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A Case Control Study of Bacterial Species and Colony Count in Milk of Breastfeeding Women with Chronic Pain

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Abstract

Background: An infectious etiology for chronic breast pain in breastfeeding women continues to be debated. Although recent data suggest that *Staphylococcus aureus* and coagulase-negative *Staphylococcus* (CNS) may cause chronic breast pain, no studies have used quantitative cultures to address this question. In this study we compared bacterial species and colony counts between breastfeeding women with (cases) and without (controls) chronic pain.
Subjects and Methods: We enrolled 114 breastfeeding women in a prospective cohort study. Cases ($n=61$), breastfeeding women with breast pain for >1 week and no signs of acute infection, were matched with controls ($n=53$) by weeks postpartum and parity.
Results: More cases had a history of mastitis (14% vs. 2%, $p=0.036$), cracked nipples (64% vs. 17%, $p=0.001$), and other breastfeeding difficulties. *Enterobacter* species growth was less likely in cases (0% vs. 7.5%, $p=0.029$). Cases had a significantly higher growth of *S. aureus* (19.7% vs. 1.9%, $p=0.003$). CNS frequency was similar between groups (75% vs. 79%, $p=0.626$), but median colony count growth was significantly lower in cases (900 colony-forming units/mL vs. 5,000 colony-forming units/ml, $p=0.003$). Growth of CNS and *S. aureus* was negatively correlated ($r=-0.265$, $p=0.004$).
Conclusions: Higher *S. aureus* growth in cases supports a pathogenic role for *S. aureus* and reinforces the need for future antibiotic treatment studies in breastfeeding women with chronic pain. In contrast, similar CNS frequency between groups, lower CNS colony counts in cases, and a negative correlation between *S. aureus* and CNS growth suggest that neither CNS, nor its overgrowth, causes chronic breast pain.

Introduction

Breastfeeding is recognized as the ideal form of nutrition for infants.[1–3] Yet, many women do not achieve their breastfeeding goals. Schwartz et al.[4] found that among women who wean in the first 3 weeks, 32.9% wean because of pain. Although it is generally accepted that acute mastitis may be caused by infections, an infectious etiology for chronic pain remains controversial.[5–9] Historically, many women received treatment with antifungal agents for presumed yeast infections.[7] In contrast, more recent studies have focused on a potential bacterial etiology.[8,10]

Previous studies identified *Staphylococcus aureus* as a pathogenic bacterium for breastfeeding women with acute cracked nipples[11–13] and mastitis.[14,15] In addition, some studies found an increased incidence of *S. aureus* in the breastmilk of women with chronic breast or nipple pain.[6,16] Although these data support *S. aureus* as a possible pathogen, the studies do not quantify the bacterial growth and often do not report on the growth of other bacterial species.[17] Quantified bacterial cultures may illuminate whether *S. aureus* and other bacterial species cause chronic breast pain.[17]

Although coagulase-negative *Staphylococcus* (CNS) is a dominant organism in normal skin flora,[18–21] recent studies in mastitis[22,23] and chronic pain[8,10] suggest a possible pathogenic role for CNS.[22,23] Delgado et al.[23] proposed that disruption in the normal bacterial flora balance in breastmilk may lead to CNS overgrowth and mastitis. In contrast, Kvist et al.[18] found no difference in CNS growth between cases and controls. In a case report and a retrospective chart review, Eglash et al.[8] and Eglash and Proctor[10] postulated that CNS may be a cause of chronic breast pain and that antibiotics may be beneficial for a chronic lactiferous infection. However, none of the chronic pain studies used quantitative cultures to assess whether CNS is pathogenic.

