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Complete List of Authors:	Amir, Lisa; La Trobe University, Mother & Child Health Research Donath, Susan; Murdoch Childrens Research Institute, Clinical Epidemiology and Biostatistics Unit Garland, Suzanne; University of Melbourne, Microbiology and Infectious Diseases Department Tabrizi, Sepehr; The Royal Women's Hospital, Microbiology and Infectious Diseases; Bennett, Catherine; Deakin University, Deakin Population Health Cullinane, Meabh; La Trobe University, Mother & Child Health Research Payne, Matthew; La Trobe University, Mother & Child Health Research
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Title:

What role does Candida and/or Staphylococcus play in nipple and breast pain in lactation? A cohort study in Melbourne, Australia

Lisa H Amir[1], Susan M Donath[2], Suzanne M Garland[2,3], Sepehr N Tabrizi[2,3], Catherine M Bennett[4], Meabh Cullinane[1], Matthew S Payne[1,5]

[1]Mother & Child Health Research, La Trobe University, Melbourne, VIC, Australia
[2]Murdoch Childrens Research Institute, Melbourne, VIC, Australia
[3]Department of Molecular Microbiology, Microbiology and Infectious Diseases, Royal Women's Hospital and Department of Obstetrics and Gynaecology, University of Melbourne, VIC, Australia
[4]Deakin Population Health, Deakin University, Burwood, VIC, Australia
[5]School of Women's and Infants' Health, University of Western Australia, WA, Australia

Abstract

Objective: To investigate the roles of Candida species and Staphylococcus aureus in the development of "nipple and breast thrush" among breastfeeding women.

Design: Prospective longitudinal cohort study.

Setting: Two hospitals in Melbourne, Australia (one public, one private) with follow-up in the community.

Participants: 360 nulliparous women recruited at ≥ 36 weeks gestation from November 2009 to June 2011. Participants were followed-up six times: in hospital, at home weekly until four weeks postpartum and by telephone at eight weeks.

Main outcome measures: A researcher-defined proxy diagnosis of "breast thrush": burning nipple pain and breast pain (not related to mastitis); detection of Candida spp. (using culture

and PCR) from the mother's vagina, nipple or breast milk or baby's mouth; detection of S. aureus from the mother's nipple or breast milk.

Results: Women with researcher-defined nipple/breast thrush were more likely to have Candida spp. in nipple/breast milk/baby oral samples (54%) compared to other women (36%, p = 0.014). S. aureus in nipple/breast milk samples was higher in women with these symptoms than other women (78% vs 65%) (p = 0.068). Time-to-event analysis examined predictors of nipple/breast thrush up to and including the time of data collection. The crude Relative Risk of Candida spp. in nipple/breast milk/baby was 1.87 (95% CI: 1.10, 3.16, p = 0.018); the multivariate RR (adjusted for S. aureus in nipple/breast milk and nipple damage) was almost unchanged at 2.03 (95% CI: 1.19, 3.45, p = 0.009). S. aureus colonisation was not a predictor of these symptoms (RR 1.53, 95% CI: 0.88, 2.64, p = 0.13), with little change in the multivariate model. Nipple damage was also a strong predictor of these symptoms, RR 2.30 (95% CI: 1.19, 4.43, p = 0.012), with little change in multivariate model, indicating that Candida spp., S. aureus and nipple damage are operating independently.

Background

Controversy about the condition known as "breast thrush" or breast candidiasis in lactating women has led to confusion among clinicians and the community. While some clinicians may diagnose and treat this condition in breastfeeding women with deep, radiating breast pain associated with burning nipple pain,[1-6] others doubt the relationship with fungal organisms[7-11] and decry "the alarming trend towards believing that fungi are important in the aetiology of breast infection and deep breast pain associated with breast feeding, despite a lack of good quality evidence".[11] p. 485. Health professionals may tell women that the pain is "all in their head", which is reminiscent of the lack of understanding of mastalgia in the 1970s, when breast pain was thought to be a psychosomatic complaint by "neurotic" women.[12]

Unlike mastitis which is diagnosed when a breastfeeding woman experiences inflammation of the breast associated with systemic symptoms,[13] breast thrush is usually diagnosed when the breast is not erythematous or indurated , and the woman is afebrile and systemically well apart from a typical burning pain radiating into the breast and/or into the back.[4] While some authors use the term 'candida mastitis',[6 11 14] we feel this is misleading, as inflammation of the breast is not evident. Although some clinicians attribute the pain to infection with *S. aureus* and treat women with long-term antibiotics,[15] this has not been tested in trials.

The primary cause of the nipple pain or damage is often the process of breastfeeding itself: trauma from the infant's mouth due to incorrect attachment, or infant anatomy or dysfunctional suck.[16] Nipple thrush is usually diagnosed when the nipple/areola is slightly pink, sensitive to touch, and the pain described is out of proportion to the damage seen on clinical examination.[4] When the areola is described as itchy and appears red and/or crusty, the diagnosis is dermatitis/eczema rather than fungal infection.[17] A nipple with obvious damage is almost certainly colonised with *S. aureus.*[18] Nipple/breast pain associated with nipple blanching persisting for longer than a few seconds is likely to be nipple vasospasm; this condition is commonly confused with breast thrush because of the burning, radiating nature of the pain.[19 20] The pain from vasospasm is often secondary to nipple damage or infection, exacerbated by cold, and relieved by heat or nifedipine.[21] In practice, more than one cause of nipple or breast pain is commonly present,[6] which makes it difficult to construct an algorithm for "breast thrush" for research purposes.

Previous breastfeeding studies have been largely cross-sectional,[1 7 9 10] whilst one longitudinal study collected microbiological data, but no clinical information.[22] This is the first

prospective longitudinal study of the role of both *S. aureus* and *Candida* spp. in breast infections and was designed to resolve the current controversy surrounding which is the primary organism responsible for the condition known as "breast thrush": *Candida* spp. or *S. aureus*?

Methods

The CASTLE (Candida and Staphylococcus Transmission: Longitudinal Evaluation) study was a prospective longitudinal descriptive study designed to investigate the role of staphylococci and/or candida in nipple and breast pain, and the relationship between breastfeeding, postpartum health problems and maternal psychological well-being. Details have been published in the study protocol.[23] A cohort of 360 nulliparous women planning to breastfeed for at least two months were recruited at ≥ 36 weeks gestation from two hospitals in Melbourne, Australia (November 2009 to June 2011). At recruitment, nasal, nipple (both breasts) and vaginal swabs were collected and participants completed a questionnaire asking about previous staphylococcal and candida infections. Following birth, participants were followed-up six times: face-to-face in hospital, then weekly at home until four weeks postpartum. Participants filled out a questionnaire at each time point to collect information about breastfeeding problems and postpartum health problems. At each visit, maternal nasal, and nipple swabs and breast milk samples (both breasts) and infant oral and nasal swabs were collected. At a final telephone interview at eight weeks postpartum information about breastfeeding problems and postpartum health was collected.

Briefly, two nipple swabs were obtained from each nipple; a standard charcoal swab for microbiological analysis (Copan Diagnostics Inc. CA, USA) and a flocked swab for molecular analysis (Copan Diagnostics Inc. CA, USA). After first moistening in sterile saline,

both the standard and flocked nipple swabs were rolled over the nipple and areola together using a 10-point swabbing technique[24] paying particular attention to any cracks / fissures present. Oral and vaginal swabs were collected for culture of *S. aureus* and *Candida* spp. Breast milk samples were also cultured for *S. aureus*, coagulase-negative staphylococci (CoNS) and *Candida* spp; nasal swabs were collected for culture of *S. aureus* only. DNA was extracted from nipple and vaginal swabs for molecular identification of *Candida* spp. using real-time PCR.[23]

At each contact, women were asked about nipple pain and whether it was burning in quality, clinical signs and symptoms of mastitis as used in previous research (i.e. redness, fever, etc.),[25] and other types of breast pain, such as radiating ("stabbing") or non-stabbing. Researchers also collected clinical observations of nipple/areola and breast at each visit (weeks 1 to 4), including colour of nipple/areola. We had planned to develop an algorithm for "nipple or breast thrush" using symptoms and appearance ("shiny" or flaky" nipple) or pink colour.[26] However, very few women were described as having these appearances (shiny, n = 4, flaky, n = 17), while 140 women were described as having "pink" nipple/s making the appearance identifiers unlikely to be helpful in the algorithm. Therefore we used a combination of burning nipple pain and breast pain (non-mastitis) as a proxy for a clinical diagnosis of "nipple and breast thrush".

Statistical analysis was conducted using Stata Version 12. Hypothesis 1 — Women with nipple/breast thrush are more likely to have *Candida* spp. isolated than other women; hypothesis 2 — Women with nipple/breast thrush are more likely to have *S. aureus* isolated than other women. A sample of 318 women was estimated to provide adequate power. [23] Chi-squared tests were used for comparing categorical variables. We investigated incidence of nipple/breast thrush using a multivariable discrete version of the proportional hazards

regression model.[27] Outcome variable was the incidence of new cases of our breast thrush diagnosis; time-varying predictors were: the presence of *Candida* spp. , presence of *S. aureus* and mother-reported nipple damage. We present crude Relative Risks (RR), and multivariate analysis, adjusting for the presence of *Candida* spp, *S. aureus* and nipple damage.

Results relating to mastitis, other breastfeeding and postpartum problems will be published separately (papers in preparation).

Results

Fourteen women withdrew from the study after giving birth, leaving 346 women available for data collection at subsequent visits; 340 women completed the study at 8 weeks postpartum. Women who participated in the study were highly educated (77% had tertiary degree or higher) and most were married or lived with their partner (96%) (Table 1). Just over half gave birth in the private hospital (56%), 45% by Caesarean section.

Burning nipple pain was reported by 42% of women (146/346) during weeks 1 to 8, or 32% women (111/346) during weeks 2 to 8. Radiating or non-radiating breast pain (not related to engorgement/mastitis) was reported by 54% of women (186/346) during weeks 1 to 8, or 47% of women (162/346) during weeks 2 to 8. Combining these symptoms to estimate the proportion of women with both burning nipple pain and non-mastitis breast pain (at the same time): 19% of women in weeks 1 to 8 (65/346), or 15% in weeks 2 to 8 (50/346).

Candida albicans was the most commonly isolated *Candida* spp in culture with *Candida glabrata* only isolated in one nipple specimen and one milk sample (Table 2). Although "other Candida spp." were found, none speciated as *Candida krusei* or *Candida kefyr*.

Candida PCR of the nipple was positive for *Candida* spp. in 33% of women (115/346); in contrast to culture of only 3% of women (9/346) being positive for *Candida* spp.. *Candida* spp. were isolated by culture from breast milk samples from 5% women (18/346), but no molecular analyses were conducted on these samples. *S. aureus* was isolated by culture from the nipple and/or breast milk in 67% of women (231/346), and from nose and/or mouth of 73% of infants (253/346).

As burning nipple pain was very common if week 1 were included, we developed a proxy diagnosis for nipple/breast thrush if women had burning nipple pain as well as breast pain (non-mastitis) between weeks 2 and 8. There was a statistically significant association between these symptoms and *Candida* spp. in nipple/breast milk/baby (p = 0.014, see Table 3); as also for *Candida* spp. in vagina/nipple/breast milk/baby (p = 0.047, not shown). There was evidence that *S. aureus* in nipple/breast milk samples was higher in women with these symptoms than other women (78% vs 65%) (p = 0.068, Table 3).

