Etiological agents and bacterial sensitivity in subclinical mastitis in Brazil: a ten-year systematic review

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Pathogen.

Summary
Considering the high prevalence of subclinical mastitis and its impacts on milk production, thematic studies are need to provide strategic data for its control. This study aimed at investigating the most frequent microorganisms associated with subclinical mastitis in dairy cows in Brazil through compiling the occurrence of the etiological agents and their sensitivity to antibiotics. The systematic review includes articles published between 2009 and 2019. Fifty-seven articles evaluating 22,287 milk samples were selected. The number of publications and the sample size were not homogeneous among Brazilian regions. Most of the studies and sampling were conducted in Rio Grande do Sul, whereas no studies were found in some states in the north and mid-west regions. The most frequent group of pathogens was *Staphylococcus* spp. It was isolated in all studies and had an average prevalence of 49% in the analyzed samples. Resistance to penicillin was the most frequent microbial resistance found in Brazil, with an average of 66% among the isolates evaluated. Moreover, bacterial resistance to cephalexin, cefoperazone, erythromycin, gentamicin, neomycin, penicillin, tetracycline, and trimethoprim increased over the research period. Given the territorial extension, the etiological diversity, and the lack of studies with a representative sample, the compilation of scientific data must be interpreted with caution. Regions where a greater number of studies were conducted and with numerous samples, such as the South, provided a comprehensive scenario that is closer to reality. Nevertheless, although decision making on the farm cannot be replaced by scientific studies, it can be supported by such efforts.

Introduction

The dairy herd occupies a prominent position within the Brazilian economic scenario, with milk being one of the main products of national agriculture. In fact, the agribusiness of milk and dairy products plays an important role in the supply of food and the social issue, with the generation of jobs and income for the population, mainly in southern region (Beber *et al*., 2019). However, mastitis is the most frequent and costly infection of dairy farming. This is because intramammary infections result in significant economic losses associated with several factors including the reduction in milk production (more than 70% of cases), cost of treatment and veterinary medical charges (7%), disposal of milk during the treatment period (9%), increased labor (1%), and the premature disposal of animals (14%) (Sharma *et al*., 2012). Moreover, there are losses for dairy products owing to the decrease in the quality of the final product, the decrease in the industrial yield for the manufacture of derivatives, and changes in the composition of mastitic milk (Ruegg, 2017).

Antimicrobial drugs are used to treat several diseases that affect dairy cows, and clinical mastitis is one of the main diseases that require the use of these drugs (Gomes and Henriques, 2016). Despite the many benefits of these drugs, from the perspective of public health and food safety, there is concern regarding antibiotic residues in food intended for human consumption from animals treated with

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antibiotics and the potential development and transmission of antimicrobial resistance which may impact the treatment of diseases (Oliver *et al*., 2020). The appearance of multidrug-resistant strains has made it difficult to treat mastitis in cows. Thus, microbiological diagnosis of mastitis needs to be performed routinely, as it is capable of generating fast and safe results that can identify the problems affecting the herd. According to Karach *et al*., (2015), the isolation and identification of the agent contribute to the most appropriate choice of the drug to be used in therapy, thus avoiding the development of bacterial resistance to antibiotics. One of the strategies to prevent bacterial resistance is knowing the main agents involved in mastitis and their sensitivities.

This systematic review aimed at investigating the most frequent microorganisms associated with subclinical mastitis in dairy cows in Brazil, compiling data on the occurrence of the etiological agents causing subclinical mastitis and its sensitivity to antibiotics. A critical analysis of the past ten years is justified as it can provide epidemiological data for better control of subclinical mastitis. The results will allow us to develop an overview of the etiological agents and antimicrobial sensitivity of the main agents that cause mastitis, helping to provide a basis to prevent resistance to the condition and address its chronic nature.