Previous studies describing microbiologic data of women with non-mastitis pain[5,6,9,16] focused on yeast and *S. aureus* growth, often omitting details on other bacterial species and

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not assessing relative bacterial growth.[17] There have been no prospective studies quantifying bacterial colony count growth in breastfeeding women with chronic breast pain.[17] In this study, we examined bacterial species, measured colony count, and examined relative bacterial growth in breastfeeding women with chronic non-inflammatory breast pain compared with asymptomatic women. Quantifying these data will clarify if specific bacteria or bacterial imbalance is associated with chronic pain while also providing information for future antimicrobial treatment studies. Preliminary results have been published previously in abstract form.[24]

Subjects and Methods

Study population

The study was conducted at Breastfeeding Medicine of Northeast Ohio (BFMEDNEO), a referral practice for mothers in Northeast Ohio needing specialized medical evaluation for breastfeeding difficulties. This practice is located at a private suburban pediatric practice in Cleveland, OH, viewed by the community as breastfeeding friendly with a typical patient volume of approximately 350 newborns per year. During the 12-month study period, 320 mothers were evaluated for breastfeeding consults. The two most common reasons were low milk supply (32%) and breast pain (46%). Of the 148 mothers presenting with a chief complaint of breast pain, 87 were excluded because pain was less than 1 week in duration or resolved in the office following mechanical correction.

Study design

This prospective, descriptive case control study was conducted from April 2011 to April 2012. Inclusion criteria for cases were breastfeeding women 18 years old or older presenting to BFMEDNEO with breast pain lasting more than 1 week and no evidence of acute inflammation such as erythema, warmth, or induration. Women were excluded if there was clinical evidence of acute inflammation (mastitis or abscess) or plugged ducts or if their symptoms resolved in the office following correction of mechanical factors such as incorrect latch or frenotomy for infant ankyloglossia. All cases were recruited by the primary author. Controls were recruited from BFMEDNEO and the pediatric practice. Controls were matched to cases by parity and weeks postpartum. All participants gave written informed consent. The research study was approved by the Case Western Reserve University Institutional Review Board.

At the initial visit, all women completed a patient questionnaire for demographic information and clinical history. At enrollment, history, exam, and treatment data were collected. Breastmilk cultures were obtained for cases and controls. Nipple cultures were performed for cases only. Follow-up e-mail questionnaires were administered to assess pain levels, feeding patterns, and breastfeeding complications. E-mails were sent at 2 and 12 weeks for controls and 2, 4, 6, 9, and 12 weeks for cases to allow closer monitoring of pain resolution. All data were collected and managed using the REDCap electronic data capture tools[25] hosted at Case Western Reserve University.

Microbiological samples were obtained from the nipple and breast of the more painful side. If there was no difference in pain, the sample was obtained from the right breast. The culture of the nipple was performed using the tip of a culture swab moistened in AMIES culture tube transport medium. The swab was rotated over the nipple/areola in a zigzag pattern as per UNC culture protocol.[26] If there was an open wound, the swab was obtained from the open wound. The culture of breastmilk was obtained following cleaning of the nipple/areola with an alcohol swab. Milk (10–20 mL) was then hand-expressed into a sterile container.

The specimens were labeled and transported at room temperature to a local office of Quest Diagnostics near Pittsburgh, PA, for processing. In the microbiology lab, 1-mL and 10-mL specimens of breastmilk were inoculated on trypticsoy agar with sheep blood to identify Gram-positive and Gram-negative bacteria. Again, 1-mL and 10-mL specimens were inoculated on MacConkey's agar for selective identification of Gram-negative bacteria. Cultures were grown at 35–37°C for 48 hours as per laboratory protocol. Species were identified using automated biochemical identification with the Siemens (Munich, Germany) MicroScan® WalkAway® 96 instrument. Panels, including biochemical identification and antibiotics in dilutions, produced by Siemens were used. Colony counts were calculated using a 1-mL loop method.

Breastmilk culture reports had bacterial species identification and quantitative colony count measurements of growth (colony-forming units [CFU]/mL).

