

Investigation of Breastfeeding Related Microbial Diversity in Mastitis Women and Their Infants in Anbar Governorate, Iraq

Yasmeen Kateb Ahmed¹, Abbas Obaid Al-Janabi, Amer Fakhri Mizban¹

Department of Microbiology, ¹Department of Surgery, College of Medicine, University of Anbar, Ramadi, Iraq

Abstract

Background: Mastitis is an infectious disease that is, common during lactation. The primary etiological agent of mastitis is bacterial infection. However, the diversity of bacteria community in infant oral cavity, women breast, and nipple remains uncharacterized till now. **Objectives:** This study aimed to characterize the isolated bacteria and fungi in samples from lactation mastitis-affected women's breast, milk, nipple, and infant oral cavities. **Materials and Methods:** In this study, 100 women with mastitis symptoms were included 800 swabs were collected from mastitis women and their infants. Two swabs were collected from different sites of infection breast, milk, nipple, and infant oral cavity under sterile conditions. **Results:** The result showed that *Staphylococcus aureus* was the most dominant isolated bacterial species in different sample 138 (66%), followed by *Klebsiella pneumonia* 61 (29%) and *Lactobacillus acidophilus* 10 (5%). **Conclusion:** *Candida albicans* was the most frequently isolated fungal species in different sample 262 (91%), followed by *Candida tropicalis* 27 (9%). These results demonstrated that the Infant oral cavity *S. aureus* may be the main cause of mastitis infectious in lactating women in Iraq.

Keywords: Bacterial infection, *Candida*, fungal infection, mastitis, *Staphylococcus aureus*

INTRODUCTION

Breastfeeding is considered the optimum feeding regime for newborns. Human breast milk can provide nutrition for infants and play a significant role in improving infant health and immune system development.^[1] Breast milk has long been considered sterile for a long time, but recent research has shown that it is a rich source of a complex community of commensal microbes that have the potential to enhance children's health by stimulating the development of the new-born's host defence and gut microbes colonization.^[2]

The types of diverse microbiota within breast milk can vary depending on various factors, including maternal health, mode of delivery, and breastfeeding practices.^[3] Some common bacteria found in breast milk include *Staphylococcus*, *Streptococcus*, *Lactobacillus*, and *Bifidobacterium* species. In addition, it is contain fungi, eukaryotes and viruses.^[4]

Mastitis is an inflammatory breast disease that affects women with a history of breastfeeding due to milk stasis

or bacterial infection in the milk ducts and lowered post-delivery maternal immunity. Pain, swelling, redness, heat, and might fever and chills are various symptoms accompanied by Mastitis.^[5]

Mastitis can be considered be a relevant public health issue as it affects up to 33% of lactating women during the first 4 weeks after parturition, it could alter the breast milk composition and milk microbiome, so that lactational mastitis is unfit for human consumption, being one of the main causes for early weaning.^[6,7]

Streptococcus and *Staphylococcus* were both identified as normal flora in human milk and skin, but they are also one of the main mastitis etiological agents as they can

Address for correspondence: Yasmeen Kateb Ahmed,
Department of Surgery, College of Medicine,
University of Anbar, Ramadi, 31001, Iraq.
E-mail: yas20m0018@uoanbar.edu.iq

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occasionally overgrow and form thick biofilms in the milk ducts leading to milk stasis and opportunistic infections, which trigger inflammation and result in mastitis symptoms.^[8]

Bacterial infections found in human milk are commonly transferred from maternal skin into the milk duct via a crack in the nipple.^[9] Thus, skin, breast, and cracked nipples may be a source of such infection in human milk. However, the transfer of skin bacterial strains to breast or milk have not been fully studied yet.^[10] Another transmission route of infection into the mammary duct is the infant's oral cavity during breastfeeding that play a role in change microbial communities of breast milk.^[2] Other study suggested the infant oral cavity as a potential source of human milk fungi infection dominated by *Candida* species.^[11]

Previous studies have suggested that the microbial diversity of breast milk during mastitis can vary depending on various factors such as the duration and severity of the mastitis, the type of mastitis-causing bacteria, geographical location, administration of antibiotics, mode of delivery, and the mother's immune response.^[12]

MATERIALS AND METHODS

Subjects and design

A cross-sectional descriptive one-group study included 100 selected mastitis patient's women and their infants who were admitted to Al-Ramadi Teaching Hospital in Anbar Governorate from November 2022 to March 2023 to investigate the diversity of bacterial and fungal communities in breasts, nipples, and milk in women with mastitis and their infant oral cavity. Breastfeeding women with chronic disease, chronic inflammation, breast disease, and who were administered antibiotics during lactation were excluded.