Time-to-event analysis examined predictors of burning nipple and breast pain (non-mastitis) up to and including the time of data collection. (See unadjusted survival curves: Figures 1, 2 and 3). The crude Relative Risk of candida in nipple/breast milk/baby was 1.87 (95% CI: 1.10, 3.16, p = 0.018); the multivariate RR (adjusted for *S. aureus* in nipple/breast milk and nipple damage) was almost unchanged at 2.03 (95% CI: 1.19, 3.45, p = 0.009). *S. aureus* colonisation was not a predictor of these symptoms (RR 1.53, 95% CI: 0.88, 2.64, p = 0.13), with little change in the multivariate model. Mothers' report of nipple damage was also a strong predictor of these symptoms, with a RR 2.30 (95% CI: 1.19, 4.43, p = 0.012), with little change in multivariate model, which indicates that candida, *S. aureus* and nipple damage are operating independently.

Discussion

Principal findings

Unlike some cross-sectional studies which found no relationship between the presence of *Candida* spp. and the condition known as breast thrush,[9 10] we have shown that *Candida* spp. is associated with burning nipple pain and breast pain – in two analyses ('at any time' and 'time-to-event'). As in previous studies,[1 7] we uncommonly isolated *Candida* spp. on the nipple using standard microbiological culture techniques. However, *Candida* spp. were more commonly identified using more sensitive molecular techniques (real-time PCR). This test is not used in routine practice currently, and therefore it is not helpful in making the diagnosis in clinical care. Diagnostic skills are needed to make the diagnosis of nipple/breast candidiasis; clinicians should routinely consider all causes of nipple and breast pain, in the same way they consider differential diagnoses when assessing a patient with chest pain.[28]

Consistent with other studies of mothers and infants,[22 29-32] colonisation with *S. aureus* is common; at least 50% of women were colonised with *S. aureus* in nipple or milk samples by four weeks postpartum. Therefore in clinical practice, a finding of *S. aureus* on the nipple or breast milk is not evidence that the bacteria are the principal cause of the woman's pain. Our analysis indicates that candida and *S. aureus* are acting independently, despite often co-existing. The case-control study of women with a clinical diagnosis of nipple and breast thrush by Panjaitan and colleagues which only used molecular techniques from nipple swabs and excluded women with clinical signs of bacterial infection (exudate on nipple or inflamed breast), found that *S. aureus* was present in equal numbers of cases and controls.[33]

Strengths and limitations

The strength of this study is that a cohort of healthy women who had not previously breastfed was recruited prior to commencing breastfeeding and was followed closely until two months postpartum. The main limitation is that we did not have a clinical diagnosis of nipple/breast thrush and had to use a proxy diagnosis to estimate this condition. Another limitation is that we only followed the cohort for eight weeks postpartum – with microbiological data to only four weeks postpartum; women who developed problems after this time period were not captured in our data collection.

Conclusions

This large cohort study confirms that *Candida* spp. play a role in nipple and breast pain in lactating women, and "thrush in the breast" should not be dismissed as "psychosomatic" as has been stated by some clinicians. Burning nipple pain is common in breastfeeding women, and a diagnosis of *Candida* spp. infection should not be made without considering differential diagnoses.[4] Further research into the role of staphylococci in breast pain in lactating women with inflammatory symptoms and without is needed.

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Contributors: LHA conceived the study, which was designed in collaboration with all authors. SMD managed the data and conducted the statistical analyses. SMG, SNT, CMB and MSP provided microbiological expertise. MC was the project co-ordinator. MSP was the research scientist.

Competing interests: All authors have completed the ICMJE uniform disclosure form at www.icjme.org/coi_disclosure.pdf (available on request from the corresponding author) and declare that: this stud received financial support from the National Health & Medical Research Council (NHMRC) (project grant 502053, equipment grant, Health Professional Training Fellowship (LHA)), Helen Mcpherson Smith Trust, Faculty Research Grant, Faculty of Health Sciences, La Trobe University; no financial relationships with any organisations that might have an interest in the submitted work; and no other relationships or activities that could appear to have influenced the submitted work.

Ethical approval: This study was approved by the La Trobe University Human Ethics Committee (06-078); Human Research Ethics Committee of the Royal Women's Hospital (06/41); Human Research Ethics Committee of the University of Melbourne (1033949); and Medical Advisory Committee at Frances Perry House.

Data sharing: No additional data available.

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Article summary

Article Focus

- Controversy about the condition known as "breast thrush" or breast candidiasis in lactating women has led to confusion among clinicians and the community.
- Previous studies have been cross-sectional.
- This is the first prospective longitudinal study of the role of both *S. aureus* and *Candida* spp. in breast infections and was designed to resolve the current controversy surrounding which is the primary organism responsible for the condition known as “breast thrush”: *Candida* spp. or *S. aureus*?

Key Messages

- *Candida* spp. is associated with burning nipple pain and breast pain.
- Colonisation with *S. aureus* is common; at least 50% of women were colonised with *S. aureus* in nipple or milk samples by four weeks postpartum.

Strengths and Limitations

- The evidence of microbiological data from this large cohort of women over four weeks postpartum is stronger than previous smaller cross-sectional studies.
- *Candida* spp. were more commonly identified using more sensitive molecular techniques (real-time PCR) than by using standard microbiological culture techniques.
- As these techniques are not used in clinical practice currently, this is not currently useful for clinicians.

Table 1 Characteristics of nulliparous women recruited in late pregnancy

Maternal characteristics (n = 346)	n (%)
Hospital	
Royal Women's Hospital (public)	154 (44.5)
Frances Perry House (private)	192 (55.5)
Age (years - mean, SD, range)	32.7 (4.1, 19 - 44)
Marital status	
Married	229 (66)
Unmarried , living with partner	103 (30)
Not living with partner	2 (1)
Separated / divorced	1 (0)
Single	11 (3)
Education level	
Tertiary degree or higher	267 (77)
Other	79 (23)
Gestation at recruitment (weeks - mean, SD, range)	37 (1.3,34 - 42)
Gestation at birth (weeks - mean, SD, range)	39 (1.2, 36 - 42)
Breastfeeding intention (months - mean, range)	9.7 (1 - 24)
Caesarean birth	156 (45)
Baby sex – male	168 (49)
Any breast milk feeding at 8 weeks postpartum (n = 340)	320 (94)

Table 2 Results from microbiological analysis of specimens collected from 346 women and their infants (at any time point)

	Culture positive	PCR positive	Either culture/PCR positive
Nipple*			
C. albicans	13	15	19
C. glabrata	1	3	4
Candida spp.	9	115	120
Any *Candida* spp.	21	116	125
S. aureus	206 (60%)	N/A	N/A
Breast milk**			
C. albicans	9	N/A	N/A
C. glabrata	1	N/A	N/A
Candida spp.	10	N/A	N/A
Any *Candida* spp.	18	N/A	N/A
S. aureus	186 (54%)	N/A	N/A
Infant nose/mouth**			
C. albicans	15	N/A	N/A
C. glabrata	0	N/A	N/A
Candida spp.	5	N/A	N/A
Any *Candida* spp.	18	N/A	N/A
S. aureus	253 (73%)	N/A	N/A
Any *Candida* spp. in nipple/breast milk/baby	133 (38)		
Any *S. aureus* in nipple/breast milk	231 (67%)		

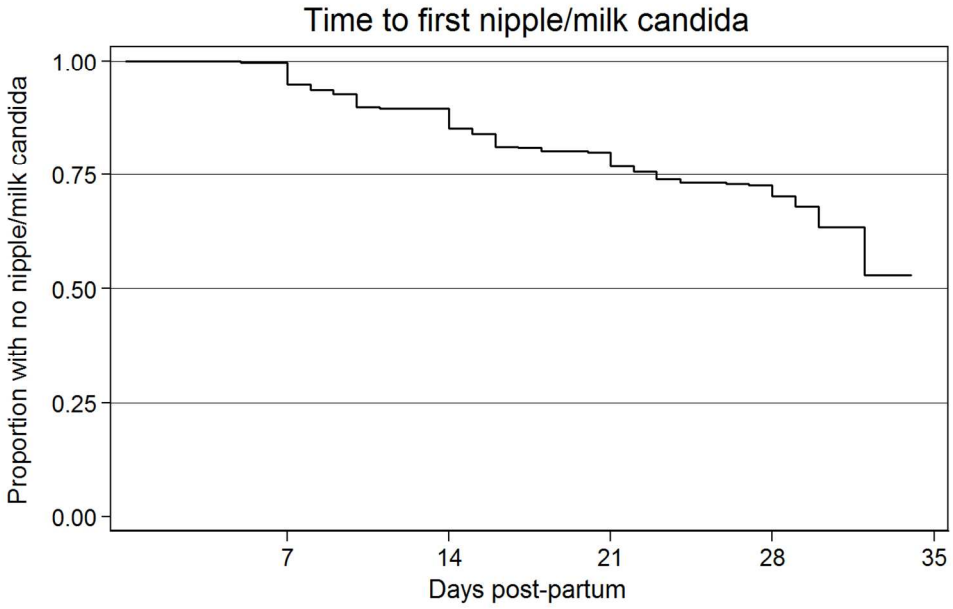
* Nipple swabs collected at late pregnancy, hospital, weeks 1, 2, 3 and 4.
** Breast milk, and infant nasal and oral swabs, collected at hospital, weeks 1, 2, 3 and 4.
N/A = not applicable.