Methods

The following systematic review with meta-analysis was planned according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) network meta-analysis reporting standards using the StArt and Biostat programs (Hutton *et al*., 2015). Observational studies that assessed etiological agents of subclinical bovine mastitis and its antimicrobial resistance/sensitivity were eligible for inclusion. The inclusion criteria were primary studies: (1) related to the proposed topic and available for online consultation in search engines using keyword *strings*; (2) published between 2009 and 2019; (3) that addressed the bovine species, (4) that used 200.000 cel/mL as somatic cell count threshold for the classification of subclinical mastitis and (5) that were conducted in Brazil. The exclusion criteria were (1) primary studies that did not specifically address the etiological agents and its antimicrobial resistance/sensitivity; (2) primary studies published outside the selected period; (3) primary studies that did not address the bovine species; (4) primary studies conducted outside Brazil, and (5) secondary studies.

The research included articles from journals and annals of scientific events published from 2009 to 2019 in SciELO, Capes Periodical Portal, Google Scholar and PubMed. In each of the databases, the keywords were searched in Portuguese and English. The terms searched were:

- Mastitis AND subclinical AND Brazil
- Etiology AND mastitis AND subclinical AND Brazil
- Antimicrobial resistance AND mastitis AND Brazil
- Antimicrobials AND mastitis AND Brazil
- Bovine mastitis AND subclinical AND Brazil
- *Staphylococcus* AND subclinical AND mastitis AND Brazil
- *Streptococcus* AND subclinical AND mastitis AND Brazil
- *Corynebacterium* AND subclinical AND mastitis AND Brazil
- Milk AND subclinical AND mastitis AND Brazil.

The data extracted from each article, when available, included: year of publication; journal; first author; Brazilian region where the study was conducted; number of animals, number of milk samples analyzed, number of culture-positive milk samples, description of the etiologic agents isolated from subclinical mastitis, antimicrobials tested and number of resistant samples to each antimicrobial class. The data obtained were tabulated and a descriptive statistical analysis of the absolute and relative frequency of the microbiological findings was performed using Microsoft Excel® and the combined Chi-Square test using BIOSTAT® to compare the prevalence of resistance in each year studied. The heterogeneity of the prevalence estimates between studies was quantified by I^2 index for the most frequent microorganisms (Higgins and Thompson, 2002).

Results

Our search strategy yielded 41,038 records (sum of all database), from which 75 studies were retained after inclusion/exclusion criteria.

Following duplicate removal, 69 articles were included in the screening step. During the screening stage, 12 articles were considered as not relevant and were excluded (5 without information regarding the geographic region; 5 without the number of animals and 3 surveys based on interviews). Fifty-seven articles, published between 2009 and 2018 with 22,287 milk samples evaluated, were selected according to the inclusion criteria. The sampling technique and representativeness of the 57 articles included can be seen in Supplementary Table 1.

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Supplementary Table I. *Sampling technique and representativeness of 55 articles published between 2009 and 2018 that met the inclusion criteria of the systematic review.*

