



The microbiology and treatment of human mastitis

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Abstract

Mastitis, which is generally described as an inflammation of breast tissue, is a common and debilitating disease which frequently results in the cessation of exclusive breastfeeding and affects up to 33% of lactating women. The condition is a primary cause of decreased milk production and results in organoleptic and nutritional alterations in milk quality. Recent studies employing culture-independent techniques, including metagenomic sequencing, have revealed a loss of bacterial diversity in the microbiome of mastitic milk samples compared to healthy milk samples. In those infected, the pathogens *Staphylococcus aureus*, *Staphylococcus epidermidis* and members of corynebacteria have been identified as the predominant etiological agents in acute, subacute and granulomatous mastitis, respectively. The increased incidence of antibiotic resistance in the causative species is also a key cause of concern for treatment of the disease, thus leading to the need to develop novel therapies. In this respect, probiotics and bacteriocins have revealed potential as alternative treatments.

Keywords Human mastitis · Microbiota · Antibiotics · Probiotics · Bacteriocins

Introduction

Over the last decade, breastfeeding has been the subject of renewed attention in developed countries because of the demonstrated health benefits to the mother–child dyad [1, 2]. Consequently, international and national health organizations encourage exclusive breastfeeding during the first 6 months of life [3–5]. However, in certain cases, exclusive breastfeeding may not be an option for mothers owing to many reasons [6], with mastitis considered as the greatest cause of undesired weaning.

Mastitis, an inflammation of breast tissue, is an acute, devitalizing condition and a potentially serious illness that may lead to breast abscess and septic fever [7]. The main causes of mastitis are milk stasis and infection [3, 8]. Milk

stasis is usually the primary cause and occurs when milk is not removed properly from the breast duct due to poor attachment of the infant, fruitless suckling and blockage of the ducts [3, 9]. It is widely accepted that most mastitic cases are related to changes in the microbiome of the mammary gland and that most mastitis-causing bacteria have the ability to form biofilms in the milk ducts which are quite narrow; this results in the impairment of milk flow and the retention of milk [10] (Fig. 1a, b). The incidence of lactational mastitis varies between 2 and 33% of lactating mothers [11, 12] and most episodes occur in the first 6 weeks postpartum [13]. According to epidemiologic studies, there are many factors which might be implicated in its occurrence [13–16]. Risk factors include age, with mothers under 21 and over 35 years having a decreased incidence [17], mastitis with a previous child [16], cracked or sore nipples, use of ointments, incorrect breastfeeding practices and peripartum antibiotherapy [8, 18, 19].

The aim of this review is to provide an overview of the latest findings in terms of the microbiological load involved in human mastitis, particularly at a time when the advances in sequencing technologies have provided an excellent platform to study both cultivable and non-cultivable microorganisms, giving a more accurate view of the microbiological dysbiosis which shapes this disease. The review also describes the available therapies to treat mastitis, the most

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the microbiota of human milk may aid in establishing the commensal GI microbiota of the infant [11, 35–38].

How or why the transition from commensal colonization to infection occurs remains to be fully understood, however, several factors have been identified which may predispose women to the development of mastitis. These range from host factors such as genetics, the presence of polymorphisms and even blood grouping, to bacterial factors, e.g., virulence factors of the bacteria present, to medical influences including the use of antibiotics and the widespread administration of iron to prenatal women [10, 37].