Table 3 Nipple/breast thrush symptoms and *Candida* spp. and *S. aureus* separately (n = 346)

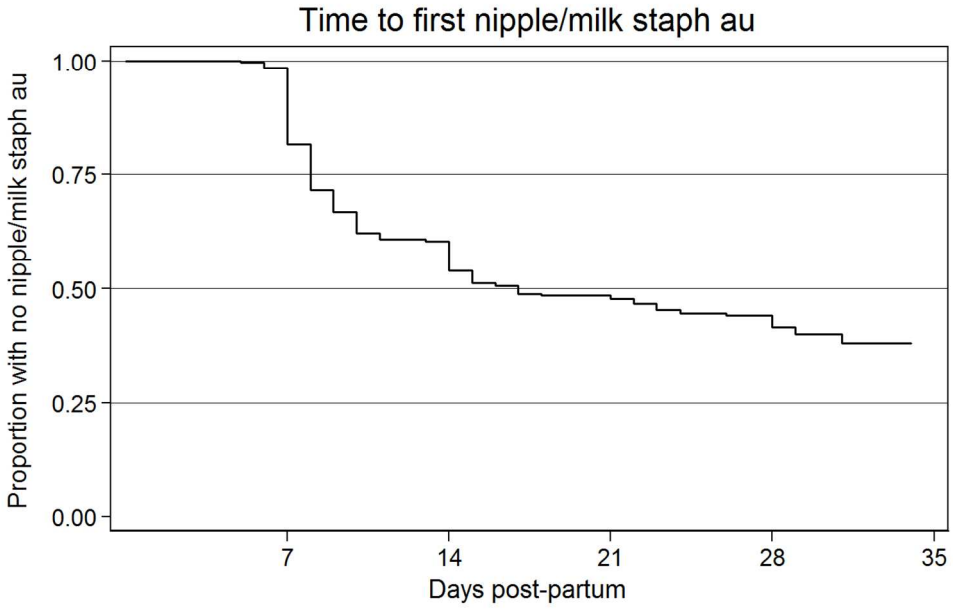
Breast thrush algorithm: Burning nipple pain weeks 2-8 plus breast pain (non-mastitis)

	Candida* spp. (nipple/breast milk/baby)	
Breast thrush algorithm	No	Yes
	(n = 213)	(n = 133)
No (n = 278)	180 (65%)	98 (35%)
Yes (n = 68)	33 (49%)	35 (52%)
	S. aureus* (nipple/breast milk)*	
Breast thrush algorithm	No	Yes
	(n = 115)	(n = 231)
No (n = 278)	97 (35%)	181 (65%)
Yes (n = 68)	18 (27%)	50 (74%)

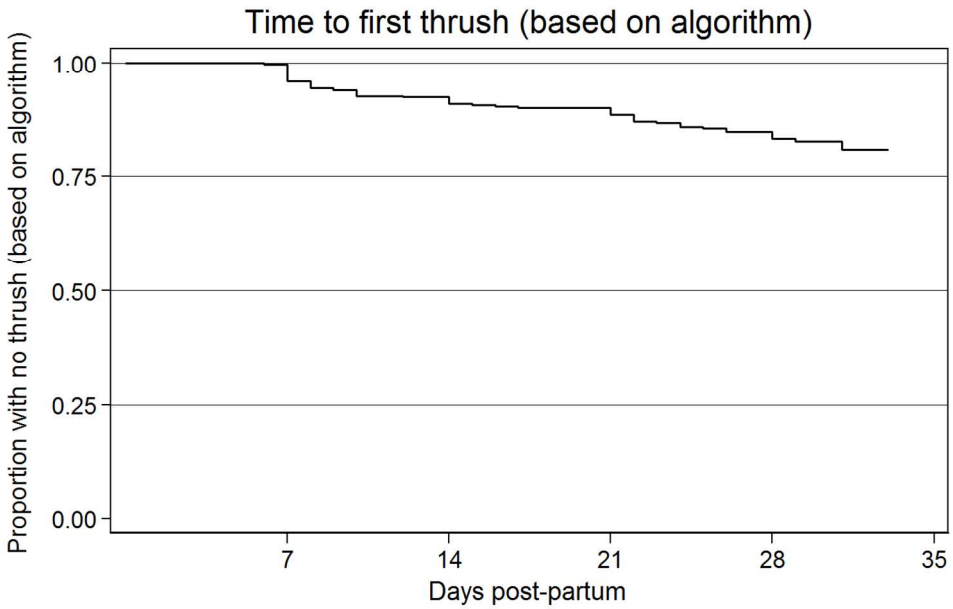
*$Chi^2(1) = 6.0734$, p = 0.014
**$Chi^2(1) = 1.7462$, p = 0.186



Survival curve for time to first nipple/milk candida



Survival curve for time to first nipple/milk S. aureus



Survival curve for time to first symptoms of "breast thrush"

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
Yes		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Yes		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Yes		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed **N/A**
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) If applicable, explain how loss to follow-up was addressed
		(*e*) Describe any sensitivity analyses
Results		
YES		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) Summarise follow-up time (eg, average and total amount)
Outcome data	15*	Report numbers of outcome events or summary measures over time
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included

		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
YES		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
YES		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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Does Candida and/or Staphylococcus play a role in nipple and breast pain in lactation? A cohort study in Melbourne, Australia

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Title:

Does Candida and/or Staphylococcus play a role in nipple and breast pain in lactation? A cohort study in Melbourne, Australia

Lisa H Amir[1], Susan M Donath[2], Suzanne M Garland[2,3], Sepehr N Tabrizi[2,3], Catherine M Bennett[4], Meabh Cullinane[1], Matthew S Payne[1,5]

[1]Mother & Child Health Research, La Trobe University, Melbourne, VIC, Australia
[2]Murdoch Childrens Research Institute, Melbourne, VIC, Australia
[3]Department of Molecular Microbiology, Microbiology and Infectious Diseases, Royal Women's Hospital and Department of Obstetrics and Gynaecology, University of Melbourne, VIC, Australia
[4]Deakin Population Health, Deakin University, Burwood, VIC, Australia
[5]School of Women's and Infants' Health, University of Western Australia, WA, Australia

Article summary

Article Focus

- Controversy about the condition known as "breast thrush" or breast candidiasis in lactating women has led to confusion among clinicians and the community.
- Previous studies have been cross-sectional.
- This is the first prospective longitudinal study to examine simultaneously both *S. aureus* and *Candida* spp. in breast infections and was designed to resolve the current controversy surrounding which is the primary organism responsible for the condition known as "breast thrush": *Candida* spp. or *S. aureus*?

Key Messages

- *Candida* spp. is associated with burning nipple pain and breast pain.

- Colonisation with *S. aureus* is common; at least 50% of women were colonised with *S. aureus* in nipple or milk samples by four weeks postpartum.

Strengths and Limitations

- The evidence of microbiological data from this large cohort of women over four weeks postpartum is stronger than previous smaller cross-sectional studies.
- *Candida* spp. were more commonly identified using more sensitive molecular techniques (real-time PCR) than by using standard microbiological culture techniques.
- As these techniques are not used in clinical practice currently, clinicans should continue to use their clinical skills to diagnose causes of nipple and breast pain in lactating women.

Abstract

Objective: To investigate *Candida* species and *Staphylococcus aureus* and the development of "nipple and breast thrush" among breastfeeding women.

Design: Prospective longitudinal cohort study.

Setting: Two hospitals in Melbourne, Australia (one public, one private) with follow-up in the community.

Participants: 360 nulliparous women recruited at ≥ 36 weeks gestation from November 2009 to June 2011. Participants were followed-up six times: in hospital, at home weekly until four weeks postpartum and by telephone at eight weeks.

Main outcome measures: Case definition "nipple and breast thrush": burning nipple pain and breast pain (not related to mastitis); detection of *Candida* spp. (using culture and PCR)

from the mother's vagina, nipple or breast milk or baby's mouth; detection of *S. aureus* from the mother's nipple or breast milk.

Results: Women with the case definition of nipple/breast thrush were more likely to have *Candida* spp. in nipple/breast milk/baby oral samples (54%) compared to other women (36%, p = 0.014). *S. aureus* was common in nipple/breast milk/baby samples of women with these symptoms as well as women without these symptoms (82% vs 79%) (p = 0.597). Time-to-event analysis examined predictors of nipple/breast thrush up to and including the time of data collection. Candida in nipple/breast milk/baby predicted incidence of the case definition (Rate Ratio 1.87 (95% CI: 1.10, 3.16, p = 0.018). We don't have evidence that *S. aureus* colonisation was a predictor of these symptoms (RR 1.53, 95% CI: 0.88, 2.64, p = 0.13). Nipple damage was also a predictor of these symptoms, RR 2.30 (95% CI: 1.19, 4.43, p = 0.012). In the multivariate model, with all three predictors, the Rate Ratios were very similar to the univariate Rate Ratios. This indicates that Candida and nipple damage are independent predictors of our case definition.

Background

Controversy about the condition known as "breast thrush" or breast candidiasis in lactating women has led to confusion among clinicians and the community. While some clinicians may diagnose and treat this condition in breastfeeding women with deep, radiating breast pain associated with burning nipple pain,[1-6] others doubt the relationship with fungal organisms[7-11] and decry "the alarming trend towards believing that fungi are important in the aetiology of breast infection and deep breast pain associated with breast feeding, despite a lack of good quality evidence".[11] p. 485.

Unlike mastitis which is diagnosed when a breastfeeding woman experiences inflammation of the breast associated with systemic symptoms,[12] breast thrush is usually diagnosed when the breast is not erythematous or indurated , and the woman is afebrile and systemically well apart from a typical burning pain radiating into the breast and/or into the back.[4] While some authors use the term 'candida mastitis',[6 11 13] we feel this is misleading, as inflammation of the breast is not evident. Although some clinicians attribute the pain to infection with *Staphylococcus aureus* (*S. aureus*) and treat women with long-term antibiotics,[14] this has not been tested in trials.

The primary cause of the nipple pain or damage is often the process of breastfeeding itself: trauma from the infant's mouth due to incorrect attachment, or infant anatomy or dysfunctional suck.[15] Nipple thrush is usually diagnosed when the nipple/areola is slightly pink, sensitive to touch, and the pain described is out of proportion to the damage seen on clinical examination.[4] When the areola is described as itchy and appears red and/or crusty, the diagnosis is dermatitis/eczema rather than fungal infection.[16] A nipple with obvious damage is almost certainly colonised with *S. aureus.*[17] Nipple/breast pain associated with nipple blanching persisting for longer than a few seconds is likely to be nipple vasospasm; this condition is commonly confused with breast thrush because of the burning, radiating nature of the pain.[18 19] The pain from vasospasm is often secondary to nipple damage or infection, exacerbated by cold, and relieved by heat or nifedipine.[20] In practice, more than one cause of nipple or breast pain is commonly present,[6] which makes it difficult to construct a case definition for "breast thrush" for research purposes.

Previous breastfeeding studies have been largely cross-sectional,[1 7 9 10] whilst one longitudinal study collected microbiological data, but no clinical information.[21] This is the first

prospective longitudinal study examining both *S. aureus* and *Candida* spp. in breast infections and was designed to resolve the current controversy surrounding which is the primary organism responsible for the condition known as "breast thrush": *Candida* spp. or *S. aureus*?

Methods

The CASTLE (Candida and Staphylococcus Transmission: Longitudinal Evaluation) study was a prospective longitudinal descriptive study; details have been published in the study protocol.[22] A cohort of 360 nulliparous women planning to breastfeed for at least two months were recruited at ≥ 36 weeks gestation from two hospitals in Melbourne, Australia (November 2009 to June 2011). At recruitment, nasal, nipple (both breasts) and vaginal swabs were collected and participants completed a questionnaire asking about previous staphylococcal and candida infections. Following birth, participants were followed-up six times: face-to-face in hospital, then weekly at home until four weeks postpartum. Participants filled out a questionnaire at each time point to collect information about breastfeeding problems and postpartum health problems. At each visit, maternal nasal, and nipple swabs and breast milk samples (both breasts) and infant oral and nasal swabs were collected. At a final telephone interview at eight weeks postpartum information about breastfeeding problems and postpartum health was collected.

Specimens were collected by research assistants. Fresh gloves were worn for each specimen. After sanitising their hands, research assistants collected nipple swabs then washed the nipple/areola region twice using sterile water wipes. Mid-stream milk was collected into a sterile container; the first drops of breast milk were expressed and discarded. Two nipple swabs were obtained from each nipple; a standard charcoal swab for microbiological analysis

(Copan Diagnostics Inc. CA, USA) and a flocked swab for molecular analysis (Copan Diagnostics Inc. CA, USA). After first moistening in sterile saline, both the standard and flocked nipple swabs were rolled over the nipple and areola together using a 10-point swabbing technique,[23] paying particular attention to any cracks / fissures present. Oral and vaginal swabs were collected for culture of *S. aureus* and *Candida* spp. Breast milk samples were also cultured for *S. aureus*, coagulase-negative staphylococci (CoNS) and *Candida* spp; nasal swabs were collected for culture of *S. aureus* only.

