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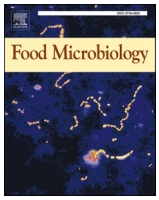

Review

# Distribution and prevalence of enterotoxigenic *Staphylococcus aureus* and staphylococcal enterotoxins in raw ruminants' milk: A systematic review

Maha Shalaby [a,b,c,*], Julien Reboud [b], Taya Forde [a], Ruth N. Zadoks [a,d], Valentina Busin [a,*]

[a] School of Biodiversity, One Health & Veterinary Medicine, University of Glasgow, Glasgow, United Kingdom
[b] James Watt School of Engineering, University of Glasgow, Glasgow, United Kingdom
[c] Food Control Department, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafr-El-Sheikh, 33516, Egypt
[d] Sydney School of Veterinary Science, Faculty of Science, The University of Sydney, NSW, 2006, Australia

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ABSTRACT

Enterotoxins produced by *Staphylococcus aureus* are a common cause of food poisoning, leading to significant gastrointestinal symptoms and even hospitalization. Following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, we searched three electronic databases for studies on detection of staphylococcal enterotoxins or enterotoxigenic *S. aureus* in raw ruminant milk. The 128 studies included in this systematic review showed a worldwide distribution of studies on staphylococcal enterotoxins and enterotoxigenic *S. aureus*, with an increase in the number from 1980 to 2021, a shift in detection methods from enterotoxins to enterotoxin genes, and a preponderance of studies from Europe and South America. Most studies focused on milk from individual animals with mastitis, especially cattle. Based on 24 studies, the within-herd prevalence of enterotoxigenic *S. aureus* in raw milk samples was 11.6%. Many studies failed to report the health status of sampled animals, or the numerator and denominator data needed for prevalence calculation. Cultural and legislative differences, economic status, diagnostic capabilities, and public awareness are all likely factors contributing to the observed distribution of studies. Our review highlighted a significant gap in quality and completeness of data reporting, which limits full assessment of prevalence and distribution of hazards posed by raw milk.

## 1. Introduction

*Staphylococcus aureus* is a pathogen that colonizes and infects various hosts, including food producing animals and humans (Peton and Le Loir, 2014). In animals, especially dairy ruminants like cattle, buffalo, goats and sheep, it is commonly reported as a cause of clinical and sub-clinical mastitis (Fagiolo and Lai, 2007; Hoekstra et al., 2019; Wellnitz and Bruckmaier, 2012). *S. aureus* is also a common cause of food poisoning, due to the production of staphylococcal enterotoxins (SEs) encoded by its enterotoxin genes (Argudín et al., 2010). At least 27 SEs have been reported so far (Merda et al., 2020) and their production is influenced by several factors, including temperature, humidity, and most importantly, bacterial density, which needs to be higher than $10^5$ colony forming units/ml in raw milk for enterotoxins to be formed (Bhatia and Zahoor, 2007; EC, 2005). Once numbers above this threshold are reached, toxin production is stimulated (Schelin et al., 2011).

Staphylococcal food poisoning (SFP) occurs following the consumption of food containing sufficient amounts of one (or more) preformed SEs (Dinges et al., 2000), even when present at a very low dose (<1 μg) (Evenson et al., 1988). SFP symptoms have a rapid onset (2–8 h) and comprise nausea, violent vomiting and abdominal cramping, with or without diarrhea (Tranter, 1990). It is usually a self-limiting disease and resolves within 24–48 h. However, in children, the elderly and people with existing morbidities, it can lead to hospitalization (Murray, 2005). Between 15% and 80% of *S. aureus* strains isolated from different food stuffs can potentially produce SEs (Fooladi et al., 2010).

The majority of SFP occurs due to consumption of food contaminated during harvesting, processing, transportation, storage, cooking or handling, as well as from inadequate cooling methods, which promote staphylococcal growth and toxin production (Hennekinne et al., 2012). Although human *S. aureus* strains are a common cause of SFP, animals with *S. aureus* carriage or infection are also recognized as an important source of contamination with enterotoxigenic *S. aureus* (Ortega et al., 2010), for example in raw milk, raw milk cheese, and raw and processed

* Corresponding author. School of Biodiversity, One Health & Veterinary Medicine, 464 Bearsden Rd, Bearsden, Glasgow, G61 1QH, United Kingdom.
*E-mail addresses:* 2427372s@student.ga.ac.uk, maha_shalaby@vet.kfs.edu.eg (M. Shalaby), vale.busin@gmail.com (V. Busin).

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meat products (Kadariya et al., 2014; Zhang et al., 2022). Even if the organism is inactivated and cannot be isolated from food stuffs, once the SEs are formed, they require prolonged boiling or autoclaving to gradually decrease their potency because they are thermostable (Johler et al., 2015; Ortega et al., 2010).

Detection of SEs can be performed using immunoassays, immunodiffusion, radioimmune assays, latex agglutination (Abril et al., 2020) and double gel diffusion assays (Robbins et al., 1974), however, commercially available diagnostic kits do not cover the full range of SEs (Féraudet Tarisse et al., 2021; Hait et al., 2018). More recently, assays have been developed for the detection of *S. aureus* enterotoxin genes, either directly from food samples using polymerase chain reaction (PCR) (Johnson et al., 1991) and loop-mediated amplification (LAMP) assays (Goto et al., 2007; Yin et al., 2016), or after a culture step to increase bacterial concentration.

SFP outbreaks have been reported worldwide, but specific incidence data are very limited, with reports available only from some subregions (WHO, 2015). Data availability is particularly limited in low- and middle-income countries for several reasons: many affected people do not seek medical attention, leading to limited availability of clinical specimens, and there is a lack of routine surveillance tools (Scallan et al., 2006). In Japan, a single outbreak led to more than 14,000 cases and one death in the summer of 2000 due to the consumption of pasteurized products containing small amounts of SEs (Ikeda et al., 2005), illustrating the potential scale of SFP outbreaks.

In order to collate epidemiological data on staphylococcal enterotoxins and enterotoxigenic *S. aureus* and assess the worldwide potential public health risk of milk-borne SFP, a systematic review of studies reporting on the investigation of *S. aureus* enterotoxin genes and/or its enterotoxins in milk was undertaken. The distribution of studies was described across time (decades), continents, animal species, health status and milk sources (individual or co-mingled), and an estimation of the prevalence of enterotoxigenic *S. aureus* and/or its related enterotoxins in raw ruminants' milk was carried out. This analysis provides epidemiological background to inform future food safety policy, identifies knowledge gaps around prevalence and distribution of SEs and enterotoxigenic *S. aureus*, and informs advice on future research methodology.

