

# Global prevalence of pathogens isolated from butter and cream samples derived from milk - a systematic review with meta-analysis

## Prevalência global de patógenos isolados em amostras de manteiga e creme derivados de leite - uma revisão sistemática com meta-análise

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### Highlights

Several pathogen groups were detected in cream and butter.

Hygienic failures and contamination were evidenced in cream and butter.

Public policies aimed at ensuring the safety of cream and butter are necessary.

### Abstract

Microbiological contamination occurring in the milk production chain represents a significant challenge for public health, especially in dairy products such as butter and cream. In this context, and considering the socioeconomic and nutritional relevance of these foods, it is essential to understand the risks

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involved and to seek effective control strategies. This study is a systematic review with meta-analysis aimed at evaluating the prevalence of pathogenic microorganisms in butter and cream. The research involved five bibliographic databases and initially included 1,275 articles. After applying the exclusion criteria, 21 articles from countries such as Brazil, India, Egypt, Ireland, Turkey, the United Kingdom, and Sweden were included, encompassing regions in Africa, Asia, Europe, and South America. The PRISMA guidelines were used for data selection and analysis. The results highlighted the presence of *Escherichia coli* and species of *Listeria*, *Staphylococcus*, and *Pseudomonas* in both types of samples, in addition to specific isolates such as *Klebsiella* spp. in cream and *Salmonella* spp. in butter. Variability in isolation and confirmation methods was identified. The research showed that hygienic failures and inadequacies in transport and storage are critical factors associated with contamination. The findings reinforce the need for standardization of microbiological analysis methods and for more efficient surveillance strategies. The low representation of continents such as North America and Oceania points to important gaps in the literature. Thus, the implementation of public policies to minimize health risks is necessary, promoting the safety of widely consumed dairy products.

**Key words:** Butter. Fresh cream. Foodborne pathogens. Food safety.

## Resumo

As contaminações microbiológicas que ocorrem na cadeia produtiva do leite, representam um desafio significativo para a saúde pública, principalmente com derivados lácteos como manteiga e creme. Nesse contexto e tendo em vista a relevância socioeconômica e nutricional que esses alimentos representam, é essencial compreender os riscos envolvidos e buscar estratégias eficazes de controle. Este estudo realizou uma revisão sistemática com meta-análise para avaliar a prevalência de microrganismos patogênicos em manteiga e creme. A pesquisa envolveu cinco indexadores bibliográficos, e contou com 1.275 artigos inicialmente, após parâmetros de exclusão, foram incluídos 21 artigos de países diversos como Brasil, Índia, Egito, Irlanda, Turquia, Reino Unido e Suécia, cobrindo continentes como África, Ásia, Europa e América do Sul. Foi utilizado as diretrizes PRISMA para seleção e análise de dados. Os resultados destacaram a presença de *Escherichia coli*, e espécies de *Listeria*, *Staphylococcus*, *Pseudomonas* nos dois tipos de amostra, além de isolados específicos como *Klebsiella* spp. em creme e *Salmonella* spp. em manteiga. Houve identificação de variabilidade nos métodos de isolamento e confirmação. A pesquisa evidenciou que falhas higiênicas e inadequações no transporte e armazenamento são fatores críticos associados à contaminação. Os achados reforçam a necessidade de padronização nos métodos de análise microbiológica e de estratégias de vigilância mais eficientes. A baixa representatividade de continentes como América do Norte e Oceania apontam para lacunas importantes na literatura. Desse modo, a implementação de políticas públicas para minimizar riscos à saúde se fazem necessárias, promovendo segurança de alimentos derivados tão consumidos.

**Palavras-chave:** Manteiga. Creme fresco. Patógenos de origem alimentar. Segurança alimentar.

## Introduction

World milk production, along with many of its derivatives, represents a strategic sector in the global food security scenario, as it is characterized as an essential source of nutrients while also representing a significant share of the socioeconomic context of many countries. The current panorama is led by regions such as Asia and the Americas, which account for the largest share of global production volume (De Klerk & Robinson, 2022; Food and Agriculture Organization [FAO], 2023).

Among dairy products, cream and butter play a prominent dietary and commercial role, and their consumption reflects a continuous and expanding global demand, with many markets showing a preference for dairy fats (Organisation for Economic Co-operation and Development / Food and Agriculture Organization of the United Nations [OECD/FAO], 2025). Butter, for example, is one of the most popular and widely consumed types of fat used in home cooking across various cultures (Meshref, 2010). Its per capita consumption is historically high and continues to grow steadily, and in terms of absolute volume, the market is led by a wide margin by India and Pakistan, which consumed more than 5.4 million and 1.2 million tons, respectively, in 2023. Regarding per capita butter consumption, Bahrain is the country with the highest consumption (19.03 g/day), followed by France (18.54 g/day) and Belgium (16.36 g/day) (FAO, 2023).