Sample collection

For detection and isolation of bacteria and fungi, 800 swab were collected from 100 mastitis women and their infants, two swab samples were taken from milk, breast, and nipple of each mastitis mother and two swab from their infant oral cavity, one swab for bacterial identification and the other one for fungal identification. 200 breast, 200 milk, 200 nipples samples, and 200 infant oral cavity samples were collected. Mothers washed their hands, breast, and nipples with sterile water to minimize breast milk contamination. Breast milks were collected from mothers by electric breast pump (Medela AG, Baar, Switzerland). The first drops were discarded, and 5 mL of milk was collected directly into a sterile tube. Nipple/breast skin samples were collected from the site of lesion or redness located on the nipple and the breast using a sterile cotton swab (Thermo Fisher Scientific, Lexena, KS) in a circular motion. Oral samples are collected from infants from the dorsum of the

tongue by sterile swab, all samples were transferred to the laboratory within 2 h and stored at -80°C until further culture.^[13]

Bacterial isolation

All samples were cultured on the primary culture medium (blood agar) incubated at 37°C for 24 h. After obtaining bacterial colonies, yellow colonies were transferred to another culture media (Mannitol Salt Agar) and incubated at 37°C for 24 h. Mannitol Salt agar was used to isolate *Staphylococci* spp. according to Hoblet guidelines.^[14] While pink mucous colonies on blood agar were transferred to MacConkey agar and incubated at 37°C for 24 h, for *Klebsiella pneumoniae* confirmation. *Staphylococcus aureus* showed positive reactions for the catalase test, while *Streptococcus* showed negative reactions.

Fungal isolation

All swab samples were inoculated on Sabouraud dextrose broth (SDB) supplemented with 20 $\mu\text{g}/\text{mL}$ of chloramphenicol and Cycloheximide at 25°C – 30°C for 4 weeks before being assessed for isolation of pathogenic fungi. Positive cultures were inspected by microscopic identification, *Candida* spp. emerging spherical to subspherical yeast-like cells with budding hyphae or pseudohyphae under a microscope using simple gram stain. The tested species were then inoculated onto the chromogenic medium CHROMagar *Candida* Plus media (Kanto Chemical Co., Chrome Agar, Vavantor, Boland) at 35°C for 72 h.^[15]

Statistical analysis

Statistical Analysis was performed using SPSS computer program version 22 (SPSS Inc., Chicago, IL). Categorical variables are expressed as frequencies and percentages. Chi-square test (χ^2) and Fisher's exact test were used for comparison of categorical data. *P* value < 0.05 was statistically significant.^[16]

Ethical considerations

The protocol was approved by the Regional Ethics Committee for AL-Ramadi Teaching Hospital in the Governorate of Anbar in September 2022 (reference number: 2022–000103). Written informed consent of all the mothers was obtained after being informed of aim of the study.

RESULTS

From all samples, the bacterial culture of breast milk samples showed low bacterial growth, 90% of samples showed no growth. While only 10% of samples showed *Lactobacillus acidophilus*. In breast samples, 30% of bacterial culture showed *S. aureus*, 20% showed *S. aureus* with *K. pneumoniae* together, and 50% showed no growth. In nipple samples, 30% of bacterial culture showed

Table 1: Bacteria isolate from different site of infection

Bacteria species	Breast milk (%)	Breast (%)	Nipple (%)	Oral cavity (%)
<i>Staphylococcus aureus</i>	0	30	30	17
<i>Klebsiella pneumonia</i>	0	0	0	0
<i>Staphylococcus aureus</i> + <i>Klebsiella pneumonia</i>	0	20	21	20
<i>Lactobacillus acidophilus</i>	10	0	0	0
No growth	90	50	49	63

Table 2: Distribution of the main isolated bacteria

Bacteria species	Number of isolates	Percentage (%)
<i>Staphylococcus aureus</i>	138	66%
<i>Klebsiella pneumonia</i>	61	29%
<i>Lactobacillus acidophilus</i>	10	5%
Total	209	100%

Table 3: Fungi isolate from different site of infection

Fungi species	Breast milk (%)	Breast (%)	Nipple (%)	Oral cavity (%)
<i>Candida albicans</i>	0	74	90	89
<i>Candida tropicalis</i>	0	6	10	11
No growth	100	20	0	0

Table 4: Distribution of the main isolated fungi

Fungi species	Number of isolates	Percent (%)
<i>Candida albicans</i>	262	91%
<i>Candida Tropicalis</i>	27	9%
Total	289	100%

S. aureus, 21% showed *S. aureus* with *K. pneumonia* together, and 49% showed no growth. In infant oral cavity samples, 17% of bacterial culture showed *S. aureus*, while 20% showed *S. aureus* with *K. pneumonia* together and 63% showed no growth as shown in Table 1. Among 209 bacterial isolates, *S. aureus* was the most dominant isolated bacterial species in different sample 138 (66%), followed by *K. pneumonia* 61 (29%) and *L. acidophilus* 10 (5%) [Table 2].