## 2. Methods

### 2.1. Search strategy

Studies were searched following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Page et al., 2021). Electronic databases searched were PubMed, Scopus, and Web of Science (which included Web of Science core collection, MEDLINE, CABI ABSTRACT and BIOSIS Previews). Search strings were developed and pre-tested to include variations of the terms (e.g. different synonyms and spellings). The search of each database was done without date restrictions. Articles retrieved from each database were transferred to an EndNote library. The full search strategy is listed in Table 1, with the last search implemented on July 1, 2021.

**Table 1**
Full search strategies for abstract collection for the systematic review of distribution and prevalence of enterotoxigenic *Staphylococcus aureus* in raw ruminants' milk.

| Database | Search strings |
|---|---|
| PubMed | All= (Search #1) AND All= (Search #2) AND All= (Search #3) |
| Scopus | Abstract, Title, Keyword= (Search #1) AND Abstract, Title, Keyword= (Search #2) AND Abstract, Title, Keyword= (Search #3) |
| Web of Science | TOPIC= (Search #1) AND TOPIC= (Search #2) AND TOPIC= (Search #3) |
| Where, | |
| Search #1 | (milk*) AND (ovine* OR sheep* OR goat* OR caprine* OR bovine* OR cow* OR cattle* OR buffalo* OR bubaline*) |
| Search #2 | (*aureus**) OR (coagulase* AND positive* AND Staphylococcus*) |
| Search #3 | (enterotoxi*) OR ((food*) AND (poisoning* OR intoxication*)) |

### 2.2. Inclusion and exclusion criteria for abstract screening

Following removal of duplicate records, article titles and abstracts were screened independently by two investigators (MS and VB) for inclusion in full text screening. They were selected for full text screening if the abstract was in English, if it was from an original study and if the article mentioned the detection of *S. aureus* enterotoxin genes or its related enterotoxins, either directly from raw milk, or through culture of *S. aureus* from milk and subsequent detection of enterotoxin genes or enterotoxin expression in the *S. aureus* isolates. They were excluded if they were not in English; were reviews, letters, editorial articles, book chapters, conference papers, theses, meta-analyses, experimental studies (e.g., induced mastitis, vaccination trials), studies optimizing or validating methods; if the milk samples were not obtained from domestic ruminants (pseudo ruminants and wildlife such as deer were not included); if the samples were only from processed (e.g. pasteurized) milk; if the studies were not specifically investigating or reporting enterotoxigenic *S. aureus* or staphylococcal enterotoxins, and if the abstract could not be retrieved. Although duplicates were removed at identification stage (Fig. 1), additional duplicates were detected and removed at screening stage. If disagreement between investigators was present at this stage, it was resolved by discussion.

### 2.3. Inclusion and exclusion criteria for full text screening

Retrieved full text articles were also screened independently by two investigators (MS and VB) prior to inclusion for data extraction and quality assessment. The criteria used for article screening were the same criteria used for abstract screening. However, in the case of full text screening, studies were only included if they reported the number of *S. aureus* isolates and/or strains carrying enterotoxin genes and/or producing enterotoxins either directly from raw milk, or through culture of *S. aureus* from milk and subsequent detection of enterotoxin genes or enterotoxin expression in the *S. aureus* isolates. If disagreement between investigators was present at this stage, a third investigator (RZ) independently reviewed these articles, with the third reviewer having the casting vote.

### 2.4. Data extraction

From each included article, the following data were extracted (if available): citation information (first author's name, journal name, volume and pages); temporal data (year of publication and year of study); geographical data (country and region); number of farms; animal species from which the samples were collected; health status of animal (healthy or mastitis, i.e., inflammation of the mammary gland) and test (s) used for mastitis diagnosis; milk source: consumption milk (bulk tank milk and/or milk available at retail, i.e., co-mingled milk) or host milk (individually collected milk), and laboratory methods used for detection of *S. aureus* enterotoxin genes and/or enterotoxins. Numerical data extracted (if available) were number of samples tested, number of *S. aureus* positive samples, number of *S. aureus* enterotoxin-positive samples and number of *S. aureus* isolates and/or strains that were carrying enterotoxin genes and/or able to produce enterotoxins. Any missing data were recorded as not reported (NR) (See https://doi.org/10.5525/gla.researchdata.1424).

### 2.5. Data analysis

Qualitative data analysis was carried out for temporal (decade of publication) and geographical distribution and distribution by animal species, milk source and health status. Geographical distribution was referenced according to the United Nations' Geoscheme UN M49, which