Microorganisms detected in lactational mastitis

Lactational mastitis is classified into different categories depending on the course (acute, subacute and granulomatous, which is explained later) or the clinical manifestations (clinical or subclinical) [10]. Mastitis is a multifactorial disease caused by a range of different pathogens and is characterized by an alteration in the mammary microbiota [27, 38, 39]. Indeed, a metagenomic analysis of 20 milk samples (half from women with mastitis, while the other half were taken from healthy women) showed a noteworthy loss of bacterial diversity in the mastitic milk samples [26]. The latter is illustrated in Table 1 which records the genera detected in healthy human milk and mastitic human milk via culture-dependent and culture-independent analyses. The majority of the available literature is based on culture-independent approaches, however, the scientific community should be handling this data with precaution as there is some argument as to the appropriate controls for such studies. Salter et al. [40] and Glassing et al. [41] have both described the potential for incorrect results in low bacterial count samples owing to contamination or other artefacts which could explain the discrepancies between the healthy core microbiome reported by Hunt et al. [25] and Murphy et al. [28]. In a more recent study, the healthy core milk microbiome consisted of the genera *Pseudomonas*, *Staphylococcus*, *Streptococcus*, *Elizabethkingia*, *Variovorax*, *Bifidobacterium*, *Flavobacterium*, *Stenotrophomonas*, *Brevundimonas*, *Chryseobacterium*, *Lactobacillus* and *Enterobacter* [28], whereas *Staphylococcus* species dominated the mastitic milk samples with *S. aureus* and *S. epidermidis* being the dominant species in the microbiome of women diagnosed with acute and subacute mastitis, respectively [26]. In another study, sequencing of 16S rDNA of 32 human mastitic milk samples revealed the presence of 17 genera and 30 distinct species [42]. *Proteobacteria* was the predominant phylum accounting for 51.3% of the total followed by *Firmicutes* (37.8%) and included representatives from the classes *Gammaproteobacteria*, *Alphaproteobacteria* and *Bacilli*. The most frequently isolated

genera included *Pseudomonas* (6 species) and *Staphylococcus* (4 species). The following species were frequently isolated; *Staphylococcus* species (87.5% of mastitic samples), *S. aureus* (75.0%), *P. aeruginosa* (53.1%) *Klebsiella pneumoniae* (43.8%) and *Brevundimonas diminuta* (38.0%). Recently, Mediano et al. [27] identified 5009 isolates from 1849 mastitic milk samples by classical, biochemical and/or molecular methods. Mean total bacterial count was 4.11 log₁₀ colony forming units (cfu)/mL with *Staphylococcus* being the most commonly isolated bacterial group (97.57%) and *S. epidermidis* being the dominant species (91.56%) followed by *S. aureus* (29.74%). Streptococci and corynebacteria comprised the second (70.20%) and third (16.60%) most dominant groups, respectively. Most recently, a 16S rRNA gene-sequencing method of 50 human milk samples (16 subacute mastitic, 16 acute mastitic and 18 healthy control samples) revealed an enrichment of genera including *Aeromonas*, *Staphylococcus*, *Ralstonia*, *Klebsiella*, *Serratia*, *Enterococcus* and *Pseudomonas* in subacute and acute mastitic samples, while *Acinetobacter*, *Ruminococcus*, *Clostridium*, *Faecalibacterium* and *Eubacterium* were consistently depleted [20] (Fig. 1c). Moreover, dramatic enrichment of aerotolerant bacteria and depletion of obligate anaerobes was observed during the infection. Lower microbial diversity was also a feature of the mastitic milk samples along with increased abundance of opportunistic pathogens including *S. aureus*, *S. epidermidis*, *S. hominis*, *K. pneumoniae*, *Ser. marcescens*, *P. aeruginosa*, *E. faecalis*, *Bacillus subtilis*, *B. cereus* and *Escherichia coli*. Marín et al. [43] analyzed the cultivable microbial diversity of 647 human mastitic milk samples using Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) technology. The total staphylococcal count was 3.7 log₁₀ cfu/mL with *Staphylococcus* genus being the most frequently isolated (94.1%) and *S. epidermidis* being the predominant species (87.6%) followed by *S. aureus* (22.1%), *S. hominis* (5.3%) and *S. lugdunensis* (3.3%). In agreement with the findings of Mediano et al. [27], streptococci were the second-most abundant group with a mean count of 3.47 log₁₀ cfu/mL. *Strep. mitis/oralis* was the most common species (40.8%), while *Strep. salivarius* and *Strep. parasanguinis* were detected with frequencies of 36.8 and 14.4%, respectively. Corynebacteria occurred in 11% of the analyzed samples with the populations of *C. tuberculostearicum* and *C. kroyenstedtii* reaching ratios of 3.6 and 2%, respectively. Similarly, Delgado et al. [44, 45] identified *Staphylococcus* as the predominant genus in mastitic milk samples using a combination of culture and molecular techniques where *S. epidermidis* was found to be the dominant species. Moreover, antibiotic resistance and the presence of the biofilm-related *icaD* gene were significantly higher in *S. epidermidis* strains isolated from mastitic milk samples [45].