Fresh cream is a fundamental garnish in traditional cuisines and is consumed and traded in high volumes, particularly in open street markets and itinerant fairs (Rios-Muñiz et al., 2022). Global and increasing cream consumption is heavily concentrated in Europe, with the four largest consumer

markets being Germany (438,000 t), France (387,000 t), Italy (308,000 t), and Poland (288,000 t). Regarding per capita consumption of fresh cream, Belgium is the country with the highest consumption (13.68 g/day), followed by Estonia (11.87 g/day) and Latvia (7.26 g/day) (FAO, 2023).

Although regional variations occur in production, storage, and even cultural adaptations such as the addition of spices (Sagdic et al., 2010; Timlin et al., 2024), the high presence of these products in the diet of different populations, coupled with their often artisanal production, poses a significant challenge for public health and the food industry (Medeiros et al., 2017).

Considering that these are products derived from raw materials of animal origin, contamination can occur at different stages of the production chain, including milking, processing, storage, and transportation (Berge & Baars, 2020; Fusco et al., 2020). Specifically, the routes of contamination differ according to the product. In butter, hygiene failures usually stem from the direct use of unpasteurized milk, inadequate cleaning of machinery, and direct contamination by handlers' hands during the maturation phase (Meshref, 2010).

In fresh cream, the greatest risk lies in post-pasteurization cross-contamination, which frequently occurs during the maturation period used to increase viscosity, as well as in manual packaging performed with poorly sanitized utensils by untrained workers (Jenkins & Henderson, 1969). In addition, street market sales substantially aggravate bacterial proliferation, as creams and butters are often left exposed in open containers without refrigeration for extended periods (Rios-Muñiz et al., 2022).

The impact of consuming these low-quality products on public health is direct and often severe. In events such as outbreaks, the complexity of tracing contamination sources is always high when food products are involved (Mohan et al., 2021). In this sense, many pathogens are frequently associated with these products, including Gram-negative bacteria, such as members of the Enterobacteriaceae and *Salmonella* families, and Gram-positive bacteria belonging to the *Listeriaceae* and *Staphylococcus* families (Berge & Baars, 2020). Because these dairy products have high moisture or fat contents, the proliferation of bacteria such as *Staphylococcus aureus* can result in the release of heat-stable enterotoxins capable of causing severe outbreaks of food poisoning (Rios-Muñiz et al., 2022; Meshref, 2010).

In addition to the production of enterotoxins, pathogens such as *Listeria monocytogenes* are of particular concern in these products, as they can survive acidic conditions and prolonged refrigerated storage of raw milk, posing a potentially fatal risk to immunocompromised individuals and pregnant women (El Marrakchi et al., 1993; Bradshaw et al., 1987). Finally, beyond the well-documented antimicrobial resistance of many Enterobacteriaceae (Berge & Baars, 2020), recent evidence has demonstrated the presence of uropathogenic *Escherichia coli* (UPEC) strains in artisanal creams, indicating that the ingestion of these contaminated derivatives may serve as a route of infection for the population (Rios-Muñiz et al., 2022).

Given the significant importance of these two products in the global food context, their distinct contamination routes, and the urgency of better understanding the

microbiological risks associated with them, this study aimed to conduct a systematic review with meta-analysis of the global prevalence of pathogenic microorganisms isolated exclusively from butter and milk-derived cream. The work also sought to explore the isolation methodologies employed, providing a basis for the implementation of microbiological control and surveillance practices in the dairy sector.