Measures

The independent variable in this study was current chronic pain (cases) versus no pain (controls). The dependent variables were bacterial growth and bacterial density. Bacterial growth was evaluated as a dichotomous variable (present or absent) created for each type of bacterial species, including *S. aureus*, CNS, *Acinetobacter*, α-hemolytic *Streptococcus*, diphtheroids, *Enterobacter*, *Enterococcus*, *Escherichia coli*, *Klebsiella*, and *Pseudomonas*. The lab reported antibiotic sensitivities for all bacteria except diphtheroids and α-hemolytic *Streptococcus*. Bacterial density was evaluated as a continuous variable reported as CFU/mL for each bacterial species.

Descriptive variables and potential covariates were collected from the study participants. Demographic characteristics were recorded at the initial visit and included maternal age (years), infant age (weeks), insurance company (coded as public or private), highest level of education completed (coded as less than college, college graduate, and graduate degree), race, gravida, parity, mode of delivery, maternal work status, antibiotics during delivery, and breastfeeding intent (<1 month, 1–3 months, 3–6 months, 6–9 months, >12 months).

History of breastfeeding problems, including history of mastitis, cracked nipples, thrush, latching difficulties, low milk supply, oversupply, and plugged ducts, were recorded both for the current child and for prior children as dichotomous variables for each problem. History of past and current treatment with antibiotics, fluconazole, and topical ointment was also collected.

Outcome data for weaning and breastfeeding complications were recorded. Weaning was defined as no infant feedings with breastmilk in the last 24 hours. Women were asked, "What percent of infant feedings in the last 24 hours was breastmilk?" Response categories included none, less than 25%, 25–75%, more than 75%, and all. If women

indicated weaning during the course of the study, they were asked reasons, including low milk supply, pain, planned on weaning, and separated from infant. Data on breastfeeding complications of plugged ducts, abscess, or mastitis were collected and coded as a single dichotomous variable.

Analyses

Of the 148 mothers presenting with the main complaint of breast pain, 87 were excluded because pain had been present for less than 1 week or resolved in the office following mechanical correction. Sixty-one cases and 57 controls were enrolled; four controls were excluded because of mishandling of the cultures in transport and lab set-up. Once collected, the data were exported from REDCap to SPSS software (SPSS, Inc., Chicago, IL) and analyzed under the supervision of the project investigator. Descriptive statistical analyses were performed to examine the distribution and normality of data. The main analyses tested the associations of case/control status and bacterial colony count, species frequency, and breastfeeding complications.

Univariate analyses testing the association of case/control status and categorical variables were examined using Pearson's χ^2 test; Fisher's exact test was used when the expected count was less than 5. In the presence of non-normally distributed data, the relationship between chronic pain and bacterial density was examined using the Mann–Whitney U test and independent-samples median test. Pearson's correlations were used to assess relationships between continuous variables (i.e., testing the association of CNS and *S. aureus* growth).

Results

Cases and controls had similar baseline characteristics with regard to maternal age, infant age, parity, type of delivery, antibiotic treatment during delivery, breastfeeding intent, returning to work, race, education, or insurance (Table 1).

Bacterial frequency

Our analysis primarily explored whether the presence or absence of bacterial growth in milk was associated with breast pain. Breastmilk bacterial cultures showed significantly higher frequency of *S. aureus* growth in breast pain cases compared with controls (19.7% vs. 1.9%, $p=0.003$) (Table 2). Conversely, controls showed higher growth of *Enterobacter* (0% vs. 7.5%, $p=0.029$). The frequency of CNS did not differ between case and control groups (75% vs. 79%, $p=0.626$). Growth of other bacterial species was similar between the two groups (Table 2). Examining the association of CNS and *S. aureus* growth in breastmilk revealed a negative correlation ($r=-0.265$, $p=0.004$) such that breastmilk growing *S. aureus* was less likely to have CNS growth. This association is more clearly seen when categorizing CNS with positive growth (i.e., CFU >0). Breastmilk with CNS growth >0 CFU was less likely to be growing *S. aureus* than breastmilk with no CNS growth (CFU $=0$) (6.8% vs. 26.9%, $p=0.005$).