Table 1 Genera detected using culture-dependent and culture-independent techniques to study the microbial diversity associated with human milk

Phylum	Genus	Culture-dependent	Culture-independent	
Firmicutes	<i>Anaerococcus</i>		H [28]	
	<i>Bacillus</i>	H [21]	M [20, 42]	
	<i>Coprococcus</i>		H [28]	
	<i>Clostridium</i>		H [20, 24, 46]	
	<i>Enterococcus</i>		H [24, 47–49]	
	<i>Erysipelotrichaceae incertae sedis</i>		H [28]	
	<i>Finegoldia</i>		H [28]	
	<i>Gemella</i>		H [25, 28, 46, 49]	
	<i>Lachnospiraceae incertae sedis</i>		H [28]	
	<i>Lactobacillus</i>	H [21, 51]	H [24, 25, 28, 37, 47–49, 52, 53]	
	<i>Lysinibacillus</i>			
	<i>Macrococcus</i>			
	<i>Peptoniphilus</i>		H [28]	
	<i>Peptostreptococcaceae incertae sedis</i>		H [28]	
	<i>Planococcus</i>			
	<i>Staphylococcus</i>	H [21, 51, 54]	H [24–26, 28, 37, 49, 53, 55]	
	<i>Streptococcus</i>	H [21, 51]	H [24, 25, 28, 48, 49, 53, 55]	
	<i>Veillonella</i>		H [25, 28, 46, 49]	
	Proteobacteria	<i>Acinetobacter</i>	H [21, 51]	H [20]
		<i>Alcaligenes</i>	H [21]	
<i>Brevundimonas</i>			H [28]	
<i>Citrobacter</i>			H [28]	
<i>Enterobacter</i>			H [28]	
<i>Erwinia</i>			H [28]	
<i>Escherichia-Shigella</i>		H [21]	H [28, 46, 49]	
<i>Hemophilus</i>			H [28]	
<i>Klebsiella</i>				
<i>Moraxella</i>		H [21]		
<i>Proteus</i>				
<i>Providencia</i>				
<i>Pseudomonas</i>		H [21]	H [25, 28, 49, 59, 60]	
<i>Ralstonia</i>			H [25]	
<i>Serratia</i>			H [25, 46, 49]	
<i>Sphingomonas</i>			H [25]	
<i>Stenotrophomonas</i>			H [28]	
<i>Variovorax</i>		H [28]		
Actinobacteria	<i>Actinomyces</i>		H [25, 28, 46]	
	<i>Atopobium</i>		H [28]	
	<i>Bifidobacterium</i>		H [24, 25, 28, 36, 37, 61]	
	<i>Brevibacterium</i>			
	<i>Corynebacterium</i>		H [25, 28]	
	<i>Granulicatella</i>		H [28]	
	<i>Leucobacter</i>			
	<i>Micrococcus</i>	H [21]		
	<i>Propionibacterium</i>		H [25, 28, 49]	
	<i>Rhodococcus</i>		H [28]	
	<i>Rothia</i>		H [25, 28]	

Table 1 (continued)

Phylum	Genus	Culture-dependent	Culture-independent
Bacteroidetes	<i>Bacteroides</i>		H [24, 28]
	<i>Chryseobacterium</i>		H [28]
	<i>Elizabethkingia</i>		H [28]
	<i>Flavobacterium</i>	H [62]	H [28]
	<i>Prevotella</i>		H [25, 28, 46]