## Material and Methods

### Type of study and data source

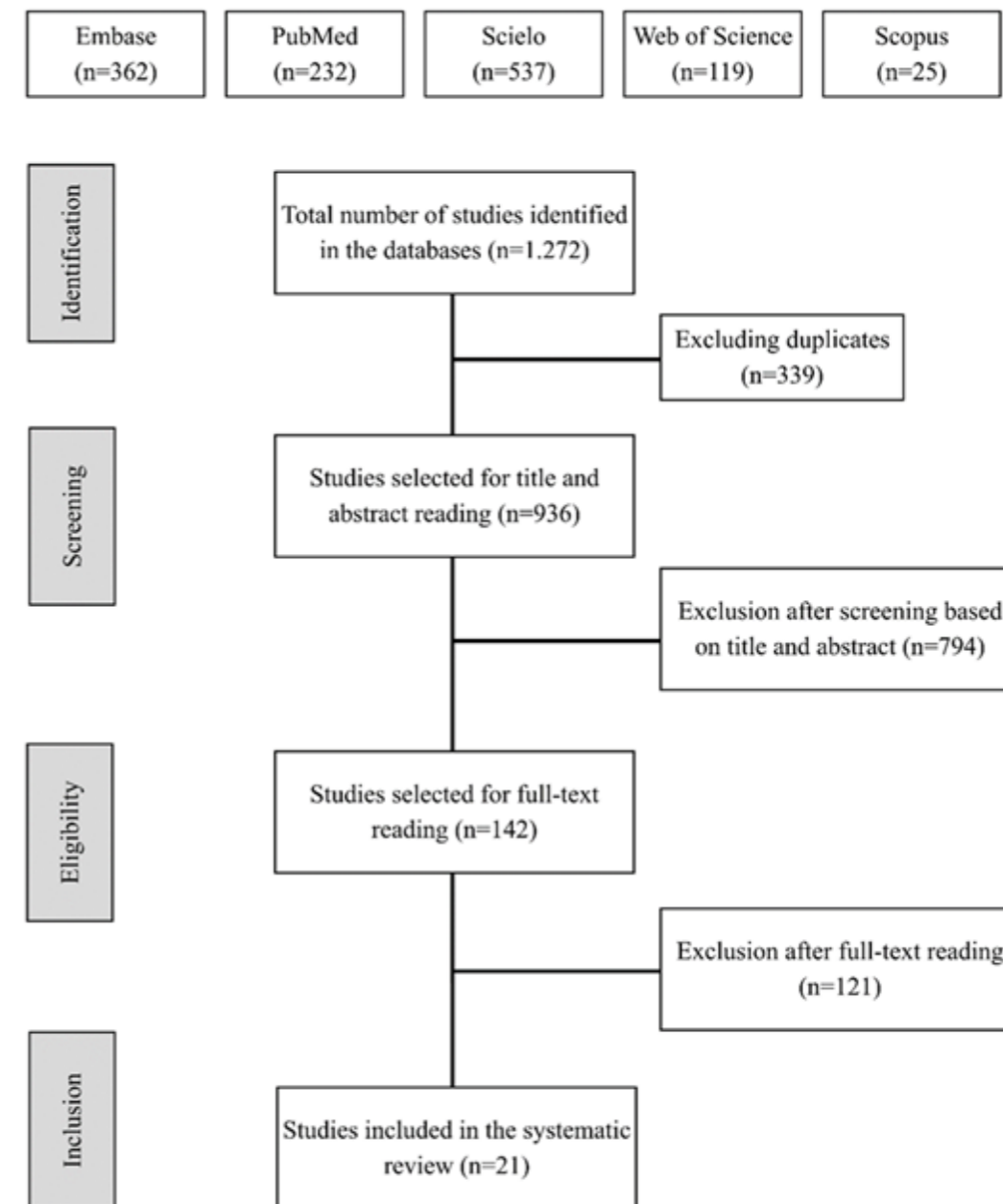
The systematic literature review with meta-analysis was based on the PRISMA guidelines, following the steps: (I) bibliographic search to identify potentially relevant articles, (II) assessment of article relevance, (III) quality assessment, and (IV) extraction of predefined data (Fig. 1). The study was registered in the International Prospective Register of Systematic Reviews (PROSPERO) under registration number CRD42024567734.

The search for published studies on pathogens isolated from cream and butter samples was conducted in April 2024. Six scientific databases were consulted: Embase, PubMed, Lilacs, SciELO, Web of Science, and Scopus. The search descriptors used were “fresh milk cream or cream or butter” and “microbiology or pathogens.” All publications retrieved were exported as BibTeX or RIS files to the Mendeley reference manager (Mendeley Desktop, version v1.19.8, Mendeley Ltd., London, UK). Only full published articles were included; no restrictions were applied regarding publication year or language.

### Criteria for inclusion and data extraction

In the preliminary screening, duplicate publications were excluded. In addition, grey literature such as theses, dissertations, undergraduate theses, review articles, and

conference abstracts were excluded. Next, titles and abstracts were independently screened by four researchers. Full-text articles were then assessed, and studies that did not meet the inclusion criteria defined for titles and abstracts were excluded (Figure 1).



**Figure 1.** Flowchart of article selection in the five scientific databases and in each phase of the systematic review.

The following inclusion criteria were applied:

- The article reported original data on microbiological analysis of butter or cream;
- The article provided information on pathogens isolated from butter or cream;
- The article included phenotypic and/or genotypic testing for the presence of pathogens.

Data extraction included geographic location (country), sample type, number of positive samples, pathogens investigated and isolated, phenotypic and genotypic prevalence, authors, and year of publication.

#### Data analysis

Meta-analyses were performed using R software, version 3.5.1 (R Core Team [R], 2019), with the “meta” package (Balduzzi et al., 2019). Based on the available quantitative data (number of samples and number of positive isolates for each microorganism), meta-analyses of microbial prevalence in dairy products (butter and cream) were conducted. This involved pooling the number of samples analyzed and the number of positive isolates for each microorganism across all included studies.

The pooled prevalence estimate and 95% confidence interval (95% CI) were calculated using the “metaprop” function of the “meta” package (Balduzzi et al., 2019). Heterogeneity, representing variability among studies, was assessed using Cochran’s Q test and Higgins and

Thompson’s  $I^2$  statistic, and incorporated using a random-effects model with the inverse variance method and restricted maximum likelihood estimation (Deeks et al., 2019). A word cloud approach was used to identify the most frequent microorganisms using the wordcloud function, generating separate outputs for each sample type.