Table 1. Demographic Characteristics of Controls and Cases

Sample characteristic	*Controls* (n=*53*)	*Cases* (n=*61*)	p *value*[a]
Age			
Mother (median years)	31.4 (18–42)	32 (24–41)	0.88
Infant (median weeks)	5.2 (0.43–85)	5.7 (1–85)	0.85
Private insurance	40 (78%)	53 (88%)	0.16
Education: college grad and higher	42 (81%)	53 (87%)	0.62
Caucasian	39 (77%)	51 (84%)	0.34
Working mother	38 (72%)	38 (62%)	0.29
Multiparous	25 (47%)	24 (39%)	0.40
Vaginal delivery	34 (64%)	37 (61%)	0.40
Antibiotics during delivery	12 (23%)	18 (30%)	0.55
Breastfeeding goal >12 months	33 (62%)	40 (67%)	0.88
Treatment history since delivery			
Antibiotics	10 (19%)	21 (34%)	0.06
Fluconazole	0 (0%)	17 (15%)	0.001
Topical antibiotics	1 (2%)	8 (7%)	0.027
History of breastfeeding problem current child			
None	23 (43%)	3 (5%)	0.001
Mastitis	1 (2%)	11 (18%)	0.005
Cracked nipples	12 (23%)	44 (76%)	0.001
Thrush	0 (0%)	10 (16%)	0.002
Latching difficulties	13 (24%)	36 (59%)	0.001
Low milk supply	16 (30%)	9 (15%)	0.047
Oversupply	1 (2%)	11 (18%)	0.005
Plugged ducts	0 (0%)	17 (28%)	0.001
Currently on			
Antibiotics	1 (0.9%)	1 (0.9%)	0.90
Fluconazole	0 (0%)	5 (8%)	0.03
Topical antibiotics	0 (0%)	13 (11%)	0.001

[a]Pearson's χ^2 test was used for dichotomous variables; the Mann–Whitney U test was used for median test.

Table 2. Breastmilk and Nipple Culture Bacterial Species Growth

Bacterial species	*Nipple culture cases*	*Breastmilk*		*Breastmilk* p *value*
		Cases (n = *61)*	*Controls* (n = *53)*	
Coagulase-negative *Staphylococcus*	50 (87%)	46(75%)	42 (79%)	0.626
S. aureus	12 (21%)	12 (19.7%)	1 (1.9%)	0.003[a]
Enterococcus sp.	1 (2%)	2 (3.3%)	1 (1.9%)	0.643
Enterobacter sp.	0	0	4 (7.5%)	0.029[a]
Diphtheroids	2 (3.5%)	5 (8.6%)	3 (5.7%)	0.547
Acinetobacter sp.	7 (12%)	3 (4.9%)	1 (2%)	0.38
Pseudomonas sp.	3 (5%)	2 (3.3%)	1 (1.9%)	0.655
α-Hemolytic *Streptococcus*	8 (14%)	13 (21%)	14 (26%)	0.523
E. coli	1 (2%)	1 (1.6%)	2 (3.8%)	0.478
Klebsiella sp.	1 (2%)	0	1 (2%)	0.276

Data are *n* (%).
[a]Significant difference.

Nipple and breastmilk bacterial species frequency was similar for cases (Table 2) and revealed a strong correlation. Those cases growing *S. aureus* on breastmilk culture were likely to grow it on nipple cultures ($r = 0.667$, $p = 0.001$). Similar correlations were noted for CNS ($r = 0.532$, $p = 0.001$).