In studies conducted in women with vulvovaginal symptoms, molecular microbiological techniques have been useful in detecting Candida in women who were negative using standard microbiology.[24 25] Therefore we planned to use molecular techniques to increase detection of *Candida* spp. in nipple specimens. [22] Due to cost constraints we did not plan to use molecular techniques for the milk specimens (up to 4000 specimens). As participants only had one or two vaginal specimens we extracted DNA from vaginal as well as nipple swabs for molecular identification of *Candida* spp. using real-time PCR.[22]

At each contact, women were asked about nipple pain ("In the last 48 hours, have you been experiencing **nipple** pain/discomfort?) and whether it was burning in quality ("If yes, would you describe your nipple pain as burning?"), clinical signs and symptoms of mastitis as used in previous research (i.e. redness, fever, etc.),[26] and other types of breast pain ("Have you had **other** breast pain in the last 2 days? No/ I have had stabbing (radiating or shooting) breast pain **only**/ I have had non-stabbing breast pain **only**/ I have had both stabbing and non-stabbing breast pain"). We also asked "Do you have nipple vasospasm? (Nipple blanches or goes white in the cold or during/after feeds) No/ Yes, for less than 5 minutes/ Yes, for more than 5 minutes/ Not sure"). Researchers also collected clinical observations of nipple/areola

and breast at each visit (weeks 1 to 4), including colour of nipple/areola. Our case definition of "nipple and breast thrush" used a combination of burning nipple pain and breast pain (non-mastitis). Francis-Morrill et al found nipple appearance ("shiny" or flaky" nipple or pink colour) to be predictive of Candida.[27] However, in our study very few women were described as having these appearances (shiny, n = 4, flaky, n = 17), while 140 women were described as having "pink" nipple/s. Adding the appearance identifiers to our case definition was not helpful. Clinically, the symptoms of nipple and breast thrush develop after the first week postpartum, and since most pain in the first week postpartum in first time mothers is likely to be due to adjusting to breastfeeding, we examined the case definition at weeks 1 to 8, and weeks 2 to 8 separately.

Statistical analysis was conducted using Stata Version 12. Hypothesis 1 — Women with nipple/breast thrush are more likely to have *Candida* spp. isolated than other women; hypothesis 2 — Women with nipple/breast thrush are more likely to have *S. aureus* isolated than other women. A sample of 318 women was estimated to provide adequate power. [22] Chi-squared tests were used for comparing categorical variables. We investigated incidence of nipple/breast thrush using a multivariable discrete version of the proportional hazards regression model.[28] Outcome variable was the incidence of new cases of our nipple and breast thrush definition; time-varying predictors were: the presence of *Candida* spp., presence of *S. aureus* and mother-reported nipple damage. We present crude Rate Ratios (RR), and multivariate analysis, adjusting for the presence of *Candida* spp, *S. aureus* and nipple damage.

Results relating to mastitis, other breastfeeding and postpartum problems will be published separately (papers in preparation).

Results

Fourteen women withdrew from the study after giving birth, leaving 346 (96%) women available for data collection at subsequent visits; 340 (94%) women completed the study at 8 weeks postpartum. Women who participated in the study were highly educated (77% had tertiary degree or higher) and most were married or lived with their partner (96%) (Table 1). Just over half gave birth in the private hospital (56%), 45% by Caesarean section.

Burning nipple pain was reported by 42% of women (146/346) during weeks 1 to 8, or 32% women (111/346) during weeks 2 to 8. Radiating or non-radiating breast pain (not related to engorgement/mastitis) was reported by 54% of women (186/346) during weeks 1 to 8, or 47% of women (162/346) during weeks 2 to 8. Combining these symptoms to estimate the proportion of women with both burning nipple pain and non-mastitis breast pain (at the same time): 19% of women in weeks 1 to 8 (65/346), or 15% in weeks 2 to 8 (50/346).

Candida albicans was the most commonly isolated *Candida* spp in culture with *Candida glabrata* only isolated in one nipple specimen and one milk sample (Table 2). Although "other Candida spp." were found, none speciated as *Candida krusei* or *Candida kefyr*. *Candida* PCR of the nipple was positive for *Candida* spp. in 33% of women (115/346); in contrast to culture of only 3% of women (9/346) being positive for *Candida* spp.. *Candida* spp. were isolated by culture from breast milk samples from 5% women (18/346), but no molecular analyses were conducted on these samples. Table 3 shows Candida isolated by culture and by PCR at each visit. *S. aureus* was isolated by culture from the nipple and/or breast milk in 67% of women (231/346), and from nose and/or mouth of 73% of infants (253/346) at some point during follow-up. There were 22 milk samples positive for Candida,

of which 10 were milk only, and 12 were positive for nipple and milk. For *S. aureus*, 425 milk samples were positive, of which 89 were positive in milk only.

Burning nipple pain was very common in week 1, primarily as women adjusted to breastfeeding, therefore we explored the relationship between *Candida* spp. and *S. aureus* using two case definitions: weeks 1-8 and weeks 2-8 (Table 4). There was a statistically significant association between these symptoms in weeks 2-8 and *Candida* spp. in nipple/breast milk/baby (p = 0.014); as also for *Candida* spp. in vagina/nipple/breast milk/baby (p = 0.047, not shown). *S. aureus* was common in nipple/breast milk/baby samples of women with these symptoms as well as women without these symptoms (82% vs 79%) (p = 0.597, Table 4).

As women with nipple vasospasm describe a burning, radiating pain, we also analysed the case definition excluding women with vasospasm (see Table 4). Only two women were removed from the analysis (n= 48), which made little difference to the results.

Time-to-event analysis examined predictors of our case definition of nipple/breast thrush (burning nipple and breast pain [non-mastitis]) up to and including the time of data collection in the first four weeks. (See unadjusted survival curves: Figures 1, 2 and 3 and Table 5). Candida in nipple/breast milk/baby predicted incidence of the case definition (Rate Ratio 1.87 (95% CI: 1.10, 3.16, p = 0.018). Thus, for women with Candida in nipple/milk/baby at any time point, the rate of subsequently developing the case definition was increased by 87%, compared to women without Candida The evidence for *S. aureus* colonisation as a predictor of these symptoms was not strong (RR 1.53, 95% CI: 0.88, 2.64, p = 0.13). Mothers' report of nipple damage was a predictor of these symptoms, with a RR 2.30 (95% CI: 1.19, 4.43, p

= 0.012). In the multivariate model, with all three predictors, the Rate Ratios were very similar to the univariate Rate Ratios. This indicates that Candida and nipple damage are independent predictors of our case definition.

Discussion

Principal findings

Unlike some cross-sectional studies which found no relationship between the presence of *Candida* spp. and the condition known as breast thrush,[9 10] we have shown that *Candida* spp. is associated with burning nipple pain and breast pain – in two analyses ('at any time' and 'time-to-event'). As in previous studies,[1 7] we uncommonly isolated *Candida* spp. on the nipple using standard microbiological culture techniques. However, *Candida* spp. were more commonly identified using more sensitive molecular techniques (real-time PCR). This test is not used in routine practice currently, and therefore it is not helpful in making the diagnosis in clinical care at this time. *Candida* spp. were also rarely isolated in breast milk, using standard techniques. It is possible that the isolation rate would have been higher if we had used the technique of Morrill and colleagues, who added iron to inactivate milk lactoferrin.[29] However, Hale et al were unable to identify Candida in breast milk of women with 'Candida mastitis' using culture and another specific technique (presence of 1→3-β-D-glucan). [10] The 16 cases had 'sore, inflamed, or traumatized nipples, intense stabbing or burning pain that radiated into the axilla often persisting after feeding, and painful feeding without alternate diagnosis'.[10] Possible explanations for Hale et al's findings are that the women had other causes of their pain (from the traumatised nipple, from maternal vasospasm, or infant posterior tongue-tie or other mechanical causes of pain); that Candida was present in the nipple (samples were only collected from the milk), or that Candida is not present in the milk of women with this syndrome. The question as to whether Candida is present in the

lactiferous ducts is still open to debate; what we have shown is a link between Candida and nipple/breast pain – independent of the presence of nipple damage or *S. aureus*.

Candida spp. are commensal organisms, and therefore the presence of *Candida* spp. does not always imply infection. Recent understanding of vulvovaginitis postulates that the threshold number of organisms for symptomatic vaginitis varies for different groups of women; women with infrequent vaginitis have a higher threshold.[30] Furthermore, the symptoms associated with vaginitis may be caused by the host neutrophil response; small numbers of organisms may promote an aggressive inflammatory response in some women.[30]

Consistent with other studies of mothers and infants,[21 31-34] colonisation with *S. aureus* is common; at least 50% of women were colonised with *S. aureus* in nipple or milk samples by four weeks postpartum. Therefore in clinical practice, a finding of *S. aureus* on the nipple or breast milk is not evidence that the bacteria are the principal cause of the woman's pain. Our analysis indicates that candida and *S. aureus* are acting independently, despite often co-existing. The case-control study of women with a clinical diagnosis of nipple and breast thrush by Panjaitan and colleagues which only used molecular techniques from nipple swabs and excluded women with clinical signs of bacterial infection (exudate on nipple or inflamed breast), found that *S. aureus* was present in equal numbers of cases and controls.[35]

Clinical implications

Diagnostic skills are needed to make the diagnosis of nipple/breast candidiasis; clinicians should routinely consider all causes of nipple and breast pain, in the same way they consider differential diagnoses when assessing a patient with chest pain.[36] Women with nipple damage

or with nipple vasospasm describe pain that is burning in quality; in the past this has often been misdiagnosed as Candida infection.[37] The pain clinically associated with Candida infection is persistent, ranges from mild to severe, and is not relieved by the use of nipple shields or expressing/pumping, or applying heat. When the pain is related directly to infant feeding the cause is likely to be mechanical, and when the pain is relieved by heat, vasospasm is the likely cause.[19] We found that nipple damage was associated with burning nipple and radiating breast pain, so clinicians should be cautious about diagnosing infection (whether fungal or bacterial) in every woman with nipple damage.

Strengths and limitations

The strength of this study is that a cohort of healthy women who had not previously breastfed was recruited prior to commencing breastfeeding and was followed closely until two months postpartum. It is the first prospective longitudinal study to examine simultaneously both *S. aureus* and *Candida* spp. in a cohort of breastfeeding women and their infants.