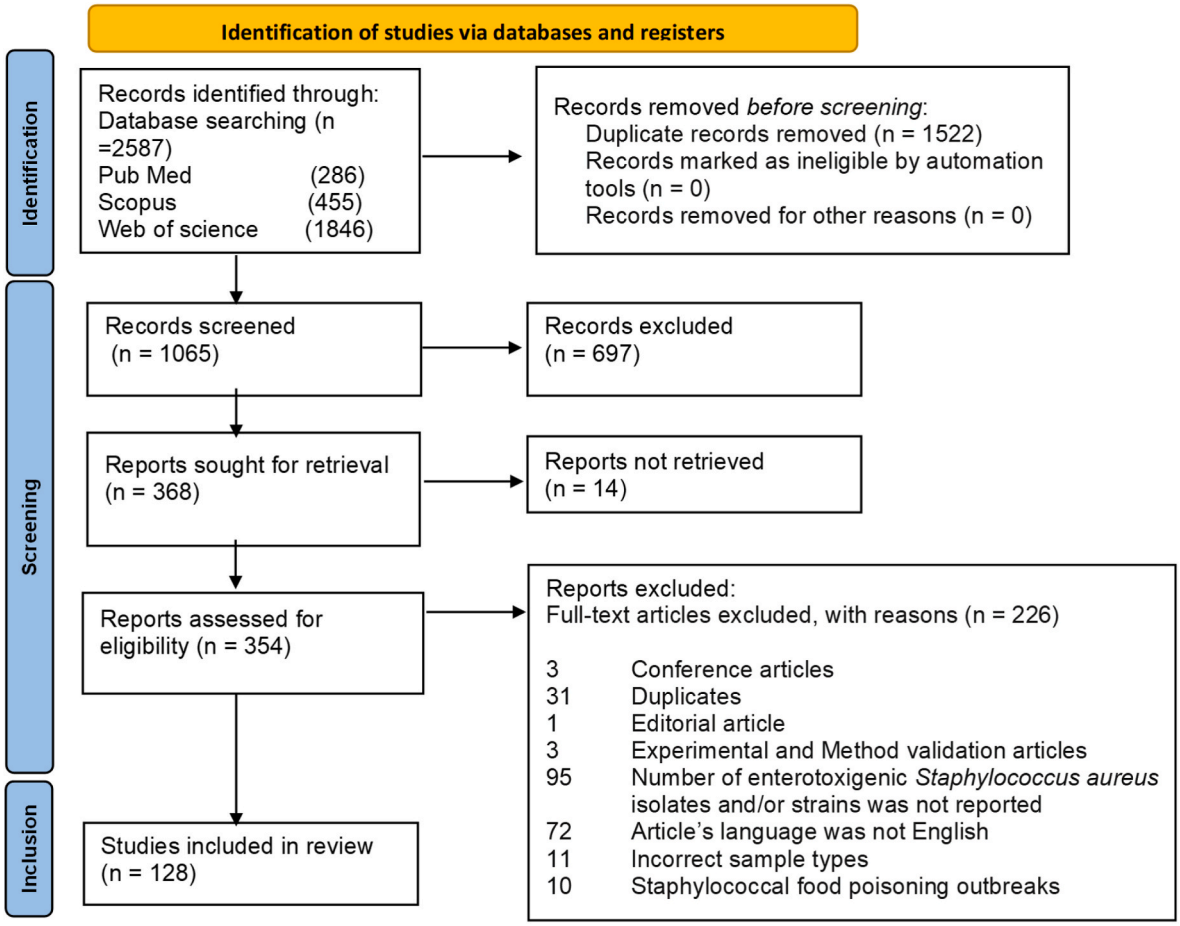


**Fig. 1.** PRISMA flowchart showing identification, screening, and inclusion of articles retrieved for the systematic review of distribution and prevalence of enterotoxigenic *Staphylococcus aureus* or staphylococcal enterotoxins in raw ruminants' milk.

provides country codes for statistical use (https://unstats.un.org/unsd/methodology/m49/).

For those studies for which all the relevant data were provided, the within-study prevalence of enterotoxigenic *S. aureus* in raw ruminants' milk was calculated as the number of milk samples positive for *S. aureus* enterotoxins or milk samples positive for isolates and/or strains carrying enterotoxin genes and/or milk samples positive for isolates and/or strains producing enterotoxins divided by the total number of milk samples*100. Prevalence data were pooled based on the detection method (detection of enterotoxins in milk or produced by *S. aureus* cultured from milk, or detection of enterotoxin genes in milk or in *S. aureus* cultured from milk) and compared based on categories of temporal (decade of publication), or geographical origin, animal species, milk source and health status. Statistical analysis was performed in Excel (Microsoft, Seattle, WA), using a one-way ANOVA to compare prevalence across categories, followed by a Tukey Kramer post hoc test to determine which categories were significantly different ($P < 0.05$), based on the studentized range distribution (q value).

### 2.6. Quality assessment

The quality of included studies was assessed following the Joanna Briggs Institute (JBI) critical appraisal checklist for prevalence studies (Munn et al., 2015), with some modifications to suit this study. Quality assessment was based on the availability and compliance with the following criteria: (1) sampling frame (i.e., representative sampling based on breed, herd or farm), sample quality: (2) handling (i.e., disinfection before sample collection and use of sterile sampling containers) and (3) transportation (i.e., use of insulated and cooled shipping containers), sample size: (4) appropriate milk volume, (5) number of animals, (6) number of samples, (7) number of farms/collection centers, (8) description of health status (i.e., healthy and/or affected by mastitis), (9) analysis of data (i.e., completeness of data provided) and (10) validity of laboratory methods used (based on detection target, *S. aureus* enterotoxin genes and/or enterotoxins). Each study was given a score of 0 (data not available and/or not compliant) or 1 (data available and compliant), with a maximum score of 10. An overall assessment of high (score 8 or above), moderate (score between 5 and 7), or low (score 4 and below) quality was assigned to each article, however no studies were excluded based on the quality assessment (See https://doi.org/10.5525/gla.researchdata.1424).

## 3. Results

### 3.1. Literature search

The total number of retrieved articles was 2587. After removal of duplicates, 1065 articles were available for title and abstract screening. Of those, 354 studies were included for full text screening and 128 studies were included for data extraction and quality assessment. Reasons for full-text exclusion are listed in Fig. 1, without any exclusion based on quality assessment.

### 3.2. Qualitative data analysis

#### 3.2.1. Temporal distribution

Distribution of studies over time (year of publication grouped by decades) is summarized in Table 2. The highest number of studies was in

**Table 2**
Temporal distribution of included studies (year of publication grouped by decades) in the systematic review of distribution and prevalence of enterotoxigenic *Staphylococcus aureus* in raw ruminants' milk.

| Decade of publication | Total number of studies | *Staphylococcus aureus* enterotoxin genes | *Staphylococcus aureus* enterotoxins | *Staphylococcus aureus* enterotoxin genes and its enterotoxins |
|---|---|---|---|---|
| 1980–1990 | 9 | 0 | 9 | 0 |
| 1991–2000 | 12 | 1 | 11 | 0 |
| 2001–2010 | 31 | 20 | 5 | 6 |
| 2011–2021 | 76 | 60 | 4 | 12 |
| Total | 128 | 81 | 29 | 18 |

the last decade (2011–2021, n = 76), with *S. aureus* enterotoxin genes being the only target in the majority of studies during that period (n = 60).

### 3.2.2. Geographical distribution

Distribution was worldwide (Fig. 2), with the highest number of studies reported in South America and Europe (n = 32 from each continent), while the lowest number of studies were in Oceania (n = 1) and North America (n = 2). The highest number of studies on cows were from South America (n = 25), while studies on sheep and goats were mostly from Europe (n = 5 and 10, respectively), and those on buffalo primarily from Africa (n = 6). Most studies on host milk were from South America (n = 21), while studies on consumption milk were most common in Europe (n = 19). In Asia and South America, most studies focused on milk from animals with mastitis (n = 15 and 19, respectively) whereas in Europe and Africa, most studies focused on milk of unknown health status (n = 21 and 12, respectively). There were 13 studies in which the region and/or the country were not reported. Full geographical distribution of included studies is reported in this link https://doi.org/10.5525/gla.researchdata.1424.

### 3.2.3. Distribution by animal species, health status and milk source

The highest number of studies were on cow milk (n = 91), followed by goat (n = 24) and sheep milk (n = 14), while buffalo milk (n = 10) was the least commonly reported on. Studies on milk from animals with mastitis (n = 59) and studies without specification of the animals' health status (n = 58) were comparable in number (Fig. 3). The majority of studies focused on individual host milk samples (n = 78) rather than comingled milk for consumption. The full distribution of included studies is reported in this link (https://doi.org/10.5525/gla.researchdata.1424).