H indicates healthy mammary gland while M indicates mastitis

Acute mastitis

Acute mastitis is characterized by breast redness, fever, pain and malaise [10]. Due to the intensity of these symptoms, acute mastitis is typically the sole type of mastitis that is accurately diagnosed despite representing a relatively small proportion of human mastitis cases. A metagenomic study of human milk samples by Jimenez et al. [26] analyzed the microbiome of 20 milk samples including 10 healthy, 5 acute and 5 subacute mastitic samples. Variation in the predominant bacterial genus was observed in milk samples obtained from healthy women (*Pseudomonas*, *Bacteroides*), however, *Staphylococcus* was the dominant genus in both acute and subacute mastitis samples. Women who presented with acute mastitis were found to have higher levels of *S. aureus* (approximately 10^6 cfu/mL) in their milk compared to those with subacute mastitis. The wide range of toxins produced by strains of *S. aureus* have previously been implicated in bovine mastitis and may also be responsible for the symptoms observed in acute mastitis in humans [56]. Comparatively, *S. epidermidis* was found to be the most abundant staphylococcal species in women with subacute mastitis. Interestingly, Patel et al. [20] recently reported that acute mastitis samples harbored significantly more *Aeromonas*, *Klebsiella*, *Ralstonia*, *Proteus* and *Leptospira* at genus level and significantly higher levels of *Aeromonadaceae*, *Burkholderiaceae*, *Brucellaceae* and *Streptococcaceae* at family level when acute mastitis, subacute mastitis and healthy milk samples were compared (Fig. 1c).

Subacute mastitis

The symptoms of subacute mastitis are distinctly more subtle than acute resulting in a lower rate of diagnosis, whether this is due to misdiagnosis or a lack of understanding by women experiencing these symptoms is not described. Where an official diagnosis has been established, the symptoms of subacute mastitis include a sharp, needling pain and a burning sensation in the breast [10].

As indicated previously, Jimenez et al. [26] proposed that *S. epidermidis* was the predominant species of *Staphylococcus* present in subacute mastitic milk samples ($n=5$).

An additional, and more extensive study of 20 women with subacute mastitis also found *S. epidermidis* to be the most dominant species (by 85%). Other species of *Staphylococcus* including *S. hominis*, *S. pasteurii*, *S. warneri* and *S. haemolyticus*, were also identified in subacute mastitic samples albeit at much lower levels than *S. epidermidis* [44]. At genus level, *Staphylococcus* was also found to be more enriched in subacute mastitis milk samples along with *Erwinia*, *Bacillus*, *Pantoea*, *Cronobacter* and *Pseudomonas* when compared to acute mastitic and healthy milk samples [20].

Collectively, the species of *Staphylococcus* found in subacute mastitic milk samples can be referred to as coagulase-negative staphylococci (CNS). Many species of CNS are healthy skin commensals; however, they can also be implicated in nosocomial infections, particularly *S. epidermidis* [63].

In addition to CNS, streptococci, more specifically viridans streptococci, may play a role in mastitis although the strains involved appear to be host-dependent. For example, *Strep. uberis* and *Strep. dysgalactiae* have been identified in bovine mastitis [50], yet they are not involved in human mastitis. On the other hand, *Strep. mitis* and *Strep. salivarius* prevail in human mastitis [57]. The combination of viridans streptococci and CNS have the potential to form thick biofilms leading to confinement of the lumen through which the milk passes. This pressure leads to the symptoms of subacute mastitis described previously [10].

Granulomatous mastitis

Idiopathic granulomatous mastitis (IGM) is a relatively rare, inflammatory disease which presents as a painful, tender lump or mass in the breast. The presence of a mass and the secondary symptoms of IGM, skin thickness, abscess formation and nipple retraction, often lead to a clinical misdiagnosis of breast malignancy [64, 65]. IGM is predominantly observed in women of reproductive age, approximately 2–6 years following pregnancy, however, it has also been reported in prepubescent girls, elderly women and men [66].