	Reference	State	Type of sampling
1	Alencar *et al*. (2014)	Rio de Janeiro	Purposive sampling, independent
2	Amorim *et al*. (2016)	Pernambuco	Purposive sampling, independent
3	Andrade *et al*. (2010)	Paraná	Convenience, random, independent
5	Assis *et al*. (2017)	Espírito Santo	Convenience, random, independent
6	Bandeira *et al*. (2013)	R.Grande do Sul	Purposive sampling, independent
7	Brito *et al*. (2014)	Maranhão	Convenience, random, independent
8	Carvalho *et al*. 2018	Maranhão	Convenience, random, independent
9	Casanova *et al*. (2016)	Santa Catarina	Purposive sampling, independent
10	Castro *et al*. (2012)	Rio de Janeiro	Quota sampling, independent
11	Chagas *et al*. (2012)	Minas Gerais	Convenience, random, independent
12	Costa *et al*. (2013)	Minas Gerais	Convenience, random, independent
13	Costa *et al*. (2013)	Santa Catarina	Snowball sampling, independent
14	Costa *et al*. (2015)	Santa Catarina	Convenience, dependend
15	Cunha *et al*. (2015)	Minas Gerais	Convenience, random, independent
16	deSantana Neres *et al*. (2015)	Sergipe	Convenience, independent
17	Dias *et al*. (2011)	Minas Gerais	Quota sampling, independent
18	Farias *et al*. (2013)	R.Grande do Sul	Purposive sampling, independent
19	Ferreira *et al*. (2010)	Piauí	Quota sampling, independent
20	Filho *et al*. (2016)	Paraná	Convenience, random, independent
21	Freitas *et al*. (2018)	R.Grande do Sul	Purposive sampling, independent
22	Gonçalves *et al*. (2018)	São Paulo	Purposive sampling, independent
23	Jardim *et al*. (2014)	Paraná	Convenience, random, independent
24	Jobim *et al*. (2010)	Paraná	Convenience, random, independent
24	Jobim *et al*. (2010)	R.Grande do Sul	Convenience, random, independent
24	Jobim *et al*. (2010)	Santa Catarina	Convenience, random, independent
25	Junior *et al*. (2015)	São Paulo	Purposive sampling, independent
26	Kaiser *et al*. (2015)	R.Grande do Sul	Purposive sampling, independent
27	Karach *et al*. (2016)	Paraná	Convenience, random, independent
28	Kolling *et al*. (2011)	R.Grande do Sul	Purposive sampling, independent
29	Krewer *et al*. (2013)	Bahia	Convenience, random, independent
29	Krewer *et al*. (2013)	Pernambuco	Convenience, random, independent
30	Lange *et al*. (2017)	Paraná	Convenience, random, independent
31	Martins *et al*. (2010)	Mato Grosso	Convenience,representative, independent
31	Martins *et al*. (2014)	Piauí	Purposive sampling, independent
32	Martins *et al*. (2015)	Goiás	Convenience, random, independent
33	Melo *et al*. (2013)	Pernambuco	Convenience, random, independent
34	Niero 2018	Santa Catarina	Purposive sampling, independent
35	Oliveira *et al*. 2009	Sergipe	Convenience, independent
36	Oliveira *et al*. (2010)	Pará	Convenience, random, independent
37	Oliveira *et al*. (2012)	Bahia	Convenience, random, independent
38	Oliveira *et al*. (2013)	Paraná	Simple random sampling, independent
39	Peters *et al*. (2016)	R.Grande do Sul	Purposive sampling, independent
40	Rall *et al*. (2014)	São Paulo	Purposive sampling, independent
41	Ribeiro *et al*. 2009	São Paulo	Purposive sampling, independent
42	Ruiz *et al*. (2011)	Pernambuco	Convenience, random, independent

	Reference	State	Type of sampling
43	Saab *et al*. (2014)	Paraná	Convenience, random, independent
44	Saeki *et al*. (2011)	São Paulo	Convenience, independent
45	Santos *et al*. (2010)	Paraná	Convenience, random, independent
46	Senhorelo *et al*. (2013)	Espírito Santo	Convenience, random, independent
47	Silva *et al*. (2011)	Bahia	Convenience, random, independent
48	Silva *et al*. (2012)	Pernambuco	Purposive sampling, independent
49	Soethe *et al*. (2015)	Paraná	Convenience, random, independent
50	Souza *et al*. (2016)	Minas Gerais	Convenience, random, independent
51	Ulsenheimer *et al*. 2018	R.Grande do Sul	Purposive sampling, independent
52	Valmorbida *et al*. (2017)	Santa Catarina	Purposive sampling, independent
53	Vesco *et al*. (2017)	R.Grande do Sul	Purposive sampling, independent
54	Zanette *et al*. (2010)	Santa Catarina	Quota sampling, independent
55	Zimermann *et al*. (2017)	Paraná	Purposive sampling, independent

The number of publications was not homogeneous (G test = 31.67; $P < 0.01$) among Brazilian states, with a greater occurrence of studies in Paraná, Rio Grande do Sul, and Santa Catarina. Thus, 49% of the selected studies were conducted in the southern region (Fig. 01). The sample size evaluated in the articles among Brazilian states was not uniform (X^2 = 8249.88; $P <$ 0.01). The states with the largest number of milk samples analyzed were Rio Grande do Sul, with 23% of the samples, followed by Minas Gerais (16%), and Paraná (12%) (Fig. 1).