## 3.3. Quantitative analysis

The overall prevalence of enterotoxigenic *S. aureus* in raw ruminants' milk was 11.6%, based on 24 studies which reported (or provided sufficient data to calculate) the prevalence. These comprised 10 studies focusing on enterotoxigenic genes, 8 studies on expressed enterotoxins from isolates, 3 studies on enterotoxigenic genes and expressed enterotoxins from isolates and 3 studies on the detection of toxins from milk samples. The highest calculated prevalence was reported in the third decade (2001–2010) (16.2%), in Europe (27.5%), from goat milk (31.3 %) and consumption milk samples (14.2%). The lowest calculated prevalence was in the first decade (1980–1990) (9.1%), in Oceania (2.4%), from cow milk (12.2%), healthy (7.2%) and host milk samples (11.8%). Although there were numerical differences (Fig. 4), there were no statistically significant differences in prevalence among the categories of interest (decade, region, animal species, health status and milk source).

## 3.4. Quality assessment

From the included studies (n = 128), 37 were assessed as high-, 52 as moderate- and 39 as low-quality. The breakdown of each category is summarized in https://doi.org/10.5525/gla.researchdata.1424.

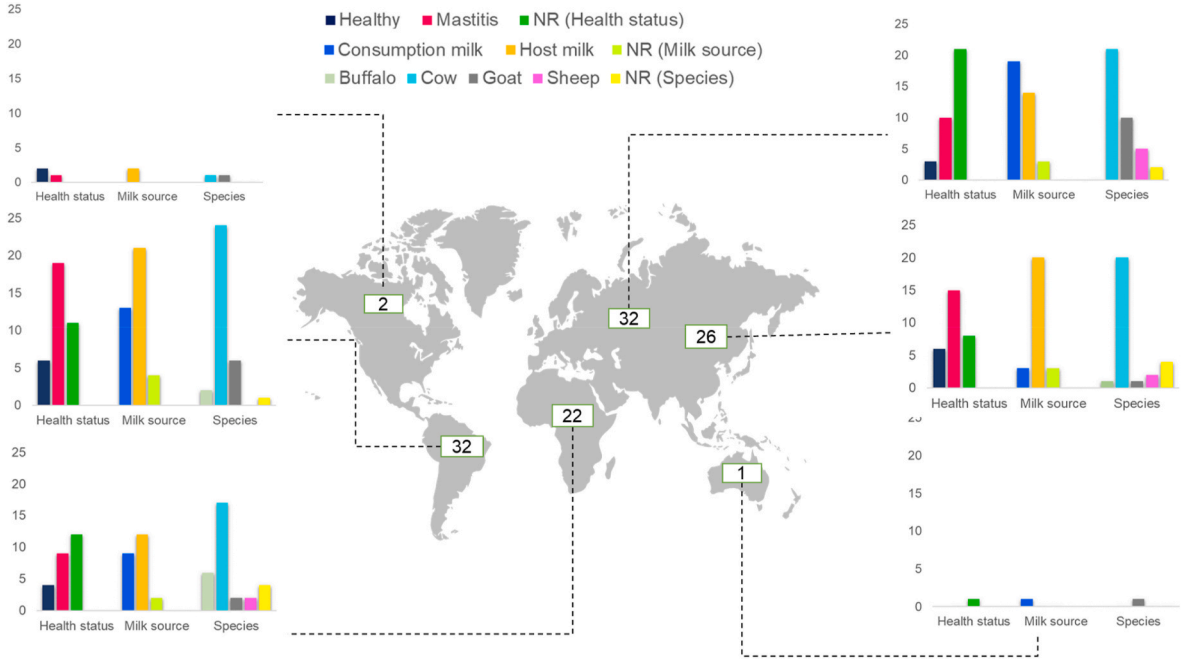


**Fig. 2.** Geographical distribution of studies included in the systematic review on the distribution and prevalence of enterotoxigenic *Staphylococcus aureus* or staphylococcal enterotoxins in raw ruminants' milk, with breakdown by health status, milk source and animal species. NR = Not reported, Host milk = individually collected milk samples, Consumption milk = bulk tank milk and/or co-mingled milk available at retail. Eighteen studies covered multiple categories and were included in this calculation more than once (e.g., if a study reported on healthy and mastitis milk in sheep and goats, it was counted under categories: healthy, mastitis, sheep and goat).

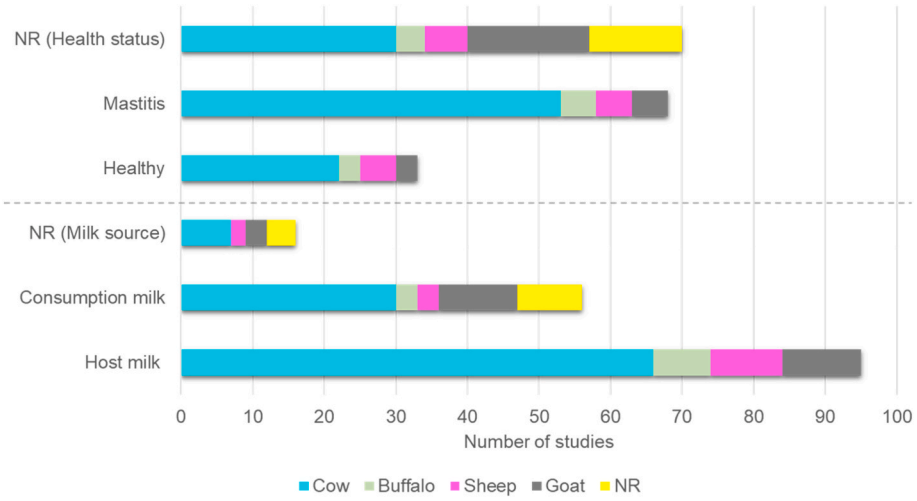


**Fig. 3.** Distribution of studies included in the systematic review of distribution and prevalence of enterotoxigenic *Staphylococcus aureus* or staphylococcal enterotoxins in raw ruminants' milk by animal species, health status and milk source. NR = Not reported, Host milk = individually collected milk samples, Consumption milk = bulk tank milk and/or co-mingled milk available at retail. Eighteen studies covered multiple categories and were included in this calculation more than once (e.g., if a study reported on healthy and mastitis milk in sheep and goats, it was counted under categories: healthy, mastitis, sheep and goat).