The main limitation is that we did not have a clinical diagnosis of nipple/breast thrush and had to use a case definition based on two symptoms to estimate this condition. Participants responded to questions about pain and nipple blanching, and research assistants reported nipple appearance, but these measures could not substitute for a clinical assessment. Furthermore infant oral anatomy was not examined to exclude tongue-tie, and breastfeeds were not observed. We are not implying that all women with burning nipple and breast pain had a clinical diagnosis of nipple/breast thrush. We hypothesise that Candida is associated with nipple/breast pain in some women, in a similar manner to the relationship between Candida and vulvovaginal symptoms: Candida is a commensal in some women, while other women experience significant pain when only small numbers of organisms are present.[30]

Another limitation is that we only followed the cohort for eight weeks postpartum – with microbiological data to only four weeks postpartum; women who developed problems after this time period were not captured in our data collection.

Conclusions

This large cohort study provides evidence that *Candida* spp. play a role in nipple and breast pain in lactating women,, however, burning nipple pain is common in breastfeeding women, and a diagnosis of *Candida* spp. infection should not be made without considering differential diagnoses.[4] Further research into the role of staphylococci in breast pain in lactating women with inflammatory symptoms and without is needed. Animal models, as have been used in vulvovaginal candidiasis,[30]may be required to fully understand the pathogenesis of this condition. Future researchers may consider the RCTs for treatment or clearance of Candida.

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Contributors: LHA conceived the study, which was designed in collaboration with all authors. SMD managed the data and conducted the statistical analyses. SMG, SNT, CMB and MSP provided microbiological expertise. MC was the project co-ordinator. MSP was the research scientist.

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Ethical approval: This study was approved by the La Trobe University Human Ethics Committee (06-078); Human Research Ethics Committee of the Royal Women's Hospital

(06/41); Human Research Ethics Committee of the University of Melbourne (1033949); and Medical Advisory Committee at Frances Perry House.

Data sharing: No additional data available.

Table 1 Characteristics of nulliparous women recruited in late pregnancy

Maternal characteristics (n = 346)	n (%)
Hospital	
Royal Women's Hospital (public)	154 (44.5)
Frances Perry House (private)	192 (55.5)
Age (years - mean, SD, range)	32.7 (4.1, 19 - 44)
Marital status	
Married	229 (66)
Unmarried , living with partner	103 (30)
Not living with partner	2 (1)
Separated / divorced	1 (0)
Single	11 (3)
Education level	
Tertiary degree or higher	267 (77)
Other	79 (23)
Gestation at recruitment (weeks - mean, SD, range)	37 (1.3,34 - 42)
Gestation at birth (weeks - mean, SD, range)	39 (1.2, 36 - 42)
Breastfeeding intention (months - mean, range)	9.7 (1 - 24)
Caesarean birth	156 (45)
Baby sex – male	168 (49)
Any breast milk feeding at 8 weeks postpartum (n = 340)	320 (94)

Table 2 Results from microbiological analysis of specimens collected from 346 women and their infants (at any time point)

	Culture positive	PCR positive	Either culture/PCR positive
Nipple*			
C. albicans	13	15	19
C. glabrata	1	3	4
Candida spp.	9	115	120
Any *Candida* spp.	21	116	125
S. aureus	206 (60%)	N/A	N/A
Breast milk**			
C. albicans	9	N/A	N/A
C. glabrata	1	N/A	N/A
Candida spp.	10	N/A	N/A
Any *Candida* spp.	18	N/A	N/A
S. aureus	186 (54%)	N/A	N/A
Infant nose/mouth**			
C. albicans	15	N/A	N/A
C. glabrata	0	N/A	N/A
Candida spp.	5	N/A	N/A
Any *Candida* spp.	18	N/A	N/A
S. aureus	253 (73%)	N/A	N/A
Any *Candida* spp. in nipple/breast milk	131 (38%)		
Any *Candida* spp. in nipple/breast milk/baby	133 (38%)		
Any *S. aureus* in nipple/breast milk	231 (67%)		
Any *S. aureus* in nipple/breast milk/baby	277 (80%)		

* Nipple swabs collected at late pregnancy, hospital, weeks 1, 2, 3 and 4.
** Breast milk, and infant nasal and oral swabs, collected at hospital, weeks 1, 2, 3 and 4.
N/A = not applicable.

Table 3– Candida positive on PCR or culture at each visit

	Candida PCR positive	Candida PCR negative	Total
Candida culture positive	88	41	129
Candida culture negative	169	1,817	1,986
Total	257	1,858	2,115

Table 4 Case definition and *Candida* spp. (culture or PCR) and *S. aureus* separately (n = 346)

Nipple/breast thrush case definition: Burning nipple pain weeks plus breast pain (non-mastitis) **Weeks 1- 8**		
	***Candida* spp. in culture/PCR (nipple/breast milk/baby)[a]**	
Nipple/breast thrush case definition	Yes (n = 127)	No (n = 219)
Yes (n = 65)	31 (48%)	34 (21%)
No (n = 281)	96 (34%)	185 (66%)
	***S. aureus* (nipple/breast milk/baby)[b]**	
Nipple/breast thrush case definition	Yes (n =274)	No (n =72)
Yes (n = 65)	52 (80%)	13 (20%)
No (n = 281)	222 (79%)	59 (21%)
Nipple/breast thrush case definition: Burning nipple pain weeks plus breast pain (non-mastitis) **Weeks 2- 8**		
	***Candida* spp. in culture/PCR (nipple/breast milk/baby)[c]**	
Nipple/breast thrush case definition	Yes (n = 127)	No (n = 219)
Yes (n = 50)	26 (52%)	24 (48%)
No (n = 296)	101 (34%)	195 (66%)
	***S. aureus* (nipple/breast milk/baby)[d]**	
Nipple/breast thrush case definition	Yes (n = 274)	No (n = 72)
Yes (n =50)	41 (82%)	9 (18%)
No (n = 296)	233 (79%)	63 (21%)
Nipple/breast thrush case definition: Burning nipple pain weeks plus breast pain (non-mastitis), *excluding vasospasm* **Weeks 2- 8**		
	***Candida* spp. in culture/PCR (nipple/breast milk/baby)[e]**	
Nipple/breast thrush case definition, excluding vasospasm	Yes (n = 127)	No (n = 219)
Yes (n = 48)	26 (54%)	22 (46%)
No (n = 298)	101 (34%)	197 (66%)
	***S. aureus* (nipple/breast milk/baby)[f]**	
Nipple/breast thrush case definition, excluding vasospasm	Yes (n = 274)	No (n = 72)
Yes (n =48)	40 (83%)	8 (17%)

No (n = 298)	234 (79%)	64 (21%)

Nipple/breast thrush case definition: Burning nipple pain weeks plus breast pain (non-mastitis) **Weeks 1- 8**		
	***Candida* spp. in culture *only* (nipple)[g]**	
Nipple/breast thrush case definition	Yes (n = 21)	No (n = 325)
Yes (n = 65)	9 (14%)	56 (86%)
No (n = 281)	12 (4%)	269 (96%)

[a]$Chi^2(1) = 4.1587$, p = 0.041
[b]$Chi^2(1) = 0.0318$, p = 0.858
[c]$Chi^2(1) = 5.8850$, p = 0.015
[d]$Chi^2(1) = 0.2799$, p = 0.597
[e]$Chi^2(1) = 7.3142$, p = 0.007
[f]$Chi^2(1) = 0.5804$, p = 0.446
[g]$Chi^2(1) = 8.4905$, p = 0.004

Table 5 Time-to-event analysis of predictors of first symptoms of case definition

		Events[1]	**Years at risk[2]**	**Rate Ratio (95%CI)**	**p-value**	**Multivariate Rate Ratio (95%CI)**	**p-value**
Candida (nipple/milk/baby)	No	35	18.3				
	Yes	23	6.4	1.87 (1.10, 3.16)	0.018	2.03 (1.19, 3.45)	0.009
***S. aureus* (nipple/milk)**	No	19	10.5				
	Yes	39	14.2	1.53 (0.88, 2.64)	0.128	1.415 (0.80, 2.46)	0.234
Nipple damage	No	11	8.7				
	Yes	47	16.1	2.3 (1.19, 4.43)	0.012	2.39 (1.21, 4.70)	0.012

[1]Women with case definition in the first 4 weeks postpartum

[2]Total observed time between birth and first symptoms of case definition or 4 weeks postpartum (whichever occurred first)

Title:

~~What role d~~Does Candida and/or Staphylococcus play a role in nipple and breast pain in lactation? A cohort study in Melbourne, Australia

Lisa H Amir[1], Susan M Donath[2], Suzanne M Garland[2,3], Sepehr N Tabrizi[2,3], Catherine M Bennett[4], Meabh Cullinane[1], Matthew S Payne[1,5]

[1]Mother & Child Health Research, La Trobe University, Melbourne, VIC, Australia
[2]Murdoch Childrens Research Institute, Melbourne, VIC, Australia
[3]Department of Molecular Microbiology, Microbiology and Infectious Diseases, Royal Women's Hospital and Department of Obstetrics and Gynaecology, University of Melbourne, VIC, Australia
[4]Deakin Population Health, Deakin University, Burwood, VIC, Australia
[5]School of Women's and Infants' Health, University of Western Australia, WA, Australia

Abstract

Objective: To investigate ~~the roles of~~ *Candida* species and *Staphylococcus aureus* and~~in~~ the development of "nipple and breast thrush" among breastfeeding women.

Design: Prospective longitudinal cohort study.

Setting: Two hospitals in Melbourne, Australia (one public, one private) with follow-up in the community.

Participants: 360 nulliparous women recruited at ≥ 36 weeks gestation from November 2009 to June 2011. Participants were followed-up six times: in hospital, at home weekly until four weeks postpartum and by telephone at eight weeks.

Main outcome measures: ~~A researcher defined proxy diagnosis of "~~ Case definition "nipple and breast thrush": burning nipple pain and breast pain (not related to mastitis); detection of

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Candida spp. (using culture and PCR) from the mother's vagina, nipple or breast milk or baby's mouth; detection of *S. aureus* from the mother's nipple or breast milk.

Results: Women with the case definition of nipple/breast thrush were more likely to have *Candida* spp. in nipple/breast milk/baby oral samples (54%) compared to other women (36%, p = 0.014). *S. aureus* was common in nipple/breast milk/baby samples of women with these symptoms as well as women without these symptoms (82% vs 79%) (p = 0.597). Time-to-event analysis examined predictors of nipple/breast thrush up to and including the time of data collection. Candida in nipple/breast milk/baby predicted incidence of the case definition (Rate Ratio 1.87 (95% CI: 1.10, 3.16, p = 0.018). ~~The crude Relative Risk of *Candida* spp. in nipple/breast milk/baby was 1.87 (95% CI: 1.10, 3.16, p = 0.018); the multivariate RR (adjusted for *S. aureus* in nipple/breast milk and nipple damage) was almost unchanged at 2.03 (95% CI: 1.19, 3.45, p = 0.009).~~ We don't have evidence that *S. aureus* colonisation was ~~not~~ a predictor of these symptoms (RR 1.53, 95% CI: 0.88, 2.64, p = 0.13)~~, with little change in the multivariate model~~. Nipple damage was also a ~~strong~~ predictor of these symptoms, RR 2.30 (95% CI: 1.19, 4.43, p = 0.012). ~~, with little change in multivariate model, indicating that *Candida* spp., *S. aureus* and nipple damage are operating independently.~~ In the multivariate model, with all three predictors, the Rate Ratios were very similar to the univariate Rate Ratios. This indicates that Candida and nipple damage are independent predictors of our case definition.