The causes of IGM may vary and several factors have been proposed that predispose to IGM including diabetes

mellitus, the use of the oral contraceptive pill and undetected organisms amongst others [66, 67]. However, Taylor et al. [68] suggested that the presence of species of corynebacteria was linked to the development of IGM. A subsequent study by Dobinson et al. [65] demonstrated the successful isolation of several strains of corynebacteria from breast masses, namely *C. kroppenstedii*, *C. tuberculostearicum* and *C. freneyi*. Despite the strong suggestion that corynebacteria is a major causative agent of IGM, a successful treatment has not yet been established [67]. This is principally due to the fact that corynebacteria exist in lipid-filled vacuoles within the granuloma as opposed to the tissue itself thus treatment requires agents that are effective against corynebacteria as well as active in lipid environments. However, most antimicrobials are hydrophilic with weak distribution to lipid environments. A combination of these agents, e.g., clarithromycin or rifampin, with corticosteroids and/or other immunomodulatory modalities has shown some success in treating IGM [65].

Effects on milk quality

Lactational mastitis is a primary cause of decreased milk production and is also related to alterations in the cellular composition of milk. Changes in the metabolic activity of milk-producing cells along with diminished milk synthesis in the mammary gland is normally a direct result of inflammatory mediators [3, 69]. Edema of the interstitial tissues is caused by opening of the paracellular pathways during lactation as a result of protein leakage from blood and milk. Moreover, due to the opening of the paracellular pathways, levels of sodium and chloride increase, while at the same time the levels of potassium and lactose decrease [3]. Due to milk stasis, white granules may be observed in the milk which are formed from caseins hardened by salts, mainly consisting of calcium [3]. Fatty or fibrous-looking material, sometimes brown or green, is occasionally forced out from blocked ducts accompanied by alleviation of symptoms [3]. Furthermore, during mastitis, a change in the organoleptic properties can occur, in that the milk becomes salty. Generally, the saltiness is provisional and lasts approximately 1 week [70].

A recent study conducted by Say et al. [69] focused on the effects of mastitis on the macronutrient content of milk. A total of 30 lactating women were divided into two groups; one diagnosed with mastitis and one consisting of healthy women. Fat, carbohydrate and energy levels were significantly lower in the mastitic milk samples. The authors concluded that the observed differences may have emerged from reduced milk synthesis, compromised permeability of the blood–milk barrier and an increase in enzymatic or proteolytic activity associated with the inflammatory process

[71, 72]. We speculate that the reduced levels of fat, carbohydrates and energy could have a negative effect on the health of the infant with the prerequisite that the mother still breastfeeds.

Perez et al. [73] concentrated on the impact of mastitis on the biogenic amine (BAs) profile in human milk. BAs are defined as low molecular weight nitrogenous organic compounds with distinct biological activities. In the study, two groups of women participated; one ($n=40$) healthy group and the other ($n=30$) diagnosed with mastitis. Putrescine, spermidine and spermine were the predominant BAs identified in both cases, however, the concentrations of BAs in mastitic milk were higher compared to healthy milk. BAs have been shown to contribute to gut maturation and the increased levels seen in mastitic milk may potentially negatively affect the development of the infants' GI microbiota [74, 75].

Therapies

An overview of current therapies for mastitis treatment are presented in Table 2.

Antibiotics

Prior to 1948, the management of lactational mastitis consisted of binding, ice packs and discontinuance of nursing, in the hope that symptoms would not develop further. Penicillin was considered the most appropriate antibacterial available yet proved to be limited in treatment of the disease [81]. As stated already, *S. aureus* and *S. epidermidis* are found to be the main causative microorganisms of lactational mastitis with *Strep. mitis* and *Strep. salivarius* being the second and third most reported causative agents, respectively [27]. However, multidrug resistance to antibiotics and/or formation of biofilms is typical among clinical isolates of these two staphylococcal species. Accordingly, it is not surprising that 70–90% of the cases of staphylococcal mastitis in cattle are immune to antibiotherapy [82]. Resistance to methicillin and a high ability to form biofilms explains why mastitis tends to be a recurrent or chronic infection. Indeed, it has been reported that 25% of mothers who discontinue breast-feeding as a result of mastitis [3] have already received antibiotherapy (cloxacillin, clindamycin, amoxicillin-clavulanic acid and/or erythromycin) for 2–4 weeks with little success [11].