## Results

### Global distribution of included studies on butter and cream

The initial search yielded 1,275 articles, after screening and eligibility assessment, 21 studies were included in this systematic review. Countries such as Croatia, Egypt, India, Ireland, Morocco, Mexico, the United Kingdom, and Sweden were represented in studies on cream, while Belgium, Brazil, Egypt, Ethiopia, India, Iran, Italy, the United Kingdom, and Turkey were represented in studies on butter. The global distribution and number of studies per continent are shown in Table 1. Considering that three studies addressed both sample types, the data were organized into 10 butter studies and 14 cream studies (Tables 2 and 3). For each article, data on country of origin, sample type (butter or cream), number of samples collected and tested, and number of positive samples were extracted. In addition, the microorganisms investigated, confirmed counts, and isolation techniques were recorded (Tables 4 and 5).

**Table 1**  
Frequency of microbiological studies with cream and butter samples by continent

| Continent | Frequency (%) of studies with cream | Frequency (%) of studies with butter |
|-----------|-------------------------------------|--------------------------------------|
| America   | 07.14                               | 10                                   |
| Europe    | 71.43                               | 40                                   |
| Asia      | 07.14                               | 20                                   |
| Africa    | 14.29                               | 30                                   |
| Oceania   | -                                   | -                                    |
| Total     | 100                                 | 100                                  |

### Global prevalence of pathogenic microorganisms isolated in butter and cream

The prevalence analysis demonstrated a higher occurrence of microorganisms such as *Escherichia coli*, *Listeria* spp., and *Staphylococcus* spp. in butter samples. In addition, studies on cream highlighted microorganisms such as *Klebsiella* spp., *Bacillus* spp., total and thermotolerant coliforms, and a similarly high prevalence of *Escherichia coli*. The pooled prevalence estimates, most frequent microorganisms, confidence intervals, and results of Cochran’s Q test and Higgins and Thompson’s  $I^2$  statistic are presented in Tables 4 and 5.

In the meta-analysis of butter samples, *E. coli* exhibited significant heterogeneity, indicating substantial variability among studies, which may be associated with methodological differences. *Listeria* spp., in turn, displayed moderate heterogeneity, suggesting limited variation among

studies (only two studies were included). On the other hand, *Staphylococcus* spp. presented insufficient data for a more robust interpretation, as only one study was included, preventing comparative analysis.

The meta-analysis of cream studies demonstrated high heterogeneity for several microorganisms. This may be related to the larger number of included studies, with *Staphylococcus* spp. (5), *Escherichia coli* (4), *Pseudomonas* spp. (3), *Listeria* spp. (3), and *Klebsiella* spp. (2) all showing a p-value <0.001 in Cochran’s Q test. Due to the limited number and methodological diversity of the studies, it was not possible to evaluate potential publication bias in the present study, which restricts the interpretation and inference of some results.

The wordcloud function reaffirmed the microorganisms most frequently cited among the studies; the results for the highest frequencies in cream and butter studies are presented in Figures 2a and 2b, respectively.

**Table 2**  
Characteristics of the included studies for the prevalence of pathogenic microorganisms in cream samples.

| Author                              | Year | Country        | Sample type | Methodology |           | Isolated microorganisms   |
|-------------------------------------|------|----------------|-------------|-------------|-----------|---|
|                                     |      |                |             | Phenotypic  | Genotypic |   |
| Attri and Narang <sup>[17]</sup>    | 2014 | India          | Cream       | YES         | NO        | <i>Staphylococcus</i> spp.; <i>E. coli</i> ; <i>Pseudomonas</i> spp.; <i>Lactobacillus</i> ;  |
| Barrow and Miller <sup>[18]</sup>   | 1967 | United Kingdom | Cream       | YES         | NO        | <i>E. coli</i> ; <i>Staphylococcus</i> spp and <i>Brucella abortus</i>  |
| Frece et al. <sup>[23]</sup>        | 2010 | Croatia        | Cream       | YES         | YES       | <i>Listeria monocytogenes</i>   |
| Gamal et al. <sup>[19]</sup>        | 2022 | Egypt          | Cream       | YES         | YES       | <i>Pseudomonas</i> spp.   |
| Gerken et al. <sup>[26]</sup>       | 1968 | United Kingdom | Cream       | YES         | NO        | Total and thermotolerant coliforms; <i>Pseudomonas</i> spp., <i>Bacillus</i> spp; <i>Staphylococcus</i> spp.  |
| Mogford <sup>[27]</sup>             | 1970 | United Kingdom | Cream       | YES         | NO        | <i>Salmonella typhimurium</i> , <i>Brucella abortus</i> type 1; <i>Escherichia coli</i> ; <i>Staphylococcus</i> spp.  |
| Lindberg et al. <sup>[13]</sup>     | 1998 | Sweden         | Cream       | YES         | YES       | <i>Citrobacter freundii</i> ; <i>Enterobacter aerogenes</i> ; <i>Enterobacter amnigenes</i> ; <i>Enterobacter cloacae</i> ; <i>Escherichia hermannii</i> ; <i>Escherichia vulneris</i> ; <i>Hafnia alvei</i> ; <i>Klebsiella planticola</i> ; <i>Klebsiella terrigena</i> ; <i>Pantoea agglomerans</i> ; <i>Rahnella aquatilis</i> ; <i>Serratia fonticola</i> ; <i>Serratia liquefaciens</i> ; |
| El Marrakchi et al. <sup>[34]</sup> | 1993 | Morocco        | Cream       | YES         | NO        | None  |
| Mchugh et al. <sup>[35]</sup>       | 2020 | Ireland        | Cream       | YES         | YES       | <i>Anoxybacillus</i> spp.; <i>Lactococcus</i> spp.; <i>Corynebacterium</i> spp; <i>Acinetobacter</i> spp;   |
| Mclauchlin et al. <sup>[28]</sup>   | 2020 | United Kingdom | Cream       | YES         | NO        | <i>E. coli</i> ; <i>L. monocytogenes</i> ; <i>Listeria</i> spp;   |
| Colenso et al. <sup>[36]</sup>      | 1966 | United Kingdom | Cream       | YES         | NO        | Total and thermotolerant coliforms (suggestive of <i>E. coli</i> )  |