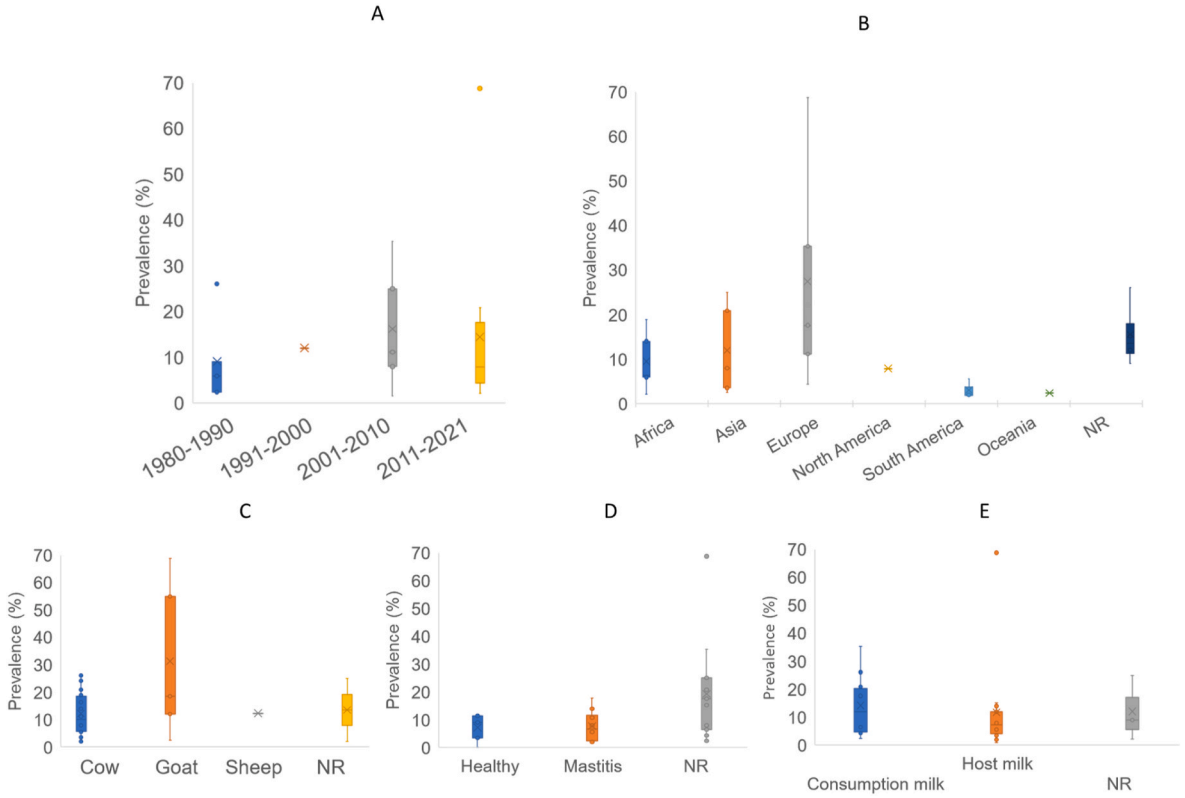


**Fig. 4.** Box plot showing the prevalence of enterotoxigenic *Staphylococcus aureus* or staphylococcal enterotoxins in raw ruminants' milk depending on: (A) temporal distribution (year of publication, grouped by decade), (B) geographical distribution (by continent), (C) animal species, (D) health status and (E) milk source. NR = Not reported, Host milk = individually collected milk samples, Consumption milk = bulk tank milk and/or co-mingled milk available at retail. Horizontal line in the box = median, X = Average.

## 4. Discussion

This systematic review collected all the retrievable studies published in English and reporting on the investigation of enterotoxigenic *S. aureus,* staphylococcal enterotoxins and staphylococcal enterotoxin genes in raw milk samples of the major domestic ruminant species: cows, goats, sheep and buffalo. The temporal and geographical distribution of these studies were described, in addition to the ruminant species sampled, their health status, and the milk source. Additionally, prevalence estimates were calculated from studies that provided sufficient data. Our review differs from a recent review of *S. aureus* prevalence in milk (raw and pasteurized) and milk products (Zhang et al., 2022) by specifically reporting on enterotoxigenic *S. aureus* prevalence in raw milk, considering both staphylococcal enterotoxins (which are

ultimately responsible for food poisoning) and their associated genes. Due to the distinct focus and consequently the differences in search strings implemented, the review by Zhang et al. does not include any of the 47 papers we included that report the detection of the enterotoxins themselves – from raw milk directly or after isolation. Moreover, none of the 24 studies used for quantitative analysis in our study were included in the quantitative analysis of Zhang et al. study for calculating the prevalence of enterotoxin genes. Our review thus represents a novel and important contribution, summarizing the available literature on enterotoxigenic *S. aureus* as a potential cause of food poisoning.

Our study revealed a steady increase (since 1980) in the number of studies on enterotoxigenic *S. aureus*, rising from 9 to 76 per decade. The increasing number of studies is primarily related to those using molecular biological assays (nucleic acid amplification) only (from 0 to 60 per decade), while those using only direct detection of SEs have decreased (from 9 to 4 per decade). This shift is likely to have been influenced by the development of molecular diagnostic techniques, which have become well established and accurate (Schmitz et al., 2022). Nucleic acid amplification tests (NAAT) are assays that provide valuable information towards the characterization of bacterial strains not only from culture, but also directly from the product of interest (Zadoks et al., 2023), which may include non-culturable *S. aureus* strains (Wu et al., 2016). In particular, the detection of *S. aureus* enterotoxin genes by NAAT has high sensitivity and specificity (Johnson et al., 1991; Letertre et al., 2003; Yin et al., 2016). However, the equipment required may be prohibitively expensive in low-resource settings (Yin et al., 2016). Moreover, NAAT are indirect methods that target the DNA and not the SEs themselves (Wu et al., 2016), and thus do not give any information about gene expression (Hennekinne et al., 2012). In the case of outbreaks and food safety emergencies, gene detection techniques are insufficient to be used alone as a confirmative method that enterotoxigenic *Staphylococcus aureus* was the cause of food poisoning (Hennekinne et al., 2012). Based on the geographic distribution of studies (Fig. 1), staphylococcal enterotoxins are primarily studied in low- and middle-income countries. Low-cost NAAT methods, e.g. paper-based microfluidic assays (Reboud et al., 2019), could provide a good alternative to equipment-based NAAT testing in those regions. Direct detection of SEs using lab-based and commercial immunoassays, similarly, exhibits high sensitivity and specificity using high quality antibodies. However, it is generally time consuming and laborious, e.g. to due to long incubation periods and multiple washing steps as needed for ELISA (Wu et al., 2016), and the sensitivity depends on the amount of detectable toxin(s) and the sample purity (Sharma et al., 2000). Moreover, reversed passive latex agglutination (RPLA) suffers from cross reactivity (Lee et al., 1978, 1980). Furthermore, the growing number of studies may not just reflect technological progress, but also an increasing awareness of the public health importance of enterotoxigenic *S. aureus* as a foodborne pathogen. The number of SFP outbreaks reported in the European Union increased from 25 outbreaks between 2007 and 2011 (EFSA, 2013) to 114 outbreaks between 2014 and 2018 (EFSA, 2019), with 117 outbreaks in 2019 and 2020 (EFSA, 2021a, b). Similarly, the number of SFP outbreaks in the United States increased from 42 outbreaks between 1993 and 1997 (Olsen et al., 2000) to 75 outbreaks between 2009 and 2015 (Dewey-Mattia et al., 2018).