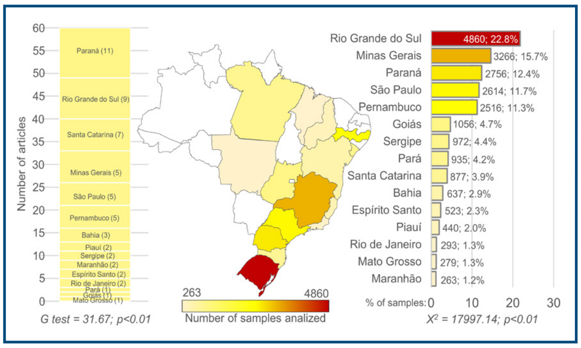

Figure 1. *Publications about subclinical mastitis in dairy cows in Brazil and sample size (milk samples) retrieved from 57 scientific articles published between 2009 and 2018.*

Of the 57 articles selected, 45 studies isolated and identified the etiologic agents that caused subclinical mastitis. The most frequent pathogens were *Staphylococcus* spp., which was isolated in all studies with an average prevalence of 49% in the samples analyzed. There was no significant heterogeneity between 45 studies (Q=82.03, df=24, p=0.88), with a heterogeneity index I^2 of 19.47%. However, when categorized by region, the distribution of *Staphylococcus* spp. was not homogeneous among the Brazilian states (X^2 = 75.40; $P < 0.01$), with a higher rate in the states of Espírito Santo (80%), Rio de Janeiro (77%), and Bahia (60%). Goiás and São Paulo had the lowest rates at 20% and 28%, respectively (Fig. 2).

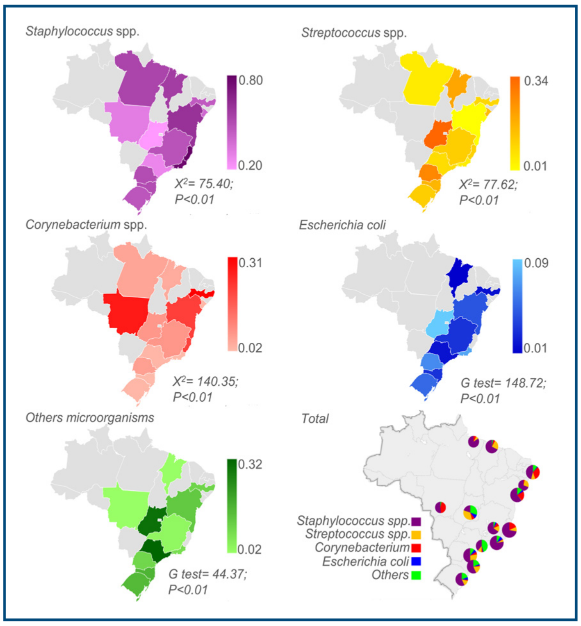

Figure 2. *Occurrence of etiologic agents of subclinical mastitis in dairy herds in Brazil retrieved from 45 scientific articles published between 2009 and 2018.*

The second most frequent pathogens were *Streptococcus* spp., identified in 76% of the identified articles, with an average occurrence of 14% in the analyzed samples and a significant heterogeneity (Q=337.70, df=98, p<0.001; I^2=70.98) between the studies retrieved. The distribution of *Streptococcus* spp. was not homogeneous among the Brazilian states (X^2 = 77.62; $P < 0.01$), with greater prevalence in the states of Goiás (34%) and Paraná (27%) (Fig. 2). The third most frequent pathogens were *Corynebacterium* spp., identified in 58% of the studies and with an average prevalence of 8% in the analyzed samples. The heterogeneity index I^2 for the prevalence of *Corynebacterium* spp. between those studies was 80.31% (Q=497.61, df=98, p<0.001). *Corynebacterium* spp. were not evenly distributed among the Brazilian states (X^2 = 140.35; P < 0.01), with greater prevalence in the states of Pernambuco (31%), Mato Grosso (27%), and Bahia and Espírito Santo (both 21%) (Fig. 2). *Escherichia coli* was the fourth most frequent pathogen, isolated in 47% of the articles and with an average occurrence of 4% in the analyzed samples. A significant heterogeneity was found regarding the prevalence of *E. coli* among the studies (Q=634.75, df=98, p<0.001; I^2=84.56).

