et al. 2021), which was lower than our study. In Mozambique, the frequency of strains positive for SEs was low (3%) in raw milk (Nhatsave et al. 2021), while none of the *S. aureus* strains isolated from traditional cheese in Turkey harbored the SE gene (Kayili and Sanlibaba 2020). The most frequently encountered enterotoxin gene carried by *S. aureus* isolates recovered from bulk milk in central Ethiopia was *sea* (36.7%), followed by *seb* (17.4%), *see* (16.5%), *tst* (14.7%), *sec-1* (11.01%), and *sed* (6.4%) (Seyoum et al. 2016). *S. aureus* isolates recovered from bulk milk and milk products in the Tigray region carried nine SE genes: *sea*, *seb*, *sec*, *sed*, *seg*, *seh*, *sei*, *sej* and *tsst-1*. The gene identified most frequently was *seg*, followed by *sei* and *tsst-1*. The most frequently observed gene in *S. aureus* strains isolated from milk of various livestock species in Kenya

was *see* (60%), followed by *sea* (25.88%) and *sec* (22.35%) (Omwenga et al. 2019). In Uganda (Asiimwe et al. 2017), *sem* (70.7%), *sei* (61%) and *seg* (51.2%) were the most frequently observed genes. In China, *S. aureus* strains isolated from retail milk of different species carry the following genes: *see* (6.5%), *sea + sec* (3.2%), *sea* (1.6%) and *sec* (1.6%) (Kou et al. 2021). In Mozambique, *S. aureus* isolates recovered from raw milk harbored only *sea* and *see* genes (Nhatsave et al. 2021).

In this study, the pooled prevalence of MRSA was higher in mastitic milk (15.9%) than raw milk (udder milk sampled without considering mastitis status) and milk products (0.73%). This finding agrees with reports from Bangladesh (Jahan et al. 2015), Turkey (Issa and Aksu 2020), China (Wang et al. 2018) and Italy (Normanno et al. 2007), which reported low MRSA occurrences of 0, 0.84, 1.4 and 3.75%, respectively, in raw milk and milk products. However, higher *mecA* gene carriage rates of 16.2, 51.6 and 56.1% were reported in Iran (Jamali et al. 2015), China (Kou et al. 2021) and Uganda (Asiimwe et al. 2017), respectively, in milk and milk products. Compared to this study, slightly lower MRSA occurrences of 1.5, 6.9, and 9.3% were reported in mastitic milk in Finland (Gindonis et al. 2013), Nepal (Shrestha et al. 2021) and Belgium (Vanderhaeghen et al. 2010). Comparable to our finding, the *mecA* gene was detected in 13.9% and 14.7% of *S. aureus* isolated from mastitic milk in Korea (Song et al. 2016) and China (Yang et al. 2020), respectively. However, higher *mecA* positivity rates of 20, 26.5 and 46.6–53.3% were reported in Bangladesh (Hoque et al. 2019), India (Annamanedi et al. 2021) and Mexico (Guzmán-rodríguez et al. 2020), respectively. A meta-analysis of the global prevalence of MRSA in mastitic milk reported a pooled prevalence of 4.3% (Zaatout and Hezil 2022) which was lower than our result. The higher pooled prevalence of MRSA in mastitic milk than raw milk and milk products in this study could be due to frequent mastitis treatment, which could encourage resistant bacteria growth, such as MRSA, as the result of natural selection.

Microbial risk assessment is a valuable assessment tool for understanding the risks posed by microorganisms and the prevention of food-borne illness. Risk assessment of *S. aureus* due to consumption of milk and milk products can be used to assist food safety regulators and manufacturers in identifying milk processing, storage, and retail practices that can encourage pathogen introduction and proliferation. This can lead to the development of more effective HACCP (Hazard Analysis Critical Control Point) programs and good manufacturing practices to ensure adequate consumer protection from the pathogen and its toxins in milk and milk products (FAO, WHO. 2006; Lee et al. 2015). Using a stochastic model,