Quantitative bacterial growth

We next examined whether quantitative bacterial growth was associated with breast pain. Breastmilk colony counts revealed *Acinetobacter*, diphtheroids, *Enterobacter*, *Enterococcus*, *E. coli*, *Klebsiella*, and *Pseudomonas* all had no growth in >90% of the samples. Subsequent analyses used the median and nonparametric tests. Analysis of median colony count for all cases and controls resulted in 0 CFU given the large number of cultures with undetectable growth, except for CNS, where the median CFU was significantly lower for cases than controls (900 vs. 5,000 CFU, $p = 0.003$) (Table 3). To control for topical antibiotic use, we examined those who did not use any topical antibiotic since delivery and those who were on topical antibiotic ointments at the initial visit. We found median CNS colony count growth continued to be significantly different at $p = 0.003$. The trend of higher CNS colony count in controls remained with a subanalysis of only those cultures with positive CNS growth ($n = 88$) (2,000 vs. 6,000 CFU, $p = 0.019$). For the whole sample, *S. aureus* distribution was significantly different between cases and controls (Table 3). However, subanalysis of cultures positive for *S. aureus* growth ($n = 13$) showed a median CFU of 17,000 for both cases and controls, which was not statistically different.

To further understand the significantly different *S. aureus* distribution in the context of a positively skewed distribution resulting in 0 median CFU, the 50th, 75th, 90th, and 95th percentile values were calculated. Table 4 reports the percentile values for both CNS and *S. aureus* given both bacteria had significantly different CFU growth and distribution between case and control cultures. For *S. aureus* growth, even though the median is 0 CFU for both groups, the percentile values illustrate where the differences occur and reveal a significant bacterial growth difference occurring at the 90th and 95th percentile.

History of breastfeeding problems, treatment, and 12-week outcomes

Cases were more likely to have a history of breastfeeding problems, including mastitis (18% vs. 2%, $p = 0.005$), cracked nipples (76% vs. 23%, $p = 0.001$), latching difficulties (59% vs.

Table 3. Median Bacterial Colony Count Growth in Breastmilk

Bacterial colony count	*CFU/mL [median (range)]*		p *value*[a]
	Controls	*Cases*	
Coagulase-negative *Staphylococcus*	5,000 (0–100,000)	900 (0–100,000)	0.003[b]
S. aureus	0 (0–17,000)	0 (0–100,000)	0.007[b]
Enterococcus	0 (0–50,000)	0 (0–50,000)	0.902
Enterobacter	0 (0–100,000)	0	0.094
Diphtheroids	0 (0–50,000)	0 (0–20,000)	0.872
Acinobacter	0 (0–20,000)	0 (0–200)	0.902
Pseudomonas	0 (0–600)	0 (0–100,000)	0.902
α-Hemolytic *Streptococcus*	0 (0–15,000)	0 (0–100,000)	0.676
E. coli	0 (0–1,000)	0 (0–200)	0.902
Klebsiella	0 (0–7,000)	0	0.944

[a]Independent-samples median test.
[b]Significant difference.
CFU, colony-forming units.

Table 4. Percentile Values for *S. aureus* and Coagulase-Negative *Staphylococcus* Colony Count Growth in Breastmilk

	Bacterial colony count (CFU/mL)										
	Controls					*Cases*					
	Range	*50%*	*75%*	*90%*	*95%*	*Range*	*50%*	*75%*	*90%*	*95%*	p *value*[a]
CNS	0–100,000	5,000	10,000	30,000	40,000	0–100,000	900	6,900	13,000	30,000	0.003[b]
S. aureus	0–17,000	0	0	0	0	0–100,000	0	0	10,000	53,000	0.007[b]

[a]Independent-samples median test.
[b]Significant difference.
CFU, colony-forming units; CNS, coagulase-negative *Staphylococcus.*

24%, $p = 0.001$), oversupply (18% vs. 2%, $p = 0.005$), plugged ducts (28% vs. 0%, $p = 0.001$), and thrush (16% vs. 0%, $p = 0.002$). Controls, however, were more likely to have a history of low milk supply (15% vs. 30%, $p = 0.047$). Breastmilk *S. aureus* growth was statistically associated with a history of plugged ducts ($p = 0.011$) but was not associated with a history of other problems.