The states of Goiás (9%) and Rio de Janeiro (8%) had the highest prevalence of *E. coli*. In 87% of the reviewed articles, other microorganisms were also isolated and associated with subclinical mastitis, such as *Candida* spp., *Micrococcus* spp., *Proteus* spp., *Alcaligenes faecalis*, *Enterobacter aerogenes*, *Klebsiella* spp., *Citrobacter* spp., *Salmonella* spp., *Yersinia* spp., *Pseudomonas* spp., *Nocardia* spp., *Trueperella* spp., and *Serratia* spp. This wide range of pathogens was more prevalent in isolates from São Paulo (32%) and Goiás (30%) (G test = 44.37; P < 0.01) (Fig. 2).

Microbial resistance

Among the 57 articles reviewed, 28 investigated the occurrence of resistance and sensitivity of isolated microorganisms against the antibiotics tested. Only studies from 11 states of the northeast, southeast, and south regions evaluated microbial resistance. These studies were conducted mainly in Rio Grande do Sul, Minas Gerais, and Santa Catarina, which together accounted for 43% of the retrieved articles. The most comprehensive samples also came from studies conducted in these three states: Rio Grande do Sul (1876 samples), Minas Gerais (552), and Santa Catarina (455) (Fig. 3).

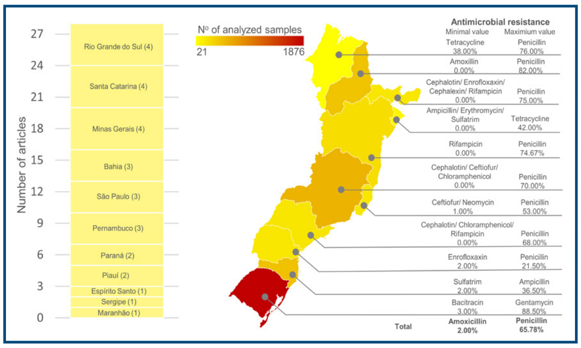

Figure 3. *Occurrence of bacterial resistance to antibiotics in dairy cows in Brazil from 28 articles published.*

The most tested antimicrobials were ampicillin, erythromycin, gentamicin, penicillin, and tetracycline. Over the years, there has been an increase in the occurrence of bacterial resistance to cephalexin, cefoperazone, erythromycin, gentamicin, neomycin, penicillin, tetracycline, and trimethoprim (Table I).

The occurrence of microorganisms resistant to penicillin varied between 34% and 76% between

Table I. *Occurrence of bacterial resistance to antibiotics in dairy cows from 57 articles published between 2009 and 2018 (n= number of isolates analyzed).*

	2010	2011	2012	2013	2015	2016	2017	2018
Amikacin	-	-	-	-	0.04a (n=69)	0.04a (n=56)	-	-
Amoxicillin	-	-	-	0.59a (n=453)	-	0.71a (n=17)	0.05b (n=313)	0.50a (n=30)
Ampicillin	0.68de (n=289)	0.27ab (n=154)	0.78e (n=242)	0.68de (n=805)	0.47c (n=232)	0.67cde (n=39)	0.18a (n=313)	0.48bcd (n=62)
Bacitracin	0.41b (n=188)	-	-	0.08a (n=194)	-	-	0.07a (n=846)	-
Cephalexin	-	0.00a (n=65)	-	0.01a (n=453)	-	0.13b (n=56)	0.74c (n=869)	0.19b (n=32)
Cephalothin	0.13b (n=188)	0.30b (n=50)	0.00a (n=83)	0.01a (n=546)	0.13b (n=153)	-	-	-
Cefoperazone	0.20a (n=101)	-	-	0.50b (n=352)	-	-	-	-
Ceftiofur	0.04a (n=101)	0.02a (n=65)	-	0.01a (n=546)	-	-	-	-

	2010	2011	2012	2013	2015	2016	2017	2018
Chloramphenicol	0.26b (n=188)	-	0.14b (n=180)	0.05a (n=194)	0.00a (n=69)	0.25b (n=32)	-	-
Enrofloxacin	0.21b (n=101)	0.02a (n=66)	0.00a (n=83)	0.01a (n=453)	-	-	0.03a (n=36)	0.09a (n=32)
Erythromycin	0.25cd (n=227)	0.16bc (n=116)	0.08ab (n=197)	0.04a (n=275)	0.39d (n=163)	0.23abcd (n=26)	0.20bc (n=120)	0.72e (n=32)
Streptomycin	0.68b (n=188)	-	-	0.12a (n=453)	-	0.24a (n=46)	-	-
Gentamicin	0.20d (n=101)	0.03ab (n=156)	0.06bc (n=242)	0.02a (n=805)	-	0.16cd (n=92)	0.75e (n=2100)	0.87e (n=30)
Neomycin	0.39b (n=101)	0.03a (n=118)	-	0.03a (n=546)	-	0.43b (n=56)	0.73c (n=1880)	-
Norfloxacin	0.26b (n=188)	0.07a (n=88)	-	0.03a (n=282)	-	0.12ab (n=26)	-	0.09ab (n=32)