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|                                       |      |                |       |     |    |   |
|---------------------------------------|------|----------------|-------|-----|----|---|
| Jenkins and Henderson <sup>[25]</sup> | 1969 | United Kingdom | Cream | YES | NO | <i>E. coli</i> ; <i>Citrobacter</i> spp.; <i>Klebsiella</i> spp.; <i>Hafnia alvei</i> ; <i>Coliforms</i> spp.; <i>Aeromonas</i> spp.; <i>Neisseria</i> spp.; <i>Acinetobacter</i> spp.; <i>Chromobacterium</i> spp.; <i>Pseudomonas</i> spp.; <i>Alkaligenes faecalis</i> ; <i>Alkalescens dispar</i> ; <i>Bacillus</i> spp.; <i>Streptococcus</i> spp.; <i>Aerococcus viridians</i> ; <i>Corynebacterium</i> spp.; <i>Micrococcus</i> spp.; <i>Staphulococcus</i> spp. |
| Rios-Muñiz et al. <sup>[29]</sup>     | 2022 | Mexico         | Cream | YES | NO | <i>E. coli</i> ; <i>Staphylococcus</i> spp.; <i>Mycobacterium</i> spp.  |
| Talbot et al. <sup>[37]</sup>         | 1967 | United Kingdom | Cream | YES | NO | <i>Brucella abortus</i>   |

**Table 3**  
Characteristics of the included studies for the prevalence of pathogenic microorganisms in butter samples

| Author                            | Year | Country        | Sample type | Methodology |           | Isolated microorganisms  |
|-----------------------------------|------|----------------|-------------|-------------|-----------|--|
|                                   |      |                |             | Phenotypic  | Genotypic |  |
| Attri and Narang <sup>[17]</sup>  | 2014 | India          | Butter      | YES         | NO        | <i>Staphylococcus</i> spp; <i>Pseudomonas</i> spp; <i>Lactobacillus</i>  |
| Cardak <sup>[22]</sup>            | 2013 | Turkey         | Butter      | YES         | NO        | <i>Listeria monocytogenes</i> ; <i>Salmonella</i> spp.   |
| Gamal et al. <sup>[19]</sup>      | 2022 | Egypt          | Butter      | YES         | YES       | <i>Pseudomonas</i> spp.  |
| Lanciotti et al. <sup>[24]</sup>  | 1992 | Italy          | Butter      | YES         | NO        | <i>Listeria monocytogens</i> , <i>Yersinia enterocolitica</i> , <i>Enterococcus faecium</i> , <i>B. polymyxa</i> , <i>B. firmus</i> , and <i>B. laterosporus</i> |
| Mclauchlin et al. <sup>[28]</sup> | 2020 | United Kingdom | Butter      | YES         | NO        | <i>Enterobacteriaceae</i> ; <i>E. coli</i>   |
| Meshref <sup>[20]</sup>           | 2010 | Egypt          | Butter      | YES         | NO        | <i>E. coli</i> ; <i>S. auerus</i> .  |
| N'Guessan et al. <sup>[38]</sup>  | 2015 | Belgium        | Butter      | YES         | NO        | <i>E. coli</i> ; <i>Listeria monocytogenes</i> .   |
| Rahimi et al. <sup>[39]</sup>     | 2014 | Iran           | Butter      | YES         | YES       | None   |
| Viana et al. <sup>[14]</sup>      | 2024 | Brazil         | Butter      | YES         | NO        | None   |
| Yilma et al. <sup>[21]</sup>      | 2007 | Ethiopia       | Butter      | YES         | NO        | <i>Enterobacter</i> spp.; <i>E coli</i> ; <i>Klebssiela</i> spp; <i>Klyuvera</i> spp.  |