Since delivery, cases were more likely to have received treatment with fluconazole (15% vs. 0%, $p = 0.001$) and topical antibiotics (7% vs. 2%, $p = 0.027$). At the time of the initial visit, cases were more likely to be using topical antibiotics than controls (7% vs. 0%, $p < 0.001$) or currently taking fluconazole (8% vs. 0%, $p = 0.03$). There was no difference in current oral antibiotic use. Breastfeeding complications did differ between cases and controls over the 12-week study. Cases were more likely to develop mastitis (28% vs. 10%, $p = 0.022$) and plugged ducts (47% vs. 17%, $p = 0.001$). Although there was no difference in overall weaning frequency between cases and controls (15% vs. 12%, $p = 0.644$), cases were more likely to wean because of pain (15% vs. 0%, $p = 0.006$).

Discussion

The primary findings of our study include higher *S. aureus* but no difference in CNS frequency between cases and controls. Upon quantifying bacterial growth, we found significantly lower CNS colony count in women with pain. Further notable findings include the higher frequency of *Enterobacter* in controls.

Our findings support a pathogenic role of *S. aureus* for women with chronic pain. These results are consistent with studies on mastitis and cracked nipples.[11,12,15]

Studies examining the association of *Candida* infections and chronic breast or nipple pain also found a higher incidence of *S. aureus*.[6,16] These studies raise the question of *S. aureus'* role as a causative agent or co-pathogen in cases treated clinically as "thrush."[6,5,16] Our study did not evaluate the role of yeast in chronic breast pain. This is a significant limitation. *Candida* will grow on blood agar, but it is not the optimal medium[27]; we designed our culture medium and study primarily to test relative bacterial growth.

When examining *S. aureus* growth relative to that of other bacteria, we found an inverse relationship between CNS and *S. aureus* growth. Given the pathogenic role of *S. aureus* in other breastfeeding complications, our findings raise the question whether CNS may be protective against *S. aureus* growth. These findings are particularly relevant given the recent interest in a pathogenic role for CNS in mastitis and chronic pain.[10,22,23,28]

Our data on CNS frequency support the recognition of CNS species as normal bacteria in breastmilk. We used quantitative analysis to further examine CNS growth and found a significantly lower median CFU count in women with pain. These findings, the first to quantitatively examine bacteria in non-mastitis breast pain, are counter to the theory that CNS overgrowth may be pathogenic and a cause of infection.[22] Our data add to the findings and address some limitations noted in the meta-analysis of microbes and deep breast pain of Betzold.[28] Further study is indicated to determine reproducibility in a more diverse patient population and to evaluate CNS virulence factors such as resistance patterns and biofilm production.[28]

Enterobacter growth was statistically more frequent in controls. There was no growth in women with pain, raising speculation that *Enterobacter* growth maybe protective against infection. Future bacterial studies on women with pain should include evaluation of *Enterobacter*.

Our testing was performed by a commercial lab using routine bacterial culture tests. Additional arrangements were made for colony count quantification and species identification of CNS, which are not standard protocol for breastmilk analysis. The breastmilk was stored and transported at room temperature. The length of time at room temperature was variable and dependent on patient visit, office specimen pick-up, and transportation time. As previous studies have shown, bacterial growth can be affected by length of time at room temperature.[19–21] The case control design helps control for these variables, but further study is indicated to identify and standardize proper collection and storage methods of breastmilk cultures.

Further study is needed to evaluate oral antibiotic treatment in women with chronic breast pain. Studies focused on *S. aureus* infection and mastitis, or cracked nipples, found fewer complications following treatment with oral antibiotics.[11,15] Given that multiple studies, including ours, have found a higher frequency of *S. aureus* among women with chronic breast pain, specific studies evaluating oral antibiotic treatment of *S. aureus* in women with chronic pain are needed.

Conclusions

When evaluating breastfeeding women for chronic breast pain, our study supports the theory that *S. aureus* may be pathogenic. In contrast, our study findings of similar CNS frequency between groups, lower CNS colony count growth in cases, and an inverse relationship between *S. aureus* and CNS growth do not support a pathogenic role for CNS. To

decrease early breastfeeding cessation secondary to pain, further prospective studies focusing on treatment options, including antimicrobial, are needed.

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Disclosure Statement

No competing financial interests exist.

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