Penicillinase-resistant penicillins such as dicloxacillin or flucloxacillin are the most suitable antibiotic therapies for treatment of the disease (Table 2). First-generation cephalosporins are also approved as a first-line therapy. More specifically, cephalexin can be administered in cases of penicillin intolerance, while in cases of allergy to penicillin, clindamycin is preferred [4, 83]. Dicloxacillin has lesser

Table 2 Overview of mastitis treatment options

Treatment options	Name of treatment	When to use/target	Status of the treatment	Duration of therapy (days)
Antibiotics	Dicloxacillin [4, 8, 76]	Staphylococcal mastitis	Standard	7–14
	Flucloxacillin [4, 76]	Staphylococcal mastitis	Standard	
	Cephalexin [4, 8, 76]	Penicillin intolerance	Standard	
	Clindamycin [4, 8, 76]	Allergic to penicillin, recurrent mastitis	Standard	
Probiotics	<i>L. salivarius</i> CECT5713 and <i>L. gasseri</i> CECT5714 [11]	Staphylococcal mastitis	Experimental stage	30
	<i>L. fermentum</i> CECT5716; <i>L. salivarius</i> CECT5713 [77]	Infectious mastitis	Experimental stage	21
	<i>L. salivarius</i> PS2 [78]	Preventive measure against mastitis	Experimental stage	~44
	<i>L. salivarius</i> PS2 [79]	Infectious mastitis	Experimental stage	21
Bacteriocins	Nisin [80]	Staphylococcal mastitis	Experimental stage	14

hepatic inimical effects than flucloxacillin [76]. However, dicloxacillin can cause phlebitis when applied intravenously; so, it should be taken orally when symptoms persist [4]. A later study demonstrated that chloramphenicol, gentamicin, ofloxacin and ciprofloxacin were the most efficacious antibiotics against mastitis pathogens [43]. With regards to duration of antibiotic therapy, there is no consensus with recommendations varying between 7 and 14 days. As outlined by Reddy et al. [84], where symptoms endure, culturing of the milk and an assessment of the antibiotic resistance of the microbes should be performed due to the escalation of penicillin-resistant, methicillin-resistant (MRSA) and oxacillin-resistant *S. aureus* (ORSA); although it would be of greater benefit if milk cultures were performed as soon as possible to establish a proper diagnosis and to establish the most adequate treatment depending on the main bacterial agent(s) involved. This would greatly help to avoid misuse of antibiotics.

It is broadly acknowledged that antibiotics are the culprit for the disruption in human microbiota which can result in antibiotic-associated diarrhea, urogenital and oral infections [37]. Studies have demonstrated an increased vulnerability to infectious, allergic and inflammatory diseases because of the dysregulation of host immune homeostasis due to altered microbiota. The latter is attributed to the excessive use of antibiotics [85, 86]. The administration of antibiotics during pre- and post-delivery stages, has been proven to adversely affect breastfed infants as antibiotics can modify the maternal microbiota which is of critical importance to the human GI microbiota in early life [87]. Arvola et al. [88] demonstrated that infants with deferred colonization or low bifidobacterial counts may be at risk of further gastrointestinal or allergic conditions, while de Weerth et al. [89] found low *Bifidobacterium* and *Lactobacillus* counts in the stools

of infants diagnosed with colic. Moreover, Kummeling et al. [90] reported that antibiotic exposure via breastfeeding in infants was associated with a higher risk of recurrent wheeze during the first 2 years of life. Consequently, there is a need to develop strategies to replace antibiotics and in this context, probiotics may provide a feasible solution.