Data for the Amoxacillin + Clavulanic acid association in 2014 was omitted due to only one entry; a, b,c,d: Proportions followed by equal letters did not differ over the years by the chi-square test with 5% significance.

2010 and 2016 and increased to 88% in 2017 ($P < 0.05$), reaching the highest level of resistance among the isolates (Table I). Other antimicrobials that started to show increasing values ($P < 0.05$) of microbial resistance as of 2017 were gentamicin and neomycin, and erythromycin, in 2018. The increase in these three agents may be linked to their widespread use in dairy farms, which results in contributing to the selective pressure of microorganisms resistant to them (Tomazi and dos Santos, 2020). Amikacin and ceftiofur were the antimicrobial drugs with the lowest prevalence of resistance and without variations ($P > 0.05$) in studies conducted between 2010 and 2016. Resistance to amikacin remained at 4% during 2015 and 2016. Resistance to ceftiofur was 4% in 2010; 2% in 2011; and 1% in 2015.

Discussion

Regarding the most frequent pathogen reported in this systematic review (pooled prevalence of 49%), Staphylococci are one of the pathogens most frequently isolated in cases of intramammary infection within dairy herds. This estimate is similar to the study done by Ashraf and Imran (2020), who conducted a review about the prevalence of various bacterial species worldwide.

Algharib *et al*., (2020) stated that *S. aureus* is an agent that is difficult to treat owing to its high resistance in the udder, which consequently, influences the efficiency of the antibiotics administered. This is due to a mechanism used by the pathogen to invade and colonize the animal's mammary gland; the microorganism invades the mammary gland through the teat canal and colonizes its epithelium, attaching to the epithelial cells of the mammary gland and forming so-called "bacterial pockets." As well as the results of this systematic review, most studies and literature reviews also consider Streptococcus spp. as the second group of microorganisms of importance in the etiological agents causing mastitis in ruminants. In most herds, *Streptococcus agalactiae*, *Streptococcus uberis*, and *Streptococcus dysgalactiae* are the main isolated species (Santos *et al*., 2018). *Streptococcus uberis* is an important agent of subclinical infections and clinical episodes of bovine mastitis worldwide (Hillerton, 2020). Santos *et al*., (2018) reported that the *Streptococcus dysgalactiae* is one of the most common pathogens of bovine mastitis, causing great economic losses.

Regarding *Corynebacterium* spp., the third most frequent reported in this systematic review, the species isolated the most in bovine mastitis is *C. bovis* (Karach *et al*., 2015). They have low pathogenicity and high contagiousness, being transmitted mainly during milking, and are considered one of the causes of contagious mastitis. It is detected mainly in the subclinical form of the disease, which in a certain manner guarantees protection to the mammary gland against other more pathogenic cells. The isolation rates of this pathogen are high in herds with problems related to the cleaning of teats, especially post-dipping (Gonçalves *et al*., 2016). The fourth most frequent pathogen was *E.coli*. Neethan *et al*., (2017) also indicate *E. coli* as the main coliform (environmental microorganism) causing subclinical mastitis, with symptoms ranging from mild (with inflammatory signs in the mammary gland) to acute, with systemic signs such as ruminal stasis, dehydration, and shock, which can even lead to the death of the affected animal. Although it mainly causes clinical mastitis, the microorganism has also been investigated in cases of subclinical mastitis. They are usually transient infections and are associated with acute or super-acute clinical conditions, which can be fatal. It is important to note that older cows, those at the beginning of lactation, and those with higher yields are most susceptible to the severe manifestation of mastitis by coliforms (Byomi *et al*., 2020; Hamali *et al*., 2017). We observed a high heterogeneity for *Streptococcus* spp, *Corynebacterium* spp and *E. coli* regarding the prevalence estimates between the retrieved studies, probably due to diversity in farm practices (hygienic milking, dry cow therapy and therapeutic actions) together with herd characteristics (genetic, stage of lactation) and agroclimatic conditions (Bangar *et al*., 2015).