(Makita et al. 2012) estimated the annual incidence rate of SFP to be 20 per 1000 people in raw milk and 5.4 cases per 10,000 people in traditional fermented milk. The risk of SFP was low in traditional fermented milk. Another quantitative risk assessment model (Weldeabezgi et al. 2019) reported 11 and 24 illnesses per 100 consumers from a single exposure to *S. aureus* for raw milk purchased from farm gates and MCCs, respectively. However, a single serving of traditionally fermented milk was not linked to a likely risk of illness in this study. In this study, the annual likely risk of illness from *S. aureus* due to consumption of raw milk and milk products was 24.2 and 48.3% in milk collected from dairy farms and MCCs, respectively. This means that nearly a quarter of farm gate and half of MCC milk consumers might experience illnesses annually. Traditionally fermented milk was found to be less risky, with 2.5% (2540 illnesses out of 100,000 consumers yearly) estimated illness per serving-per year. A study in Korea reported a low risk of *S. aureus* food-borne illness from the consumption of cheese, with probabilities of 0.286 and 0.818 *S. aureus* illness occurring per 1 million people per year (Lee et al. 2015). In Cuba, the risk of illness from *S. aureus* enterotoxin due to consumption of artisan fresh cheese was 46.9% (Vasallo et al. 2021), which was higher than the result in the two studies in Ethiopia.

In the current systematic review, penicillin G exhibited the highest percentage of pooled AMR of 92%. Similarly, *S. aureus* isolates showed high resistance rates to penicillin G in India (100%) (Thaker et al. 2013), Greece (99.3%) (Papadopoulos et al. 2018), India (98%, 86%) (Sudhanthiramani et al. 2015; Shylaja et al. 2018), China (72–85.2%) (Kou et al. 2021; Liu et al. 2017; Zhao et al. 2020) and Turkey (84.71%) (Kayili and Sanlibaba 2020). Relatively lower penicillin G resistance rates of 18.2%, 43% and 47.3% were reported in Brazil (Girardini et al. 2016), Mozambique (Nhatsave et al. 2021) and Iran (Jamali et al. 2015), respectively. Pooled resistance to ampicillin (82%) in this systematic review was comparable with the findings from China (79.6%) (Liu et al. 2017), India (88%, 74.42%) (Sudhanthiramani et al. 2015; Shylaja et al. 2018), and Turkey (72.94%) (Kayili and Sanlibaba 2020). However, a comparatively lower resistance rate was reported in India (40%) (Thaker et al. 2013). Pooled oxacillin resistance (33.4%) in our systematic review was consistent with a report in China (37.1%) (Kou et al. 2021), but a higher rate was reported in Turkey (63.53%) (Kayili and Sanlibaba 2020). However, a comparatively lower resistance to oxacillin was reported in Iran (16.2%) (Jamali et al. 2015). *S. aureus* has an extraordinary ability to develop resistance to antimicrobials. Many strains of *S. aureus* are resistant to β -lactam antibiotics due to

the production of an enzyme called β -lactamase that hydrolyzes the critical β -lactam bond and destroys the drug's antibacterial activity (Fuda et al. 2005). In line with this, pooled AMR in this review was found to be highest for β -lactams such as penicillin G, amoxicillin and ampicillin. The combination of penicillin and streptomycin or intramammary infusions containing β -lactams has been widely used to treat clinical mastitis in Ethiopia, which may explain the high AMR. Irrational drug use, repetitive use of few drugs and drug administration without sufficient diagnosis were reported in Ethiopia, which might have contributed to the occurrence of drug-resistant *S. aureus* strains (Kifle and Tadesse 2014; Gemedo et al. 2020; Gebeyehu et al. 2021). It has been reported that appropriate usage of antimicrobials in food producing animals is important to control and prevent the spread of drug-resistant isolates (Jessen et al. 2017).

The pooled estimate of tetracycline-resistant *S. aureus* (43.1%) in the current systematic review was higher than the findings in Greece (28.2%) (Papadopoulos et al. 2018), Mozambique (16%) (Nhatsave et al. 2021) and China (11.3%) (Kou et al. 2021), while Brazil (Girardini et al. 2016) recorded much lower resistance (2.3%). A slightly higher resistance was reported in Iran (56.1%) (Jamali et al. 2015). Tetracycline is the most commonly used antibiotic for the treatment of bacterial infections in animals in Ethiopia, as it is the cheapest and most readily available drug (Tufa et al. 2018), which could explain the reason for the higher resistance. In this review, antibiotics that are not used in veterinary medicine in Ethiopia, such as cephalothin (20.6%), vancomycin (19.8%), kanamycin (18.2%), gentamycin (15.7%), chloramphenicol (15.3%) and ciprofloxacin (3.8%), have shown lower resistance rates than those that are frequently used.