According to the fungal culture of breast milk samples, our results showed that no fungal species were found. In breast samples, *C. albicans* was isolated from 74% of the samples and *C. tropicalis* was isolated from 6% of the samples, whereas 20% of the samples showed no growth. In nipple samples, all samples showed fungal growth, *C. albicans* was isolated from 90% of the samples, whereas *C. tropicalis* isolated from 10% samples. Also, in infant oral cavity all samples showed fungal growth, *C. albicans* was isolated from 89% of the samples, whereas *C. tropicalis* isolated from 11% samples [Table 3]. Among 289 fungi isolates, *C. albicans* was the most frequently isolated fungal species in different sample 262 (91%), followed by *C. tropicalis* 27 (9%) [Table 4].

Our results showed that there is no relation between types of isolated bacteria and infection sites $P < 0.05$. There is no difference in the microbiota components in different infection sites [Table 5]. However, our data showed a significant association between types of isolated fungi and infection sites $P = 0.0476$, there is a significant difference in the growth of fungi between the four infection sites. *C. albicans* were the most dominant fungi compared to other *candida* spp [Table 6].

DISCUSSION

Breastfeeding is the perfect natural food that supports the growing and development of infants.^[17] Breast milk is also an important source of biologically beneficial probiotic microbiota that enhances the initiation and development of newborn immune system.^[18] Researchers have investigated that the composition of the microbiome of the breast milk is influenced by multiple factors including maternal infection during lactation.^[6]

Mastitis is one of the most common problems that compromise breastfeeding during the early postpartum weeks. Mastitis is an inflammation that affects the lobes of the mammary gland, commonly resulting from an infection, and often leads to early weaning. The origin of mastitis is milk stasis associated with bacterial growth of some species in the milk.^[19]

In our study, *L. acidophilus* accounted for most of the microbiota in breast milk of women suffering from mastitis which is the same as the findings from Jiménez et al.^[20] who isolated *Lactobacillus* species from 6 milk samples of 10 mastitis women. On the other hand, many studies revealed the *Staphylococcus* mainly *S. epidermidis* and *S. aureus*, seem to be the dominant species present in breast milk of women with mastitis.^[21,22]

Lactobacillus species belonging to the commensal species that is, commonly found in the human body, including the oral and intestinal cavity, breast milk and has been suggested to have a beneficial effect on the host's health. Some studies have suggested that *Lactobacillus* species isolated from breast milk may play a protective role against mastitis caused by *S. aureus*.^[20,23]

The low prevalence of *Lactobacillus* species in the milk samples obtained from the women with mastitis in our study suggests that its presence may not be related to the

Table 5: Comparison between the bacterial growth in the different site of infection

Infection sites	Bacteria species					P value
	<i>Staphylococcus aureus</i> N = 77	<i>Klebsiella pneumoniae</i> N = 0	<i>Staphylococcus aureus</i> + <i>Klebsiella pneumoniae</i> N = 62	<i>Lactobacillus acidophilus</i> N = 10	No growth N = 252	
Breast milk	0	0	0	10	90	0.945
Breast Nipple	30	0	20	0	50	
Oral cavity	30	0	21	0	49	
	17	0	21	0	63	

P: P value of Chi-square test for comparing bacterial growth in various infection sites

Table 6: Comparison between the fungal growth in the different site of infection

Infection sites	Fungal species			P value
	<i>Candida albicans</i> (N = 253)	<i>Candida tropicalis</i> (N = 27)	No growth (N = 120)	
Breast milk	0	0	100	0.0476*
Breast Nipple	74	6	20	
Oral cavity	90	10	0	
	89	11	0	

P: P value of Fisher exact test for comparing fungal growth in various infection sites

development progression of mastitis in all cases. Jiménez *et al.*^[20] also observed that *Lactobacillus* composition in breast milk is usually includes a low number of *lactobacillus* species, while the non-growth of 90% of breast milk samples may support the hypothesis of breast milk is sterile and germ-free.

In consistent with our results Williams *et al.*^[24] studied the microbiomes composition from maternal milk and infant oral cavity samples in the first 6 month postpartum, and observed a high relative abundances of *Staphylococcus* in oral cavity samples from a infants mouth. In the same line, Costello *et al.*^[25] evaluated the composition bacterial communities in multiple body sites of neonate including saliva and oral cavity associated and detected that the most abundant phylum were the Firmicutes (71.6%) followed by Proteobacteria (21.4%), and the most abundant bacteria were as follows: *Staphylococcus*, *Streptococcus*, and *Klebsiella*.