**Table 4**  
Characteristics of the studies included in the systematic review, a guide for characterizing the meta-analysis

| Article ID* | References - Cream          | Article ID | References - Butter      |
|-------------|-----------------------------|------------|--------------------------|
| 1           | Attri and Narang, 2014      | 1          | Attri & Narang, 2014     |
| 2           | Barrow and Miller, 1967     | 15         | Cardak, 2013             |
| 3           | Jenkins and Henderson, 1969 | 5          | Gamal et al., 2022       |
| 4           | Frece et al., 2010          | 16         | Lanciotti et al., 1992   |
| 5           | Gamal et al., 2022          | 11         | McLauchlin et al., 2020  |
| 6           | Gerken et al., 1968         | 17         | Meshref, 2010            |
| 7           | Mogford, 1971               | 18         | N'Guessan et al., 2015   |
| 8           | Lindberg et al., 1998       | 19         | Rahimi et al., 2014      |
| 9           | El Marrakchi et al., 1993   | 20         | Silva Viana et al., 2024 |
| 10          | Mchugh et al., 2020         | 21         | Yilma et al., 2007       |
| 11          | Mclauchlin et al., 2020     |            |                          |
| 12          | Colenso et al., 1966        |            |                          |
| 13          | Talbot et al., 1967         |            |                          |
| 14          | Rios-Muñiz et al., 2022     |            |                          |

\* The article ID serves as an identifier for the references in Table 5.

**Table 5**  
Meta-analysis of the prevalence of pathogenic microorganisms isolated from butter and cream samples

| Microorganism              | N of studies | N of samples | Butter                |                       |                |                |           |                |
|----------------------------|--------------|--------------|-----------------------|-----------------------|----------------|----------------|-----------|----------------|
|                            |              |              | N of positive samples | Pooled prevalence (%) | 95% CI         | I <sup>2</sup> | Cochran Q | References     |
| <i>Escherichia coli</i>    | 4            | 500          | 254                   | 39.77                 | 25.15 - 56.46% | 88.7%          | <0.001    | 11; 17; 18; 21 |
| <i>Listeria</i> spp.       | 2            | 373          | 108                   | 24.59%                | 10.59 - 47.32  | 35.9%          | 0.2115    | 15; 18         |
| <i>Salmonella</i> spp.     | 2            | 42           | 1                     | 4.96%                 | 0.79 - 25.54%  | 19.6%          | 0.2647    | 15; 20         |
| <i>Staphylococcus</i> spp. | 1            | 60           | 14                    | 23.33%                | 13.38 - 36.04% | -              | -         | 17             |
| <i>Pseudomonas</i> spp.    | 1            | 25           | 4                     | 16%                   | 6.14 - 35.69%  | -              | -         | 5              |

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| Microorganism                   | N of studies | N of samples | N of positive samples | Cream                 |                |                |           |                |
|---------------------------------|--------------|--------------|-----------------------|-----------------------|----------------|----------------|-----------|----------------|
|                                 |              |              |                       | Pooled prevalence (%) | 95% CI         | I <sup>2</sup> | Cochran Q | References     |
| <i>Staphylococcus</i> spp.      | 5            | 3918         | 88                    | 4.04%                 | 1.34 - 11.55%  | 95.3%          | <0.001    | 2; 3; 6; 7; 14 |
| <i>Escherichia coli</i>         | 4            | 495          | 203                   | 30.89%                | 14.26 - 54.59% | 96.6%          | <0.001    | 2; 3; 11; 14   |
| <i>Pseudomonas</i> spp.         | 3            | 265          | 27                    | 12.45%                | 1.75 - 53.17%  | 95.4%          | <0.001    | 3; 5; 6        |
| <i>Listeria</i> spp.            | 3            | 300          | 52                    | 6.66%                 | 0.88 - 36.54%  | 89.6%          | <0.001    | 4; 9; 10       |
| <i>Brucella abortus</i>         | 2            | 279          | 7                     | 3.74%                 | 0.69 - 17.92%  | 80.1%          | 0.0251    | 2; 12          |
| <i>Bacillus</i> spp.            | 2            | 235          | 100                   | 43.43%                | 16.7 - 74.62%  | 95.8%          | <0.001    | 3; 6           |
| <i>Klebsiella</i> spp.          | 2            | 181          | 50                    | 18.62%                | 3.46 - 59.33%  | 91.5%          | <0.001    | 3; 8           |
| <i>Citrobacter</i> spp.         | 2            | 181          | 32                    | 12.83%                | 3.14 - 40.02   | 83.5%          | 0.0137    | 3; 8           |
| <i>Hafnia alvei</i>             | 2            | 181          | 8                     | 3.86%                 | 0.22 - 42.63   | 86.9%          | 0.0057    | 3; 8           |
| <i>Total coliforms</i>          | 2            | 681          | 272                   | 39.96%                | 36.34 - 43.69% | 0%             | 0.3497    | 6; 12          |
| <i>Thermotolerant coliforms</i> | 2            | 681          | 158                   | 18.5%                 | 8.78 - 34.88%  | 87.5%          | 0.0047    | 6; 12          |
| <i>Acinetobacter</i>            | 1            | 129          | 2                     | 1.55%                 | 0.19 - 5.49%   | -              | -         | 3              |
| <i>Corynebacterium</i> spp.     | 1            | 129          | 1                     | 0.78%                 | 0.02 - 4.24%   | -              | -         | 3              |