Background

Controversy about the condition known as "breast thrush" or breast candidiasis in lactating women has led to confusion among clinicians and the community. While some clinicians may diagnose and treat this condition in breastfeeding women with deep, radiating breast pain

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associated with burning nipple pain,[1-6] others doubt the relationship with fungal organisms[7-11] and decry "the alarming trend towards believing that fungi are important in the aetiology of breast infection and deep breast pain associated with breast feeding, despite a lack of good quality evidence".[11] p. 485. ~~Health professionals may tell women that the pain is "all in their head", which is reminiscent of the lack of understanding of mastalgia in the 1970s, when breast pain was thought to be a psychosomatic complaint by "neurotic" women.[12]~~

Unlike mastitis which is diagnosed when a breastfeeding woman experiences inflammation of the breast associated with systemic symptoms,[12] breast thrush is usually diagnosed when the breast is not erythematous or indurated , and the woman is afebrile and systemically well apart from a typical burning pain radiating into the breast and/or into the back.[4] While some authors use the term 'candida mastitis',[6 11 13] we feel this is misleading, as inflammation of the breast is not evident. Although some clinicians attribute the pain to infection with ~~S~~*Staphylococcus~~ ~~aureus* (*S. aureus*) and treat women with long-term antibiotics,[14] this has not been tested in trials.

The primary cause of the nipple pain or damage is often the process of breastfeeding itself: trauma from the infant's mouth due to incorrect attachment, or infant anatomy or dysfunctional suck.[15] Nipple thrush is usually diagnosed when the nipple/areola is slightly pink, sensitive to touch, and the pain described is out of proportion to the damage seen on clinical examination.[4] When the areola is described as itchy and appears red and/or crusty, the diagnosis is dermatitis/eczema rather than fungal infection.[16] A nipple with obvious damage is almost certainly colonised with *S. aureus*.[17] Nipple/breast pain associated with nipple blanching persisting for longer than a few seconds is likely to be nipple vasospasm; this condition is commonly confused with breast thrush because of the burning, radiating nature

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of the pain.[18 19] The pain from vasospasm is often secondary to nipple damage or infection, exacerbated by cold, and relieved by heat or nifedipine.[20] In practice, more than one cause of nipple or breast pain is commonly present,[6] which makes it difficult to construct a case definition~~n algorithm~~ for "breast thrush" for research purposes.

Previous breastfeeding studies have been largely cross-sectional,[1 7 9 10] whilst one longitudinal study collected microbiological data, but no clinical information.[21] This is the first prospective longitudinal study examining~~of the role of~~ both *S. aureus* and *Candida* spp. in breast infections and was designed to resolve the current controversy surrounding which is the primary organism responsible for the condition known as "breast thrush": *Candida* spp. or *S. aureus*?

Methods

The CASTLE (Candida and Staphylococcus Transmission: Longitudinal Evaluation) study was a prospective longitudinal descriptive study~~: designed to investigate the role of staphylococci and/or candida in nipple and breast pain, and the relationship between breastfeeding, postpartum health problems and maternal psychological well-being. D~~ details have been published in the study protocol.[22] A cohort of 360 nulliparous women planning to breastfeed for at least two months were recruited at ≥ 36 weeks gestation from two hospitals in Melbourne, Australia (November 2009 to June 2011). At recruitment, nasal, nipple (both breasts) and vaginal swabs were collected and participants completed a questionnaire asking about previous staphylococcal and candida infections. Following birth, participants were followed-up six times: face-to-face in hospital, then weekly at home until four weeks postpartum. Participants filled out a questionnaire at each time point to collect information about breastfeeding problems and postpartum health problems. At each visit, maternal nasal,

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and nipple swabs and breast milk samples (both breasts) and infant oral and nasal swabs were collected. At a final telephone interview at eight weeks postpartum information about breastfeeding problems and postpartum health was collected.

Specimens were collected by research assistants. Fresh gloves were worn for each specimen. After sanitising their hands, research assistants collected nipple swabs then washed the nipple/areola region twice using sterile water wipes. Mid-stream milk was collected into a sterile container; the first drops of breast milk were expressed and discarded. ~~Briefly, t~~Two nipple swabs were obtained from each nipple; a standard charcoal swab for microbiological analysis (Copan Diagnostics Inc. CA, USA) and a flocked swab for molecular analysis (Copan Diagnostics Inc. CA, USA). After first moistening in sterile saline, both the standard and flocked nipple swabs were rolled over the nipple and areola together using a 10-point swabbing technique,[23] paying particular attention to any cracks / fissures present. Oral and vaginal swabs were collected for culture of *S. aureus* and *Candida* spp. Breast milk samples were also cultured for *S. aureus*, coagulase-negative staphylococci (CoNS) and *Candida* spp; nasal swabs were collected for culture of *S. aureus* only.

In studies conducted in women with vulvovaginal symptoms, molecular microbiological techniques have been useful in detecting Candida in women who were negative using standard microbiology.[24 25]~~.~~ Therefore we planned to use molecular techniques to increase detection of *Candida* spp. in nipple specimens.[22] Due to cost constraints we did not plan to use molecular techniques for the milk specimens (up to 4000 specimens). As participants only had one or two vaginal specimens we extracted DNA ~~was extracted~~ from vaginal as well as nipple ~~and vaginal~~ swabs for molecular identification of *Candida* spp. using real-time PCR.[22]

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At each contact, women were asked about nipple pain ("In the last 48 hours, have you been experiencing **nipple** pain/discomfort?) and whether it was burning in quality ("If yes, would you describe your nipple pain as burning?"), clinical signs and symptoms of mastitis as used in previous research (i.e. redness, fever, etc.),[26] and other types of breast pain ("Have you had **other** breast pain in the last 2 days? No/ I have had stabbing (radiating or shooting) breast pain **only**/ I have had non-stabbing breast pain **only**/ I have had both stabbing and non-stabbing breast pain"), ~~such as radiating ("stabbing") or non-stabbing~~. We also asked "Do you have nipple vasospasm? (Nipple blanches or goes white in the cold or during/after feeds) No/ Yes, for less than 5 minutes/ Yes, for more than 5 minutes/ Not sure"). Researchers also collected clinical observations of nipple/areola and breast at each visit (weeks 1 to 4), including colour of nipple/areola. Our case definition of ~~We defined~~ "nipple and breast thrush" used ~~as~~ a combination of burning nipple pain and breast pain (non-mastitis). Francis-Morrill et al found nipple appearance ("shiny" or flaky" nipple~~)~~ or pink colour) to be predictive of Candida.[27] However, in our study very few women were described as having these appearances (shiny, n = 4, flaky, n = 17), while 140 women were described as having "pink" nipple/s. Adding ~~making~~ the appearance identifiers ~~unlikely to be helpful in~~to our case definition was not helpful.~~the algorithm. Therefore we used a combination of burning nipple pain and breast pain (non-mastitis) as a proxy for a clinical diagnosis of "nipple and breast thrush".~~ Clinically, the symptoms of nipple and breast thrush develop after the first week postpartum, and since most pain in the first week postpartum in first time mothers is likely to be due to adjusting to breastfeeding, we examined the case definition at weeks 1 to 8, and weeks 2 to 8 separately.

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Statistical analysis was conducted using Stata Version 12. Hypothesis 1 — Women with nipple/breast thrush are more likely to have *Candida* spp. isolated than other women; hypothesis 2 — Women with nipple/breast thrush are more likely to have *S. aureus* isolated than other women. A sample of 318 women was estimated to provide adequate power. [22] Chi-squared tests were used for comparing categorical variables. We investigated incidence of nipple/breast thrush using a multivariable discrete version of the proportional hazards regression model.[28] Outcome variable was the incidence of new cases of our nipple and breast thrush definition~~diagnosis~~; time-varying predictors were: the presence of *Candida* spp., presence of *S. aureus* and mother-reported nipple damage. We present crude Rate~~elative~~ Ratios~~isks~~ (RR), and multivariate analysis, adjusting for the presence of *Candida* spp, *S. aureus* and nipple damage.

Results relating to mastitis, other breastfeeding and postpartum problems will be published separately (papers in preparation).

Results

Fourteen women withdrew from the study after giving birth, leaving 346 (96%) women available for data collection at subsequent visits; 340 (94%) women completed the study at 8 weeks postpartum. Women who participated in the study were highly educated (77% had tertiary degree or higher) and most were married or lived with their partner (96%) (Table 1). Just over half gave birth in the private hospital (56%), 45% by Caesarean section.

Burning nipple pain was reported by 42% of women (146/346) during weeks 1 to 8, or 32% women (111/346) during weeks 2 to 8. Radiating or non-radiating breast pain (not related to engorgement/mastitis) was reported by 54% of women (186/346) during weeks 1 to 8, or

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47% of women (162/346) during weeks 2 to 8. Combining these symptoms to estimate the proportion of women with both burning nipple pain and non-mastitis breast pain (at the same time): 19% of women in weeks 1 to 8 (65/346), or 15% in weeks 2 to 8 (50/346).

Candida albicans was the most commonly isolated *Candida* spp in culture with *Candida glabrata* only isolated in one nipple specimen and one milk sample (Table 2). Although "other Candida spp." were found, none speciated as *Candida krusei* or *Candida kefyr*. *Candida* PCR of the nipple was positive for *Candida* spp. in 33% of women (115/346); in contrast to culture of only 3% of women (9/346) being positive for *Candida* spp.. *Candida* spp. were isolated by culture from breast milk samples from 5% women (18/346), but no molecular analyses were conducted on these samples. Table 3 shows Candida isolated by culture and by PCR at each visit. *S. aureus* was isolated by culture from the nipple and/or breast milk in 67% of women (231/346), and from nose and/or mouth of 73% of infants (253/346) at some point during follow-up. There were 22 milk samples positive for Candida, of which 10 were milk only, and 12 were positive for nipple and milk. For *S. aureus*, 425 milk samples were positive, of which 89 were positive in milk only.

~~As b~~Burning nipple pain was very common ~~if~~in week 1, primarily as women adjusted to breastfeeding ~~were included~~, therefore we explored the relationship between *Candida* spp. and *S. aureus* using two case definitions: weeks 1-8 and weeks 2-8 (Table ~~3~~4). ~~we developed a proxy diagnosis for nipple/breast thrush if women had burning nipple pain as well as breast pain (non-mastitis) between weeks 2 and 8.~~ There was a statistically significant association between these symptoms ~~-~~ in weeks 2-8 and *Candida* spp. in nipple/breast milk/baby ($p = 0.014$~~, see Table 3~~); as also for *Candida* spp. in vagina/nipple/breast milk/baby ($p = 0.047$, not shown). ~~There was evidence that~~ *S. aureus* was common in nipple/breast milk/baby

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samples of ~~was higher in w~~women with these symptoms as well as ~~than other~~ women without these symptoms (82~~78~~% vs 79~~65~~%) (p = 0.597~~0.068~~, Table ~~3~~4).

As women with nipple vasospasm describe a burning, radiating pain, we also analysed the case definition excluding women with vasospasm (see Table 4). Only two women were removed from the analysis (n= 48), which made little difference to the results.