The geographical distribution of studies was worldwide, with similarly high numbers of studies from Europe and South America (32 each). In Europe, we hypothesize that this may be driven, at least in part, by food safety concerns. According to the European Food Safety Authority (EFSA), consumption and marketing of raw milk in Europe is not prohibited, but consumers are advised to boil milk before consumption to avoid any microbiological hazards (EFSA, 2015). High diagnostic capacity compared to developing countries (Harvey et al., 2012) and high economic status of European countries (OECD, 2022), may also contribute to the high number of studies reported in Europe. However, the same number of studies was reported from South America, which has a lower economic status than Europe (OECD, 2022), with the vast majority of studies reported from Brazil (n = 27). Brazil is one of the largest (sixth) dairy producers in the world (Andrighi et al., 2019). More than 30% of the raw milk produced in Brazil is used for cheese production (Farina et al., 2005) and about 55% of cheeses are fresh, produced without any heat treatment (Nogueira, 2021). Fresh cheeses have been generally implicated as high-risk products for SFP (Alves et al., 2018; Cortimiglia, 2015). Raw milk is also sold in retail stores (Farina et al., 2005), despite trading raw milk in Brazil being prohibited by the law (Argentina, 1980). Possibly linked to these consumption practices, enterotoxigenic *S. aureus* is one of the most common foodborne pathogens in Brazil, having caused more than 15,000 illnesses between 2000 and 2018 (Finger et al., 2019). Those factors are likely to have contributed to the high number of studies reported in Brazil. In contrast, a very low number of studies was reported in Oceania (n = 1); this may be due in part to restrictions on sales of raw milk, e.g. marketing of raw milk in New South Wales (NSW) and Australia was made illegal following several food poisoning outbreaks associated with its consumption (NSW, 2014). In Australia, the incidence of hospitalization due to food poisoning has declined since 2000 (Kirk et al., 2014), and almost 30% of reported staphylococcal outbreaks were associated with commercially prepared food (Pillsbury et al., 2013). The low number of studies reported in North America (n = 2), similarly, could be due to restrictions on trade and consumption of raw milk in a considerable number of US states (Koski et al., 2022).

Most studies were on cow milk samples, followed by those from goats and sheep, while the lowest number of studies was on buffalo milk samples. Cow milk production represents 85% of the total worldwide milk production and is the most popular milk consumed (Gerosa and Skoet, 2012). Studies on small ruminants' milk (goats and sheep) were mainly reported from Europe. Although Europe does not produce as much small ruminant milk as Africa or Asia (Gonzales-Barron et al., 2017), goat and sheep milk production is an important sector (Silanikove et al., 2016) and the relative overrepresentation of European studies may be linked to socioeconomic aspects of public health and the diagnostic capacities discussed above. Studies on buffalo milk were mainly reported from Asia and Africa, which would be expected as the highest population and production of buffalo milk is in Asia, followed by Africa (Gilbert et al., 2018). The low number of studies on buffalo milk may reflect its relative contribution to global milk production, which was estimated at 15% (Zicarelli, 2020). It may also be exacerbated by the lower socioeconomic status (Grace, 2015) in Asia and Africa, which limits conducting and/or publication of studies (Whitworth et al., 2008).

Most of the included studies were conducted on milk samples from animals with mastitis but many studies did not report the health status of the milk samples tested, revealing a significant data gap. Most eligible studies were on individually collected milk samples (host milk), as opposed to bulk tank or other comingled milk samples (consumption milk), which generally should exclude milk from animals with clinical mastitis, especially where this is mandated by law. Exclusion of milk from clinically affected animals, however, is not sufficient to eliminate the risk of milk-borne SFP as *S. aureus* is one of the main causes of both clinical and subclinical mastitis in domesticated ruminants. Both types of mastitis may be caused by enterotoxigenic *S. aureus* strains, especially those producing enterotoxin C (Rajic-Savic et al., 2015; Zhang et al., 2022). Although milk from clinically affected animals can be excluded from consumption, and must be excluded in many countries, milk from sub-clinically affected animals cannot easily be excluded, reinforcing the need for diagnostic tests to detect SE or SE genes and the potential value of point-of-need tests to assess the safety of raw milk for human consumption.

Although the majority (n = 89) of studies were of high or moderate quality, the quantitative analysis identified important gaps that should be addressed in future research. One of the most critical gaps was the failure to report an essential detail for prevalence calculation, namely the total number of collected samples (denominator data). In some studies, the results from raw milk samples were combined with the

results from other samples, without specification of the origin of the enterotoxigenic *S. aureus* strains (i.e., whether the isolates were from raw milk samples or from other sample types), and other studies did not specify whether enterotoxigenic *S. aureus* strains were collected from one individual or comingled samples.

This systematic review showed the distribution of studies investigating enterotoxigenic *S. aureus* in raw ruminants' milk around the world. Cultural and legislative differences, like habits or restrictions regarding raw milk consumption, as well as differences in economic status, diagnostic capabilities and public awareness between countries are all likely factors contributing to the observed predominance of studies in Europe and South America. Our data showed a considerable shift in the laboratory methods over time and would suggest the value of investing further in developing rapid and accurate NAAT methods that could be used at the point-of-need. Our study also highlighted a significant gap in data reporting. As a result, we advise researchers to focus on including critical data for prevalence calculations in their publications.

## Author contributions

MS, VB, RNZ, and JR contributed to the concept and the design of the work. MS searched and populated the database, whilst VB and RNZ participated in the screening. MS wrote the first draft and subsequent modifications of the manuscript. All authors contributed to manuscript revisions, read, and approved the submitted version.

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## Declaration of competing interest

The authors declare no conflict of interest.

## Data availability

All data are available in Appendix A.

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## Appendix A. Supplementary data

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## References

Abril, A., Gonzalez-Villa, T., Barros-Velázquez, J., Cañas, B., Sánchez-Pérez, A., Calo-Mata, P., Carrera, M., 2020. Staphylococcus aureus exotoxins and their detection in the dairy industry and mastitis. Toxins 2020 12, 537.

Alves, V.F., Nino-Arias, F.C., Pitondo-Silva, A., de Araújo Frazilio, D., de Oliveira Gonçalves, L., Toubas, L.C., Torres, I.M.S., Oxaran, V., Dittmann, K.K., De Martinis, E.C.P., 2018. Molecular characterisation of *Staphylococcus aureus* from some artisanal Brazilian dairies. Int. Dairy J. 85, 247–253.

Andrighi, D., Frizzo, A.F., Junior, I.A.N., Starikoff, K.R., 2019. Milk consumption habits on dairy for farmers of family farming. Braz. J. Vet. Res. Anim. Sci. 56, e155455-e155455.