Regarding microbial resistance, it can be inferred that some of the most used agents in intramammary therapies were evaluated by only a few of the selected studies. An example is the third generation cephalosporins, identified as the second most frequently used class of antimicrobials in Brazil (Tomazi and dos Santos, 2020). We recommend that studies on microbial resistance select the agents most used in the geographic region studied so that the results better reflect reality. Penicillin is one of the main antibiotics used for intramammary treatments not only in Brazil but also in other countries (Tomazi and dos Santos, 2020). In the United States, more than 70% of isolates obtained from mastitis caused by *S. aureus* are resistant to penicillin, whereas in Ireland, the level of resistance is around 85% (Cazoto *et al*., 2011). The widespread use of an agent is one of the causes of bacterial resistance (Freitas *et al*., 2018). Another cause of resistance to penicillins is owing to *Staphylococcus* spp., the main genus associated with subclinical mastitis, being able to develop resistance to most antimicrobials. Resistance to beta-lactams, as is the case with penicillins, can occur via two main mechanisms: through the production of beta-lactamases, encoded by the blaZ gene and the change in the antimicrobial action site owing to the production of a modified low-affinity penicillin-binding protein (PBP2a or PBP2), encoded by the mecA gene (Soares *et al*., 2012). Indeed, in a systematic review that addressed article from 5 continents, Molineri *et al*. (2021) found that the highest overall prevalence of resistant *S. aureus* was against penicillin.

Although belonging to the third generation cephalosporin class, the second most frequently used class in Brazil between 2014 and 2016, among the isolates tested, low microbial resistance was shown to ceftiofur, with values ranging between 4% and 1% (Tomazi and dos Santos, 2020). In line with our results, Molinieri *et al*. (2021) stated that ceftiofur and cephalothin presented the lowest overall prevalence of antimicrobial-resistant *S. aureus* from article retrieved between the years 1969–2020. This result contradicts the common understanding that associates the duration of use with greater resistance (Dyar *et al*., 2017). One of the facts that may have led to this result is that after 2013, we found no studies evaluating resistance to ceftiofur. In this review, few studies evaluated the effectiveness of Amikacin on bovine mastitis isolates. However, authors such as Fim Junior *et al*., (2015) and Souza *et al*., (2016) reported 92.3% and 96.0% sensitivity of the isolates, respectively, thus demonstrating the effectiveness of Amikacin with their results.

The scarcity of studies investigating the use of this antimicrobial drug may be because gentamicin is one of the main aminoglycosides used in veterinary medicine, more specifically in the treatment of mastitis.

Limitations

It is worth noting that analyzing microbial resistance with information obtained from published scientific articles has its limitations. One of them is the temporal and geographical limitation, since, from an epidemiological point of view, the monitoring of publications over the years does not guarantee a significant sample at the national level, and these data are not from a single region of Brazil. Moreover, the compilation of several studies conducted in one specific period is not representative.

Furthermore, some geographical areas with lack of studies pose challenges to obtaining high-quality survey, contributing with bias that can affect the reliability of the findings, mainly when extrapolating the results to other regions. Thus, we strengthen the need of more studies at regional level with properly methodologies regarding the sample representativeness. Another limitation is related to the seasons, since the time of year is related to antimicrobial treatment in dairy herds (Tomazi and dos Santos, 2020). The articles selected in this study did not provide enough data to analyze this variable.

Conclusions

With the etiological diversity found in this review, our results strengthen the knowledge of the microbiological agent and antibiotic-resistance patterns of pathogens isolated from subclinical mastitis in dairy cows at regional level. The spread of bacterial resistance can be prevented using the culture test and antibiogram, and that although decision making in a farm cannot be replaced by scientific studies, it can be supported by such efforts. Nevertheless, the identification of the microbiological agent is essential to the most appropriate therapy. When possible, etiology should be determined before treatment to avoid microbial resistance.

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