Time-to-event analysis examined predictors of our case definition of nipple/breast thrush (burning nipple and breast pain [~~(~~non-mastitis]~~)~~) up to and including the time of data collection in the first four weeks. (See unadjusted survival curves: Figures 1, 2 and 3 and Table 5). Candida in nipple/breast milk/baby predicted incidence of the case definition (~~The crude~~ Rate~~elative~~ Ratio~~isk of candida in nipple/breast milk/baby was~~ 1.87 (95% CI: 1.10, 3.16, p = 0.018). Thus, for women with Candida in nipple/milk/baby at any time point, the rate of subsequently developing the case definition was increased by 87%, compared to women without Candida~~; the multivariate RR (adjusted for *S. aureus* in nipple/breast milk and nipple damage) was almost unchanged at 2.03 (95% CI: 1.19, 3.45, p = 0.009).~~ The evidence for *S. aureus* colonisation as~~was not~~ a predictor of these symptoms was not strong (RR 1.53, 95% CI: 0.88, 2.64, p = 0.13)~~, with little change in the multivariate model~~. Mothers' report of nipple damage was ~~also~~ a ~~strong~~ predictor of these symptoms, with a RR 2.30 (95% CI: 1.19, 4.43, p = 0.012)~~, with little change in the multivariate model, which~~ In the multivariate model, with all three predictors, the Rate Ratios were very similar to the univariate Rate Ratios. This indicates that Candida~~, *S. aureus*~~ and nipple damage are ~~operating~~ independent~~ly~~ predictors of our case definition.

Discussion

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Principal findings

Unlike some cross-sectional studies which found no relationship between the presence of *Candida* spp. and the condition known as breast thrush,[9 10] we have shown that *Candida* spp. is associated with burning nipple pain and breast pain – in two analyses ('at any time' and 'time-to-event'). As in previous studies,[1 7] we uncommonly isolated *Candida* spp. on the nipple using standard microbiological culture techniques. However, *Candida* spp. were more commonly identified using more sensitive molecular techniques (real-time PCR). This test is not used in routine practice currently, and therefore it is not helpful in making the diagnosis in clinical care at this time. *Candida* spp.~~Candida~~ were also rarely isolated in breast milk, using standard techniques. It is possible that the isolation rate would have been higher if we had used the technique of Morrill and colleagues, who added iron to inactivate milk lactoferrin.[29] However, Hale et al were unable to identify Candida in breast milk of women with 'Candida mastitis' using culture and another specific technique (presence of 1→3-β-D-glucan). [10] The 16 cases had 'sore, inflamed, or traumatized nipples, intense stabbing or burning pain that radiated into the axilla often persisting after feeding, and painful feeding without alternate diagnosis'.[10] Possible explanations for Hale et al's findings are that the women had other causes of their pain (from the traumatised nipple, from maternal vasospasm, or infant posterior tongue-tie or other mechanical causes of pain); that Candida was present in the nipple (samples were only collected from the milk), or that Candida is not present in the milk of women with this syndrome. The question as to whether Candida is present in the lactiferous ducts is still open to debate; what we have shown is a link between Candida and nipple/breast pain – independent of the presence of nipple damage or *S. aureus*.

Candida spp. are commensal organisms, and therefore the presence of *Candida* spp. does not always imply infection. Recent understanding of vulvovaginitis postulates that the threshold

number of organisms for symptomatic vaginitis varies for different groups of women; women with infrequent vaginitis have a higher threshold.[30] Furthermore, the symptoms associated with vaginitis may be caused by the host neutrophil response; small numbers of organisms may promote an aggressive inflammatory response in some women.[30]

~~Diagnostic skills are needed to make the diagnosis of nipple/breast candidiasis; clinicians should routinely consider all causes of nipple and breast pain, in the same way they consider differential diagnoses when assessing a patient with chest pain.[28]~~

Field Code Changed

Consistent with other studies of mothers and infants,[21 31-34] colonisation with *S. aureus* is common; at least 50% of women were colonised with *S. aureus* in nipple or milk samples by four weeks postpartum. Therefore in clinical practice, a finding of *S. aureus* on the nipple or breast milk is not evidence that the bacteria are the principal cause of the woman's pain. Our analysis indicates that candida and *S. aureus* are acting independently, despite often co-existing. The case-control study of women with a clinical diagnosis of nipple and breast thrush by Panjaitan and colleagues which only used molecular techniques from nipple swabs and excluded women with clinical signs of bacterial infection (exudate on nipple or inflamed breast), found that *S. aureus* was present in equal numbers of cases and controls.[35]

Clinical implications

Diagnostic skills are needed to make the diagnosis of nipple/breast candidiasis; clinicians should routinely consider all causes of nipple and breast pain, in the same way they consider differential diagnoses when assessing a patient with chest pain.[36] Women with nipple damage or with nipple vasospasm describe pain that is burning in quality; in the past this has often been misdiagnosed as Candida infection.[37] The pain clinically associated with Candida

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infection is persistent, ranges from mild to severe, and is not relieved by the use of nipple shields or expressing/pumping, or applying heat. When the pain is related directly to infant feeding the cause is likely to be mechanical, and when the pain is relieved by heat, vasospasm is the likely cause.[19] We found that nipple damage was associated with burning nipple and radiating breast pain, so clinicians should be cautious about diagnosing infection (whether fungal or bacterial) in every woman with nipple damage.

Strengths and limitations

The strength of this study is that a cohort of healthy women who had not previously breastfed was recruited prior to commencing breastfeeding and was followed closely until two months postpartum. It is the first prospective longitudinal study to examine simultaneously both *S. aureus* and *Candida* spp. in a cohort of breastfeeding women and their infants.

The main limitation is that we did not have a clinical diagnosis of nipple/breast thrush and had to use a case definition based on two symptoms~~proxy diagnosis~~ to estimate this condition. Participants responded to questions about pain and nipple blanching, and research assistants reported nipple appearance, but these measures could not substitute for a clinical assessment. Furthermore infant oral anatomy was not examined to exclude tongue-tie, and breastfeeds were not observed. We are not implying that all women with burning nipple and breast pain had a clinical diagnosis of nipple/breast thrush. We hypothesise that Candida is associated with nipple/breast pain in some women, in a similar manner to the relationship between Candida and vulvovaginal symptoms: Candida is a commensal in some women, while other women experience significant pain when only small numbers of organisms are present.[30]

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Another limitation is that we only followed the cohort for eight weeks postpartum – with microbiological data to only four weeks postpartum; women who developed problems after this time period were not captured in our data collection.

Conclusions

This large cohort study provides evidence~~confirms~~ that *Candida* spp. play a role in nipple and breast pain in lactating women, ~~and "thrush in the breast" should not be dismissed as "psychosomatic" as has been stated by some clinicians. B~~, however, burning nipple pain is common in breastfeeding women, and a diagnosis of *Candida* spp. infection should not be made without considering differential diagnoses.[4] Further research into the role of staphylococci in breast pain in lactating women with inflammatory symptoms and without is needed. Animal models, as have been used in vulvovaginal candidiasis,[30] ~~-~~may be required ~~-~~to ~~To~~ fully understand the pathogenesis of this condition.~~,~~ Future researchers may consider the ~~use of animal models~~ RCTs for treatment or clearance of Candida.

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Contributors: LHA conceived the study, which was designed in collaboration with all authors. SMD managed the data and conducted the statistical analyses. SMG, SNT, CMB and MSP provided microbiological expertise. MC was the project co-ordinator. MSP was the research scientist.

Competing interests: All authors have completed the ICMJE uniform disclosure form at www.icjme.org/coi_disclosure.pdf (available on request from the corresponding author) and declare that: this stud received financial support from the National Health & Medical Research Council (NHMRC) (project grant ~~502053~~541907, equipment grant, Health Professional Training Fellowship (LHA)), Helen Mcpherson Smith Trust, Faculty Research Grant, Faculty of Health Sciences, La Trobe University; no financial relationships with any

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organisations that might have an interest in the submitted work; and no other relationships or activities that could appear to have influenced the submitted work.

Ethical approval: This study was approved by the La Trobe University Human Ethics Committee (06-078); Human Research Ethics Committee of the Royal Women's Hospital (06/41); Human Research Ethics Committee of the University of Melbourne (1033949); and Medical Advisory Committee at Frances Perry House.

Data sharing: No additional data available.

Article summary

Article Focus

- Controversy about the condition known as "breast thrush" or breast candidiasis in lactating women has led to confusion among clinicians and the community.
- Previous studies have been cross-sectional.
- This is the first prospective longitudinal study to examine simultaneously both ~~of the role of both~~ *S. aureus* and *Candida* spp. in breast infections and was designed to resolve the current controversy surrounding which is the primary organism responsible for the condition known as "breast thrush": *Candida* spp. or *S. aureus*?

Key Messages

- *Candida* spp. is associated with burning nipple pain and breast pain.
- Colonisation with *S. aureus* is common; at least 50% of women were colonised with *S. aureus* in nipple or milk samples by four weeks postpartum.

Strengths and Limitations

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- The evidence of microbiological data from this large cohort of women over four weeks postpartum is stronger than previous smaller cross-sectional studies.
- *Candida* spp. were more commonly identified using more sensitive molecular techniques (real-time PCR) than by using standard microbiological culture techniques.
- As these techniques are not used in clinical practice currently, clinicans should continue to use their clinical skills to diagnose causes of nipple and breast pain in lactating womenthis is not currently useful for clinicians.

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Table 1 Characteristics of nulliparous women recruited in late pregnancy

Maternal characteristics (n = 346)	n (%)
Hospital	
Royal Women's Hospital (public)	154 (44.5)
Frances Perry House (private)	192 (55.5)
Age (years - mean, SD, range)	32.7 (4.1, 19 - 44)
Marital status	
Married	229 (66)
Unmarried , living with partner	103 (30)
Not living with partner	2 (1)
Separated / divorced	1 (0)
Single	11 (3)
Education level	
Tertiary degree or higher	267 (77)
Other	79 (23)
Gestation at recruitment (weeks - mean, SD, range)	37 (1.3,34 - 42)
Gestation at birth (weeks - mean, SD, range)	39 (1.2, 36 - 42)
Breastfeeding intention (months - mean, range)	9.7 (1 - 24)
Caesarean birth	156 (45)
Baby sex – male	168 (49)
Any breast milk feeding at 8 weeks postpartum (n = 340)	320 (94)

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Table 2 Results from microbiological analysis of specimens collected from 346 women and their infants (at any time point)

	Culture positive	PCR positive	Either culture/PCR positive
Nipple*			
C. albicans	13	15	19
C. glabrata	1	3	4
Candida spp.	9	115	120
Any *Candida* spp.	21	116	125
S. aureus	206 (60%)	N/A	N/A
Breast milk**			
C. albicans	9	N/A	N/A
C. glabrata	1	N/A	N/A
Candida spp.	10	N/A	N/A
Any *Candida* spp.	18	N/A	N/A
S. aureus	186 (54%)	N/A	N/A
Infant nose/mouth**			
C. albicans	15	N/A	N/A
C. glabrata	0	N/A	N/A
Candida spp.	5	N/A	N/A
Any *Candida* spp.	18	N/A	N/A
S. aureus	253 (73%)	N/A	N/A
Any *Candida* spp. in nipple/breast milk	131 (38%)		
Any *Candida* spp. in nipple/breast milk/baby	133 (38%)		
Any *S. aureus* in nipple/breast milk	231 (67%)		
Any *S. aureus* in nipple/breast milk/baby	277 (80%)		

* Nipple swabs collected at late pregnancy, hospital, weeks 1, 2, 3 and 4.
** Breast milk, and infant nasal and oral swabs, collected at hospital, weeks 1, 2, 3 and 4.
N/A = not applicable.