Argentina, P.d.l.N., 1980. Quality grading of milk. Decree-Law 6640/63, as amended by the Laws 20 330 and 22 263. Industria Lechera 60, 11–13.

Argudín, M.Á., Mendoza, M.C., Rodicio, M.R., 2010. Food poisoning and *Staphylococcus aureus* enterotoxins. Toxins 2, 1751–1773.

Bhatia, A., Zahoor, S., 2007. *Staphylococcus aureus* enterotoxins: a review. J. Clin. Diagn. Res. 3, 188–197.

Cortimiglia, C., 2015. Short communication: prevalence of *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA) in bulk tank milk of dairy goat farms from Northern Italy. J. Dairy Sci. 98, 2307–2311.

Dewey-Mattia, D., Manikonda, K., Hall, A.J., Wise, M.E., Crowe, S.J., 2018. Surveillance for foodborne disease outbreaks—United States, 2009–2015. Morb. Mortal. Wkly. Rep. - Surveillance Summ. 67, 1.

Dinges, M.M., Orwin, P.M., Schlievert, P.M., 2000. Exotoxins of *Staphylococcus aureus*. Clin. Microbiol. Rev. 13, 16–34.

EC, 2005. Commission Regulation (EC) No 2073.

EFSA, 2013. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2011. EFSA J. 11, 3129.

EFSA, 2015. Scientific opinion on the public health risks related to the consumption of raw drinking milk. EFSA J. 13, 3940.

EFSA, 2019. The European Union one health 2018 zoonoses report. EFSA J. 17, e05926.

EFSA, 2021a. The European Union one health 2019 zoonoses report. EFSA J. 19, e06406.

EFSA, 2021b. The European Union one health 2020 zoonoses report. EFSA J. 19, e06971.

Evenson, M.L., Hinds, M.W., Bernstein, R.S., Bergdoll, M.S., 1988. Estimation of human dose of staphylococcal enterotoxin A from a large outbreak of staphylococcal food poisoning involving chocolate milk. Int. J. Food Microbiol. 7, 311–316.

Fagiolo, A., Lai, O., 2007. Mastitis in buffalo. Ital. J. Anim. Sci. 6, 200–206.

Farina, E.M., Gutman, G.E., Lavarello, P.J., Nunes, R., Reardon, T., 2005. Private and public milk standards in Argentina and Brazil. Food Pol. 30, 302–315.

Féraudet Tarisse, C., Goulard-Huet, C., Nia, Y., Devilliers, K., Marcé, D., Dambrune, C., Lefebvre, D., Hennekinne, J.-A., Simon, S., 2021. Highly sensitive and specific detection of staphylococcal enterotoxins SEA, SEG, SEH, and SEI by immunoassay. Toxins 13, 130.

Finger, J.A., Baroni, W.S., Maffei, D.F., Bastos, D.H., Pinto, U.M., 2019. Overview of foodborne disease outbreaks in Brazil from 2000 to 2018. Foods 8, 434.

Fooladi, A.I., Tavakoli, H., Naderi, A., 2010. Iranian J. Microbiol. IJM 2, 137.

Gerosa, S., Skoet, J., 2012. Milk availability: trends in production and demand and medium-term outlook. ESA Working Paper 12-01. https://doi.org/10.22004/ag.econ.289000.

Gilbert, M., Nicolas, G., Cinardi, G., Van Boeckel, T.P., Vanwambeke, S., Wint, W.G.R., Robinson, T.P., 2018. Global Buffaloes Distribution in 2010 (5 Minutes of Arc), V3 ed. Harvard Dataverse.

Gonzales-Barron, U., Gonçalves-Tenório, A., Rodrigues, V., Cadavez, V., 2017. Foodborne pathogens in raw milk and cheese of sheep and goat origin: a meta-analysis approach. Curr. Opin. Food Sci. 18, 7–13.

Goto, M., Hayashidani, H., Takatori, K., Hara-Kudo, Y., 2007. Rapid detection of enterotoxigenic *Staphylococcus aureus* harbouring genes for four classical enterotoxins, SEA, SEB, SEC and SED, by loop-mediated isothermal amplification assay. Lett. Appl. Microbiol. 45, 100–107.

Grace, D., 2015. Food safety in low and middle income countries. Int. J. Environ. Res. Publ. Health 12, 10490–10507.

Hait, J.M., Nguyen, A.T., Tallent, S.M., 2018. Analysis of the VIDAS® Staph Enterotoxin III (SET3) for detection of staphylococcal enterotoxins G, H, and I in foods. J. AOAC Int. 101, 1482–1489.

Harvey, A., Brand, A., Holgate, S.T., Kristiansen, L.V., Lehrach, H., Palotie, A., Prainsack, B., 2012. The future of technologies for personalised medicine. Biotechnol. J. 29, 625–633.

Hennekinne, J.-A., De Buyser, M.-L., Dragacci, S., 2012. Staphylococcus aureus and its food poisoning toxins: characterization and outbreak investigation. FEMS Microbiol. Rev. 36, 815–836.

Hoekstra, J., Rutten, V.P., Van den Hout, M., Spaninks, M.P., Benedictus, L., Koop, G., 2019. Differences between *Staphylococcus aureus* lineages isolated from ovine and caprine mastitis but not between isolates from clinical or subclinical mastitis. J. Dairy Sci. 102, 5430–5437.

Ikeda, T., Tamate, N., Yamaguchi, K., Makino, S.-i., 2005. Mass outbreak of food poisoning disease caused by small amounts of staphylococcal enterotoxins A and H. Appl. Environ. Microbiol. 71, 2793–2795.

Johler, S., Weder, D., Bridy, C., Huguenin, M.-C., Robert, L., Hummerjohann, J., Stephan, R., 2015. Outbreak of staphylococcal food poisoning among children and staff at a Swiss boarding school due to soft cheese made from raw milk. J. Dairy Sci. 98, 2944–2948.

Johnson, W., Tyler, S., Ewan, E., Ashton, F., Pollard, D., Rozee, K., 1991. Detection of genes for enterotoxins, exfoliative toxins, and toxic shock syndrome toxin 1 in *Staphylococcus aureus* by the polymerase chain reaction. J. Clin. Microbiol. 29, 426–430.

Kadariya, J., Smith, T.C., Thapaliya, D., 2014. *Staphylococcus aureus* and staphylococcal food-borne disease: an ongoing challenge in public health. BioMed Res.Int. 2014.

Kirk, M., Ford, L., Glass, K., Hall, G., 2014. Foodborne illness, Australia, circa 2000 and circa 2010. Emerg. Infect. Dis. 20, 1857.

Koski, L., Kisselburgh, H., Landsman, L., Hulkower, R., Howard-Williams, M., Salah, 2022. Foodborne illness outbreaks linked to unpasteurised milk and relationship to changes in state laws–United States, 1998–2018. Epidemiol. Infect. 150, e183.