Probiotics

The WHO/FAO describes probiotics as “live microorganisms that when administered in adequate amounts confer a health benefit on the host” [91, 92]. It is now known that the infant gut is colonized by bacteria from human milk [28, 38, 47, 48, 93]. Consequently, modulation of maternal gut microbiota during pregnancy and lactation could have a direct impact on infant health [94]. Indeed, recent studies illustrate that the mammary gland accommodates its own microbiota during late pregnancy and lactation [94]. More specifically, strains isolated from breast milk can be good candidates for use as probiotics [11, 77] as they fulfill several criteria including human origin, adaptation to mucosal substrates and a history of safe extended consumption by sensitive individuals such as infants [78].

An initial study by Jimenez et al. [11] investigated the potential of two lactobacilli strains as an alternative therapy for staphylococcal mastitis, namely *L. salivarius* CECT5713 and *L. gasseri* CECT5714 [Table 2]. Of a total of 20 women presenting with the condition, ten received the two *Lactobacillus* strains (10 log₁₀ cfu of each) for a 4-week period and ten received the excipient only for the same period. On day 0, mean staphylococcal counts in both groups were similar, but by day 30, the mean staphylococcal count in the probiotic group was lower when compared to the control group (2.96 log₁₀ cfu/

mL versus $4.79 \log_{10}$ cfu/mL, respectively). By day 14, clinical signs were no longer observed in the probiotic group although mastitis persisted in the control group. A subsequent study by Arroyo et al. [77] also investigated the potential of *L. salivarius* CECT5713 and the strain *L. fermentum* CECT5716 in a much larger group of subjects, 352 women presenting with infectious mastitis. Women received either *L. salivarius* CECT5713 ($n = 127$, $9 \log_{10}$ cfu), or *L. fermentum* CECT5716 ($n = 124$, $9 \log_{10}$ cfu) or antibiotic therapy ($n = 101$) for 3 weeks. By day 21, milk bacterial counts were lower in women receiving probiotics compared to those on antibiotic therapy. The authors state that women in the probiotic groups ‘improved more’ and had lower recurrence of mastitis than those receiving antibiotic therapy. The outcomes of both these studies are further discussed by Fernandez et al. [10].

A more recent study examined the potential of *L. salivarius* PS2 to hinder infectious mastitis in pregnant women who were previously diagnosed with mastitis [78]—108 women were divided into two groups. Those in the probiotic group ingested $9 \log_{10}$ cfu/day of *L. salivarius* PS2 from approximately week 30 of pregnancy until delivery while those in the control group received a placebo. Following the end of the study, 41% of the participating women were diagnosed with mastitis; although the number of women from the probiotic group suffering from mastitis (25%) was significantly lower than the placebo group (57%). The bacterial counts of women with subacute mastitis from the placebo group were significantly higher when compared to the probiotic group (4.61 and $3.83 \log_{10}$ cfu/mL, respectively). Comparable results were noted in the group of women with acute mastitis where those who belonged in the placebo group had higher bacterial mean counts (4.51 – $5.53 \log_{10}$ cfu/mL) than those who belonged in the probiotic group (3.29 – $4.29 \log_{10}$ cfu/mL). This study demonstrates that *L. salivarius* PS2 could be an effective alternative to forestall infectious mastitis.

Espinosa-Martos et al. [79] endeavored to correlate microbiological, biochemical or immunological markers in milk, blood or urine with the beneficial outcome observed during probiotic treatment of mastitis. A total of 31 women took part in the study. Among them, 23 women had clinical symptoms of mastitis while the remaining ($n = 8$) were healthy. Over a period of 21 days, both groups received three daily doses (10^9 cfu) of *L. salivarius* PS2. In the mastitic group, the probiotic intake resulted in a notable reduction of mean bacterial ($1.1 \log_{10}$ cfu/mL) counts in milk. Moreover, clinical symptoms were alleviated in the probiotic group after day 7 while the somatic cell count significantly declined after the intake of probiotics. The effect of the probiotic strain on the total bacterial counts in human milk is in agreement with previous studies [11, 77].