T~~New t~~able 3– Candida positive on PCR or culture at each visit~~.~~

	Candida PCR positive	Candida PCR negative	Total
Candida culture positive	88	41	129
Candida culture negative	169	1,817	1,986
Total	257	1,858	2,115

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Table ~~34~~ ~~Nipple/breast thrush symptoms~~Case definition and *Candida* spp. (culture or PCR) and *S. aureus* separately (n = 346)

Nipple/breast thrush case definition: Burning nipple pain weeks plus breast pain (non-mastitis) **Weeks 1- 8**		
	***Candida* spp. in culture/PCR (nipple/breast milk/baby)[a]**	
Nipple/breast thrush case definition	Yes (n = 127)	No (n = 219)
Yes (n = 65)	31 (48%)	34 (21%)
No (n = 281)	96 (34%)	185 (66%)
	***S. aureus* (nipple/breast milk/baby)[b]**	
Nipple/breast thrush case definition	Yes (n =274)	No (n =72)
Yes (n = 65)	52 (80%)	13 (20%)
No (n = 281)	222 (79%)	59 (21%)
Nipple/breast thrush case definition: Burning nipple pain weeks plus breast pain (non-mastitis) **Weeks 2- 8**		
	***Candida* spp. in culture/PCR (nipple/breast milk/baby)[c]**	
Nipple/breast thrush case definition	Yes (n = 127)	No (n = 219)
Yes (n = 50)	26 (52%)	24 (48%)
No (n = 296)	101 (34%)	195 (66%)
	***S. aureus* (nipple/breast milk/baby)[d]**	
Nipple/breast thrush case definition	Yes (n = 274)	No (n = 72)
Yes (n =50)	41 (82%)	9 (18%)
No (n = 296)	233 (79%)	63 (21%)
Nipple/breast thrush case definition: Burning nipple pain weeks plus breast pain (non-mastitis), *excluding vasospasm* **Weeks 2- 8**		
	***Candida* spp. in culture/PCR (nipple/breast milk/baby)[e]**	
Nipple/breast thrush case definition, excluding vasospasm	Yes (n = 127)	No (n = 219)
Yes (n = 48)	26 (54%)	22 (46%)
No (n = 298)	101 (34%)	197 (66%)
	***S. aureus* (nipple/breast milk/baby)[f]**	
Nipple/breast thrush case definition, excluding vasospasm	Yes (n = 274)	No (n = 72)
Yes (n =48)	40 (83%)	8 (17%)
No (n = 298)	234 (79%)	64 (21%)
Nipple/breast thrush case definition: Burning nipple pain weeks plus breast pain (non-mastitis) **Weeks 1- 8**		
	***Candida* spp. in culture *only* (nipple)[g]**	
Nipple/breast thrush case	Yes	No

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definition		(n = 21)	(n = 325)
	Yes (n = 65)	9 (14%)	56 (86%)
	No (n = 281)	12 (4%)	269 (96%)

[a]$Chi^2(1)$ =4.1587, p = 0.041
[b]$Chi^2(1)$ = 0.0318, p = 0.858
[c]$Chi^2(1)$ = 5.8850, p = 0.015
[d]$Chi^2(1)$ = 0.2799, p = 0.597
[e]$Chi^2(1)$ = 7.3142, p = 0.007
[f]$Chi^2(1)$ = 0.5804, p = 0.446
[g]$Chi^2(1)$ = 8.4905, p = 0.004

Table 5 Time-to-event analysis of predictors of first symptoms of case definition

		Events[1]	Years at risk[2]	Rate Ratio (95%CI)	p-value	Multivariate Rate Ratio (95%CI)	p-value
Candida (nipple/milk/baby)	No	35	18.3				
	Yes	23	6.4	1.87 (1.10, 3.16)	0.018	2.03 (1.19, 3.45)	0.009
***S. aureus* (nipple/milk)**	No	19	10.5				
	Yes	39	14.2	1.53 (0.88, 2.64)	0.128	1.415 (0.80, 2.46)	0.234
Nipple damage	No	11	8.7				
	Yes	47	16.1	2.3 (1.19, 4.43)	0.012	2.39 (1.21, 4.70)	0.012

[1]Women with case definition in the first 4 weeks postpartum

[2]Total observed time between birth and first symptoms of case definition or 4 weeks postpartum (whichever occurred first)

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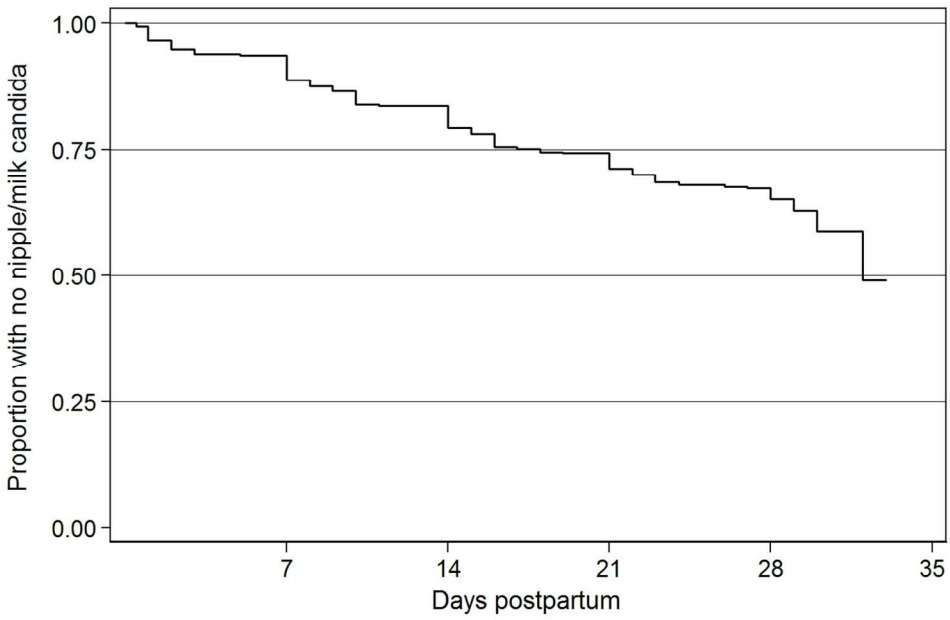
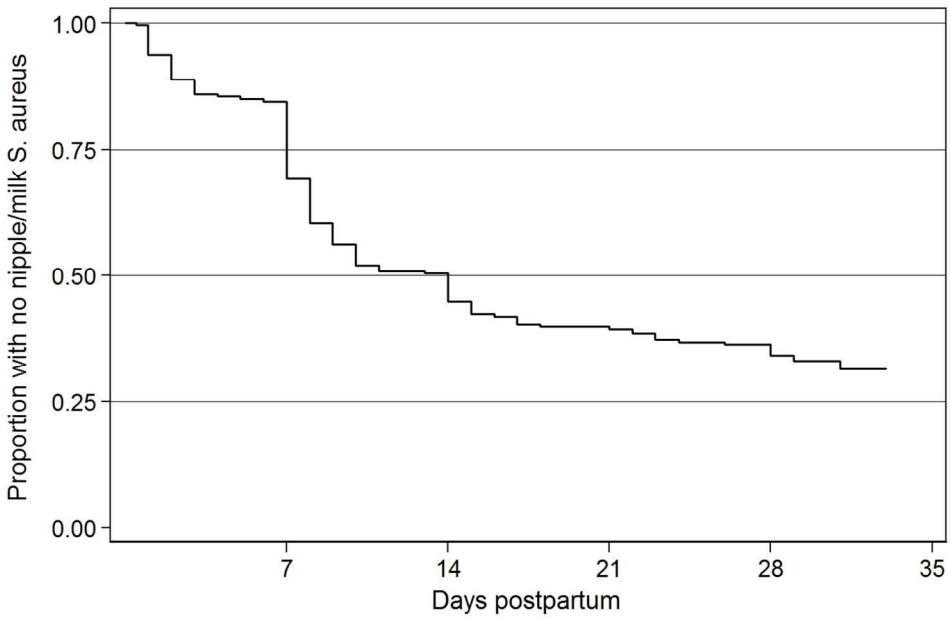
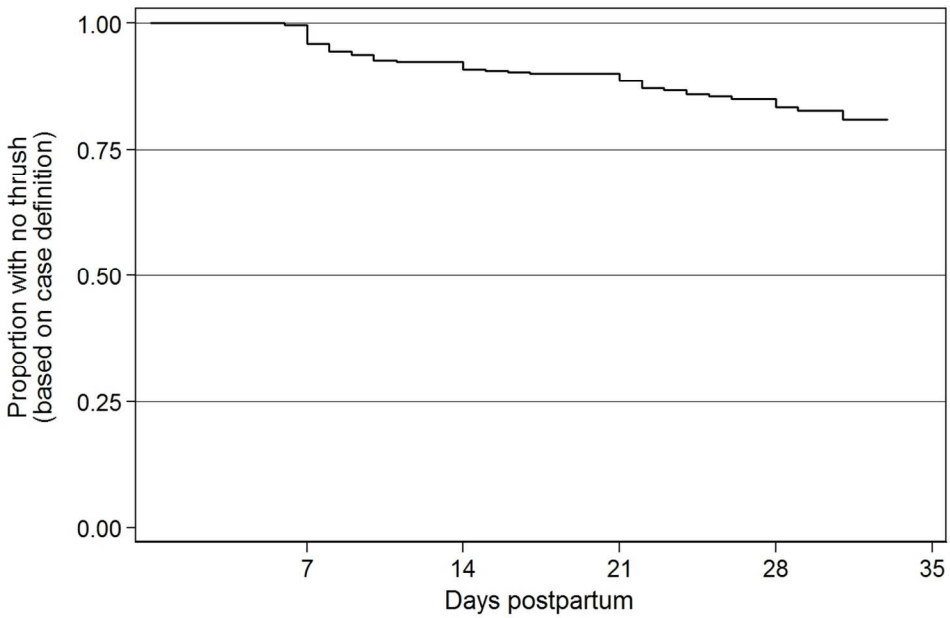


Survival curve for first time to nipple/milk Candida
134x90mm (300 x 300 DPI)



Survival curve for time to first nipple/milk S. aureus
134x90mm (300 x 300 DPI)



Survival curve for time to first symptoms of case definition of "breast thrush"
134x90mm (300 x 300 DPI)

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
Yes		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Yes		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Yes		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed **N/A**
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) If applicable, explain how loss to follow-up was addressed
		(*e*) Describe any sensitivity analyses
Results		
YES		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
		(c) Summarise follow-up time (eg, average and total amount)
Outcome data	15*	Report numbers of outcome events or summary measures over time
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included

		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
YES		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
YES		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.