Staphylococcus in the infant's mouth is probably linked to the higher levels of *Staphylococcus* in the mother's skin may be due to contact during breastfeeding. Therefore, it is not surprising to detect comparable bacteria in both the mother's breast and nipple, as well as the infant's oral samples.^[26]

In the past, it was believed that milk bacteria were due to contamination from either the mother's skin or the infant's oral cavity. However, our findings indicate that the development of mastitis may be more complex than the presence of *S. aureus* in human milk and further studies are needed to determine the role and pathogenesis of such infection across the mastitis, this hypothesis was supported by McLoughlin *et al.*^[27]

During lactation, the nipple and surrounding breast region enter in the infant's mouth facilitating the transmission of maternal skin-associated bacteria to the infant's oral cavity.^[28] Based on the physiology of infant suckling, bacteria from the child's mouth can enter the mammary gland from the infant's oral cavity via the nipple. This mechanism may illustrate the prevalence of these organisms in the infant oral cavity and nipple as well as surrounding breast region. *Staphylococcus* and *Streptococcus* infection may be due to mothers poor hygiene practices before breastfeeding.^[29]

The growth inhibition of bacteria in nearly 50% of the samples is also noteworthy. It could be due to administration of antibiotic during pregnancy or lactation.

Lactating mother's breast and Infants' oral cavities can be colonized by several different species of *Candida* as part of skin normal flora, but overgrowth may cause it to become symptomatic resulting in breast candidiasis. Previous studies have suggested fungal infection, mainly *C. albicans* as an etiological agent of mastitis. Also, some maternal health issue have been mentioned as a causes of mastitis such as blocked milk ducts, damaged nipples, and or breast trauma.^[30]

According to our results, breast, nipple, and oral cavity samples showed higher prevalence of fungal growth, mainly *C. albicans* the most dominant compared to *C. tropicalis*.

Many studies have revealed the prevalence of *C. albicans* in nipple, breast, and infant oral cavity of breastfeeding women accompanied with nipple pain, breast mycosis and inflammation,^[30-32] these evidence support the etiological

role of *C. albicans* in the pathogenesis of mastitis for women during lactation.

Limited studies have concerned the relation between mastitis and nipple, breast, and infant oral cavity fungal infection mainly the role of *Candida albicans*. However, Zöllne *et al.*^[33] showed that extensive colonization by *Candida* spp. both on lactating mother's breasts and in infant's oral cavities that result in plugged duct mastitis.

The growth inhibition of fungal species in the milk samples while *Candida* spp. were detected in swabs from nipples, breast, and the oral cavity of the child is an interesting finding. It may suggest that the presence of *Candida* in the breast or oral cavity of the child does not always result in colonization or infection of the milk, additionally, a study showed that immune system of the mother may play a critical role in preventing the overgrowth of potentially pathogenic microorganisms in the milk, *Lactobacilli* in mother milk prevent the proliferation of pathogenic microbe by competing with pathogens for nutrients; by stimulating the release of antimicrobial substances.^[34]

Our results showed a significant association between the presence of fungal infection in the nipples, breast, child mouth, and milk samples $P = 0.0476$, with *C. albicans* being the most found fungus.

In agreement with our findings, Plachouri *et al.*^[35] and Douglas^[36] investigated the prevalence of *Candida* species in nipple swabs, breast milk, and oral cavity of children, supporting the hypothesis that there is a relation between the presence of *C. albicans* in the maternal breast, nipple, and child mouth samples, causing the signs of symptoms popularly diagnosed as mammary candidiasis. which support our findings.

Our results showed that there is no difference between bacterial infection distribution in different site of infection $P < 0.05$. These results suggest that the presence of bacteria in the nipple breast, milk, and oral cavity samples may be related to each other, that could be due to a variety of factors, such as poor hygiene, breastfeeding practices. These facts lead to the hypothesis, that bacterial and fungal infections may be transmitted from the infant's mouth to the mother's nipple during breastfeeding, leading to mastitis.^[36,37]

CONCLUSION

Staphylococcus aureus and *C. albicans* are the most prevalent isolated species from women suffering mastitis in Iraq. *S. aureus* and *C. albicans* could be considered as the main etiological agent in Iraqi mastitis. Infant oral cavity infection would be the main cause of maternal mastitis infection.

Institutional Review Board statement

Not applicable.

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Conflicts of interest

There are no conflicts of interest.

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