The cefoxitin (48.2%) and streptomycin (41.1%) resistance rates estimated in this systematic review were higher than the respective 5.5% and 5.8% prevalence rates reported in Iran (Jamali et al. 2015). Higher erythromycin resistance rates of 32.3% (Kou et al. 2021) and 58.7% (Zhao et al. 2020) were reported in China, compared to 23.7% calculated in this review. In contrast, a lower resistance rate of 7.9% was recorded in Iran (Jamali et al. 2015).

The high prevalence of MDR *S. aureus* isolates (62.1%) in this systematic review nearly agrees with earlier findings of 52.94% (Qian et al. 2019) and 55.4% (Zhao et al. 2020) in China and 59.2% in Jordan (Obaidat et al. 2018). However, higher MDR rates were reported in Nigeria (96.4%) and Turkey (72.94% (Kayili and Sanlibaba 2020)), while lower rates of 5%, 12.8%, 13.3%, and 13.95% were reported in Mozambique (Nhatsave et al.

2021), Iran (Jamali et al. 2015), Greece (Papadopoulos et al. 2018) and India (Sudhanthiramani et al. 2015), respectively. The relatively high prevalence of MDR *S. aureus* observed in this systematic review could be due to irrational drug use in the treatment of infections in animals in Ethiopia (Kifle and Tadesse 2014; Gemeda et al. 2020; Gebeyehu et al. 2021).

Implications and limitations

The findings of this systematic review suggest the need for intervention in milk and milk product safety along the dairy value chain. Policy makers may use these findings as an input to make intervention measures that safeguard the public from potential risks. The limitations of this systematic review are the presence of small number of study reports on *S. aureus* contamination of camel and goat milk, few studies reported on the occurrence of MRSA, and only two studies reported on the risk assessment of *Staphylococcus aureus* associated with the consumption of milk and milk products. The other limitation of this study is the large degree of heterogeneity observed among the studies. Research articles might also be published in databases other than those that we searched or not published at all.

Conclusions

This systematic review result indicated a high contamination rate of milk and milk products by *S. aureus*. The level of contamination significantly increased from udder to MCCs or processing plants. A large proportion (58.9%) of *S. aureus* isolates carried at least one gene encoding enterotoxins, showing a high risk of spread of SFP. The report of MRSA in milk and milk products is a potential health hazard. *S. aureus* resisted antibiotics commonly used in the treatment of infections in livestock and the pooled MDR was also high. The results of this systematic review indicated the potential public health risk of consuming raw milk and milk products in Ethiopia. Therefore, we recommend implementation of good hygiene and handling practices across the dairy value chain starting from farm, udder health, milk cooling, heat treatment of milk before drinking and rational use of antibiotics in veterinary medicine to reduce the potential health risk from *S. aureus* and MRSA contamination of milk and milk products.

Abbreviations

MCC: Milk collection center; SFP: Staphylococcal food poisoning; SE: Staphylococcal enterotoxin; MRSA: Methicillin resistant *Staphylococcus aureus*; AMR: Antimicrobial resistance; MDR: Multidrug resistance; PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses; PCR: Polymerase Chain Reaction.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s40550-022-00094-5>.

Additional file 1. PRISMA checklist

Additional file 2. Forest plot of *S. aureus* prevalence in raw cow milk

Additional file 3. Forest plot of *S. aureus* prevalence in camel, goat and pasteurized milk

Additional file 4. Forest plot of *S. aureus* prevalence in local cheese and fermented milk

Additional file 5. Raw data for antimicrobial resistance pattern against *S. aureus*

Authors' contributions

AD and KA participated in the study conception, design, data extraction, data synthesis, and publication draft preparation. The draft was independently reviewed by GM and SL. All authors read and approved the final manuscript.

Funding

Not applicable.

Availability of data and materials

All data generated during this study are included in this article and its additional files.

Declarations

Competing interests

The authors declare no competing interests.

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Received: 7 April 2022 Accepted: 15 July 2022

Published online: 10 September 2022

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