**Figure 2.** Word clouds extracted from the 21 articles included in the study on the prevalence of pathogens in samples of cream (a) and butter (b).

### Isolation and confirmation methods

The vast majority of the articles addressed phenotypic methods of isolation and confirmation, with most studies employing direct plating on selective media for growth and screening evaluation, followed by biochemical assays or cultivation in specific media for particular microbial species. For the isolation of *Escherichia coli*, plating on selective and differential culture media was one of the most frequently reported techniques among the authors, especially in studies involving cream samples and in older articles within the chronological timeline.

Regarding genotypic analyses of Enterobacteriaceae, the detection of genes such as *vt1*, *vt2*, and *eaeA* was particularly notable (Lindberg et al., 1998). For the confirmation of *Listeria* spp.,

genotypic detection of the *hly* gene has been characterized as a rapid confirmation method. Emerging microorganisms of concern such as *Salmonella* spp. and *Brucella* spp. are still reported in phenotypic isolation studies involving butter and cream (Silva Viana et al., 2024).

### Discussion

The limited number of articles included in the research highlights the scarcity of investigations on the subject. This limitation was evidenced not only by the final number of studies, but also by the chronological span analyzed, which covered more than 58 years. The limited scope for comparison is further demonstrated by the low representation of continents such as Oceania and North America, which may suggest potential publication bias

regarding these specific products, especially considering that these regions generally maintain well-regulated food safety systems (Sivakumar et al., 2021).

The predominance of articles originating from countries such as India, Egypt, the United Kingdom, and Ireland for cream, and Brazil, Belgium, and Iran for butter, may be related both to the importance of these dairy products in local diets (Kataria & Singh, 2024) and to the high production of raw milk in these countries. India alone accounts for approximately 30% of global milk production, which exceeded 900 million tons in 2023. In addition, the European Union also stands out, with production approaching 150 million tons (FAO, 2023).

The presence of studies from regions such as Africa and the Middle East, particularly Egypt and Ethiopia, points to a challenging scenario in developing countries, where hygiene practices and methods of food preservation and production vary considerably (Attri & Narang, 2014; Barrow & Miller, 1967; Gamal et al., 2022; Meshref, 2010; Yilma et al., 2007).

To understand the significant prevalence of pathogens observed, it is essential to correlate contamination levels with the type of manufacturing process employed, which directly explains the origin of microorganisms in these dairy products. In artisanal and traditional production systems, frequently reported in these developing countries, contamination tends to originate primarily at the source.

Products such as homemade butter and rural creams are often produced from raw milk, manually handled without adherence to good manufacturing practices,

and sold unrefrigerated in open-air markets for extended periods. In this context, the high prevalence of thermotolerant and fecal pathogens, such as *Escherichia coli* and *Staphylococcus* spp., is directly associated with the use of untreated raw materials, fecal contamination during milking, and poor hygiene practices among handlers.

On the other hand, in industrial production systems and larger dairy industries that employ heat treatment (pasteurization), contamination shifts toward environmental or post-pasteurization recontamination. Although these systems generally produce bacteriologically superior products, the persistence of pathogens such as *Listeria* spp. (Cardak, 2013; Frece et al., 2010; Lanciotti et al., 1992), coliforms, and strains of *Staphylococcus* spp. (Attri & Narang, 2014; Barrow & Miller, 1967; Jenkins & R. J. Henderson, 1969; Gerken et al., 1968; Mogford, 1971; Mclauchlin et al., 2020; Meshref, 2010; Rios-Muñiz et al., 2022) is associated with failures in equipment sanitization, biofilm formation in cream separators, and cross-contamination during packaging.

Thus, both inadequate artisanal handling and failures in industrial processing indicate the persistence of significant public health risks, given the potential occurrence of foodborne disease outbreaks (World Health Organization [WHO], 2015).

The substantial presence of *Bacillus* spp., with a combined prevalence approaching 44%, strongly indicates environmental contamination, primarily associated with storage environments and machinery, since this bacterial group is widely distributed in soil, water, and plants. Because

these are spore-forming bacteria, they exhibit high resistance to adverse conditions such as heat, dehydration, and chemical agents, allowing them to survive industrial pasteurization processes and making them a persistent problem in the dairy industry (Tirloni et al., 2022).