Lee, A., Robbins, R., Reiser, R., Bergdoll, M., 1980. Isolation of specific and common antibodies to staphylococcal enterotoxins B, C1, and C2. Infect. Immun. 27, 431–434.

Lee, A.C.-m., Robbins, R., Bergdoll, M., 1978. Isolation of specific and common antibodies to staphylococcal enterotoxins A and E by affinity chromatography. Infect. Immun. 21, 387–391.
Letertre, C., Perelle, S., Dilasser, F., Fach, P., 2003. Detection and genotyping by real-time PCR of the staphylococcal enterotoxin genes sea to sej. Mol. Cell. Probes 17, 139–147.
Merda, D., Felten, A., Vingadassalon, N., Denayer, S., Titouche, Y., Decastelli, L., Hickey, B., Kourtis, C., Daskalov, H., Mistou, M.-Y., 2020. NAuRA: genomic tool to identify staphylococcal enterotoxins in *Staphylococcus aureus* Strains responsible for foodborne outbreaks. Front. Microbiol. 11, 1483.
Munn, Z., Moola, S., Lisy, K., Riitano, D., Tufanaru, C., 2015. Methodological guidance for systematic reviews of observational epidemiological studies reporting prevalence and cumulative incidence data. Int. J. Evid. Base. Healthc. 13, 147–153.
Murray, R., 2005. Recognition and management of *Staphylococcus aureus* toxin-mediated disease. Intern. Med. J. 35, S106–S119.
Nogueira, S., 2021. Dairy and Products Annual. Global agricultural information Network.
NSW, 2014. Raw Milk – a Reminder to All Dairy Production Licensees of Their Obligations.
OECD, 2022. OECD Econ. Outlook 2022 Issue 1.
Olsen, S.J., MacKinnon, L.C., Goulding, J.S., Bean, N.H., Slutsker, L., 2000. Surveillance for foodborne-disease outbreaks—United States, 1993–1997. Morb. Mortal. Wkly. Rep. CDC Surveillance Summ. 49, 1–62.
Ortega, E., Abriouel, H., Lucas, R., Gálvez, A., 2010. Multiple roles of *Staphylococcus aureus* enterotoxins: pathogenicity, superantigenic activity, and correlation to antibiotic resistance. Toxins 2, 2117–2131.
Page, M.J., McKenzie, J.E., Bossuyt, P.M., Boutron, I., Hoffmann, T.C., Mulrow, C.D., Shamseer, L., Tetzlaff, J.M., Akl, E.A., Brennan, S.E., 2021. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews 372. Bmj.
Peton, V., Le Loir, Y., 2014. *Staphylococcus aureus* in veterinary medicine. Infect. Genet. Evol. 21, 602–615.
Pillsbury, A., Chiew, M., Bates, J., Sheppeard, V., 2013. An outbreak of staphylococcal food poisoning in a commercially catered buffet. Comm. Dis. Intell. 37, E144–E148.
Rajic-Savic, N., Katic, V., Velebit, B., Colovic, S., 2015. Characteristics of enterotoxigenic coagulase positive staphylococci isolated from bovine milk in cases of subclinical mastitis. Procedia Food Sci. 5, 250–253.
Reboud, J., Xu, G., Garrett, A., Adriko, M., Yang, Z., Tukahebwa, E.M., Rowell, C., Cooper, J.M., 2019. Based microfluidics for DNA diagnostics of malaria in low resource underserved rural communities. Proc. Natl. Acad. Sci. USA 116, 4834–4842.
Robbins, R., Gould, S., Bergdoll, M., 1974. Detecting the enterotoxigenicity of *Staphylococcus aureus* strains. Appl. Environ. Microbiol. 28, 946–950.
Scallan, E., Jones, T.F., Cronquist, A., Thomas, S., Frenzen, P., Hoefer, D., Medus, C., Angulo, F.J., Group, F.W., 2006. Factors associated with seeking medical care and submitting a stool sample in estimating the burden of foodborne illness. Foodborne Pathogens 3, 432–438.
Schelin, J., Wallin-Carlquist, N., Thorup Cohn, M., Lindqvist, R., Barker, G.C., 2011. The formation of *Staphylococcus aureus* enterotoxin in food environments and advances in risk assessment. Virulence 2, 580–592.
Schmitz, J.E., Stratton, C.W., Persing, D.H., Tang, Y.-W., 2022. Forty years of molecular diagnostics for infectious diseases. J. Clin. Microbiol. 60, e02446-02421.
Sharma, N.K., Rees, C.E., Dodd, C.E., 2000. Development of a single-reaction multiplex PCR toxin typing assay for *Staphylococcus aureus* strains. Appl. Environ. Microbiol. 66, 1347–1353.
Silanikove, N., Leitner, G., Merin, U., 2016. Influence of animal health, breed, and diet on non-cow milk composition. Non-bovine milk and milk products 61–79.
Tranter, H.S., 1990. Foodborne staphylococcal illness. Lancet (British edition) 336, 1044–1046.
Wellnitz, O., Bruckmaier, R.M., 2012. The innate immune response of the bovine mammary gland to bacterial infection. Vet. J. 192, 148–152.
Whitworth, J.A., Kokwaro, G., Kinyanjui, S., Snewin, V.A., Tanner, M., Walport, M., Sewankambo, N., 2008. Strengthening capacity for health research in Africa. Lancet 372, 1590–1593.
WHO, 2015. WHO Estimates of the Global Burden of Foodborne Diseases: Foodborne Disease Burden Epidemiology Reference Group 2007-2015. World Health Organization.
Wu, S., Duan, N., Gu, H., Hao, L., Ye, H., Gong, W., Wang, Z., 2016. A review of the methods for detection of Staphylococcus aureus enterotoxins. Toxins 8, 176.
Yin, H., Fang, T., Wen, H., 2016. Combined multiplex loop-mediated isothermal amplification with lateral flow assay to detect sea and seb genes of enterotoxic. Staphylococcus aureus Letters in applied microbiology 63, 16–24.
Zadoks, R., Scholz, E., Rowe, S., Norris, J., Pooley, H., House, J., 2023. A framework for evaluation of on-farm mastitis diagnostics in Australia. Aust. Vet. J. 101, 142–152.
Zhang, J., Wang, J., Jin, J., Li, X., Zhang, H., Zhao, C., 2022. Prevalence, antibiotic resistance, and enterotoxin genes of *Staphylococcus aureus* isolated from milk and dairy products worldwide: a systematic review and meta-analysis. Int. Food Res. J., 111969
Zicarelli, L., 2020. Current trends in buffalo milk production. J. Buffalo Sci. 9, 121–132.