Bacteriocins

Bacteriocins are antimicrobial peptides produced by bacteria and exhibit potent activity against other bacteria including antibiotic-resistant strains [95]. They are generally stable, have low toxicity and can exhibit a narrow or broad spectrum of activity. Certain bacteriocins such as nisin have already gained acceptance as antimicrobials in the food industry where they provide protection against pathogen and food spoilage microorganisms. The lantibiotics describe a group of bacteriocins which undergo post-translational modifications and have exhibited a high potency to hinder various multidrug-resistant bacteria combined with a low propensity to generate resistance. The food-grade lantibiotic, nisin, is produced by certain strains of *Lactococcus lactis*, a common species in the breast milk of healthy women [10, 49]. Indeed, approximately 30% of isolates of this origin can produce nisin [96]. During the past 2 decades, there has been a revived interest in the use of this lantibiotic as a therapeutic agent in bovine mastitis [10]. Furthermore, another bacteriocin, i.e., lactacin 3147 has generated promising results in the treatment of bovine mastitis [97, 98]. Like nisin, lactacin 3147 is a lantibiotic with a broad spectrum of activity. Indeed, the potential of nisin in treating infectious mastitis in lactating mothers has already been demonstrated (Table 2). In a 2-week study, nisin was investigated as an alternative treatment for staphylococcal mastitis in women who had already received antibiotics for 2–4 weeks but which failed to ameliorate their condition [80]. More specifically, eight women diagnosed with staphylococcal mastitis were divided in two groups. The first group received a solution of nisin which was applied to the nipple and mammary areola, while the second group received a solution lacking nisin. On day 0, the staphylococcal counts were similar in the bacteriocin and placebo groups (5.0 ± 0.2 and $4.9 \pm 0.2 \log_{10}$ cfu/mL, respectively), while on day 14, the average counts in the nisin group ($3.2 \pm 0.4 \log_{10}$ cfu/mL) were significantly lower compared with the placebo group ($5.0 \pm 0.2 \log_{10}$ cfu/mL). At the end of the study, no clinical signs were observed in the bacteriocin group.

Conclusion and future prospects

While the number of studies addressing the microbiological load of human lactational mastitis is limited, those which have been completed provide an insight into the microbiological dysbiosis associated with the disease and hence shed light on potential new therapies. One study which employed metagenomic analysis of mastitic milk samples clearly identified a loss of bacterial diversity [26]. Unsurprisingly, *Staphylococcus* is the dominant genus associated with the disease where *S. aureus* is the main

etiologically agent in acute mastitis and *S. epidermidis* is associated with subacute mastitis. In addition, *Corynebacterium* species are now recognized as the causative agents of human granulomatous mastitis.

While the treatment of mastitis with antibiotics has, in the past, been met with mixed results, the looming threat of antibiotic resistance has significantly increased the need to identify alternative therapies. In addition, antibiotics are known to impart deleterious effects on the microbiota of human milk. In this regard, probiotics offer a viable alternative and the limited number of studies available, would seem to indicate that they can be effective against human mastitis both as prophylactics and therapeutics and in the latter case have generated similar or superior results to antibiotics in certain instances. Some of the probiotic strains tested to date have in fact been isolated from breast milk and it is now accepted that the mammary gland has its own microbiota in late pregnancy and during lactation. In this regard, it may be conceivable in the near future to generate ‘personalized’ probiotics for those at particular risk of mastitis, such as women with a known history of the disease.

The preliminary success of the bacteriocin nisin in the management of human mastitis, as well as the promising results with lactacin 3147 for the treatment of bovine mastitis may pave the way for other bacteriocins or bacteriocin-producing live strains in the treatment of human mastitis in the future. For such alternative therapies to be accepted as preventative agents or for treatment of this condition, randomized controlled trials in healthy and diseased lactating women are required to confirm efficacy, and furthermore to demonstrate that no negative effects of treatment are imparted on the quality of milk for nutrition of the nursing infant.

Undoubtedly, continued investigation into the microbiology of human mastitis is essential and will help in the identification and development of successful therapies. In this regard, there is an onus on researchers, health agencies and biotechnology companies to work in unison towards reducing the incidence of this devitalizing disease.

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Compliance with ethical standards

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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