Thermotolerant coliforms are also used as indicators of fecal contamination; as bioindicators, their presence challenges the hygienic quality standards of the final product. In this sense, their occurrence is associated with the failures previously described: in artisanal products, they originate primarily from fecal contamination, whereas in industrial products they are linked to failures during storage or post-processing transport. For *E. coli*, the high heterogeneity observed in the meta-analysis suggests that, in addition to the marked differences between artisanal and industrial production systems, variability in isolation methodologies hinders accurate inferences regarding the dataset, making direct comparisons among studies difficult.

Still within the Enterobacteriaceae family, isolates of *Klebsiella* spp. (Jenkins & Henderson, 1969; Lindberg et al., 1998; Yilma et al., 2007) were detected more frequently in cream samples. This group of opportunistic bacteria is associated with nosocomial infections and, similarly to *E. coli*, may act as a reservoir and transmitter of antimicrobial resistance genes. Their presence in food is alarming; however, their detection in these dairy products may be explained by their direct association with asymptomatic mastitis in dairy cattle, which represents one of the primary causes of clinical and subclinical infections in these herds and may

be perpetuated throughout the production chain via raw milk (Azwai et al., 2024).

Regarding Gram-positive bacteria, *Staphylococcus* spp., especially *S. aureus*, was the most predominant among the studies. This pathogen is known for producing enterotoxins that cause acute food poisoning characterized by nausea, vomiting, and diarrhea. The prominence of its isolation in these products directly reflects the type of processing employed: in artisanal manufacturing, the high staphylococcal load is associated with the use of raw milk and inadequate, unhygienic manual handling, representing primary contamination of human origin, whereas in industrial production, its presence generally indicates sanitation failures and post-pasteurization cross-contamination.

The presence of pathogens from the Listeriaceae family, including *L. monocytogenes*, is particularly concerning because this microorganism can cause severe infections such as meningitis, septicemia, and abortion in vulnerable populations (Fotopoulou et al., 2024). The origin of this pathogen also differs according to the production system: in traditional cheeses, creams, and butters, the risk arises from untreated raw materials, whereas in industrial settings, *L. monocytogenes* represents a persistent hazard due to its ability to form biofilms and survive in refrigerated processing environments. Regarding this pathogen, studies on butter showed moderate heterogeneity, reflecting methodological consistency among the reviewed studies, while the greater variability observed in cream studies may be related to the increased sample size and number of included studies.

Isolation methods remain predominantly phenotypic, demonstrating a historical reliance on economically viable and accessible techniques. However, traditional culture methods face limitations in specificity, frequently allowing identification only at the genus level, and are strongly affected by competition from the background microflora present in dairy products. More recent studies continue to rely on selective culture media as the primary screening method, as exemplified by McLauchlin et al. (2020) and Silva Viana et al. (2024).

Although less frequently employed, genetic analyses represent a fundamental advancement in meeting the demand for rapid and precise diagnosis and identification of contamination sources. This can be observed in the work of Lindberg et al. (1998), who described targeted detection of the virulence genes *vt1*, *vt2*, and *eeA* in Enterobacteriaceae, and in Gamal et al. (2022), who used multiplex PCR to screen for virulence genes such as *LasB* and *aprX* in spoilage and pathogenic species of *Pseudomonas*. Similarly, Frece et al. (2010) described the use of the *hly* gene for rapid and sensitive confirmation of *Listeria monocytogenes*, reducing the false-positive results frequently generated by traditional biochemical tests.

One area with limited representation involves studies on *Salmonella* spp. and *Brucella* spp., which are emerging microorganisms of global concern. In Brazil, legislation requires the complete absence of *Salmonella* spp. in food products. One of the studies included in this review (Silva Viana et

al., 2024), which investigated *Salmonella* spp. in Brazil, did not identify positive samples in commercial dairy products, supporting the notion that outbreaks associated with this bacterium occur mainly in domestic settings or as a result of failures in the informal supply chain. However, when artisanal and raw products are evaluated, studies such as that of Cardak (2013) warn of the concerning prevalence of *Salmonella* in butter and traditional cheese samples. Regarding *Brucella* spp., no studies published within the last 10 years were identified that specifically investigated this pathogen in cream or butter.

## Conclusion

The evaluated studies demonstrated the presence of different groups of pathogens, including *Enterobacteriaceae*, *Listeriaceae*, and *Staphylococcaceae*. This study showed that butter and cream can act as important reservoirs for numerous pathogens, particularly groups with high dissemination potential in the environment. Consequently, the analyses indicate that contamination may involve both human and animal niches, raising significant concerns regarding food quality and safety. The findings reinforce the need for more robust analytical standards that are both economically viable and capable of improving the speed and accuracy of pathogen detection. The study also highlights the importance of public policies aimed at ensuring food safety and minimizing the risks associated with contamination of these widely consumed dairy products.

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## Conflicts of interest

The authors declare no conflicts of interest.

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