PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Does Candida and/or Staphylococcus play a role in nipple and breast pain in lactation? A cohort study in Melbourne, Australia
AUTHORS	Amir, Lisa; Donath, Susan; Garland, Suzanne; Tabrizi, Sepehr; Bennett, Catherine; Cullinane, Meabh; Payne, Matthew

VERSION 1 - REVIEW

REVIEWER	Alison Stuebe, MD, MSc Assistant Professor University of North Carolina at Chapel Hill I have no competing interests to declare.
REVIEW RETURNED	12-Dec-2012

THE STUDY	the instruments used to define "nipple thrush" are not well described, and this classification appears to have been developed post hoc. The conclusions drawn are not supported by the data, and other studies casting doubt on the association between candida and breast pain are either not discussed (Hale et al) or not referenced -- eg "Andrews, J. I., D. K. Fleener, et al. (2007). "The yeast connection: is Candida linked to breastfeeding associated pain?" Am J Obstet Gynecol 197(4): 424 e421-424."
RESULTS & CONCLUSIONS	The authors sought to determine the association between breast pain and both candida and s aureus. However, their definition of breast pain is not clearly described or justified, and their techniques for assessing presence of candida and s aureus differ, without justification. The previous evidence is not adequately discussed. In addition, the authors use inflammatory language to suggest that clinicians who do not routinely diagnose and treat thrush are providing inappropriate care.
REPORTING & ETHICS	Policies regarding treatment of symptomatic women are not discussed.
GENERAL COMMENTS	This paper addresses an important clinical problem – maternal pain during breastfeeding – and draws on a rich longitudinal study design that includes assessment of maternal symptoms and collection of biological specimens to explore the association between microorganisms and pain in the first 8 weeks postpartum. The authors note in their introduction that the differential diagnosis for breast pain is broad, and includes infection, dermatitis, and vasospasm. However, a key weakness of the paper is the failure to explain how these diagnoses were considered in the analysis. Symptoms of burning pain are commonly associated with vasospasm, but there is no discussion of how mothers with vasospasm or with visible nipple damage were classified in the study. Were these women included in the “nipple thrush” group if they had burning pain or evidence of dermatitis?

	The authors further indicate that they had planned to use previously derived criteria of shiny, flaky nipple and pink color as diagnostic criteria for thrush, but found that the prevalence of shiny and flaky nipples was low, whereas pink color was very common (N=140), so they changed their case definition to “burning nipple pain and breast pain” in the absence of mastitis. However, they report that 111 women reported such pain in weeks 2 to 8. Thus the decision to ignore pink color but include burning pain seems arbitrary. Similarly, the authors chose to exclude burning pain in week 1 from their diagnostic criteria because it was “very common” (N=146) (page 7, line 18-19). Again, this appears to be arbitrary. Please justify these decisions based on the hypothesized underlying biology. Moreover, from the survival curve for time to first positive candida culture, it appears that no women at 7 days postpartum had a positive candida culture, despite the high prevalence of BBNP. Would the inclusion of symptoms at one week change the statistical significance of the observed association between candida sp and burning breast and nipple pain? The rationale for the use of a survival curve in a repeated measure study such as this one is also unclear. Presumably, some women had pain that resolved, or no longer had Candida or Staph present in follow-up. A graph demonstrating this fluctuation over time would be more informative than a survival curve. A strength of this study was recruitment of a prospective sample and the use of home visits to collect data and biological specimens. However, this approach may lead to detection of subclinical symptoms that were not distressing to the mother and did not interfere with breastfeeding. Was a threshold used for frequency and intensity of burning pain, or was any endorsement of burning pain considered symptomatic? And were women asked to consider multiple descriptors of pain character, or were they simply asked, “Is the pain burning?” The questions used to classify women as having burning nipple and breast pain should be included in the paper to clarify this issue. It is also unclear how women with burning pain were managed. Were they treated for presumed candida? If so, was the presence of candida on PCR / culture predictive of response to therapy? If not, what are the human subjects implications of identifying pathology and not offering therapy? Given the weaknesses of the case definition, there are significant concerns with terming the syndrome of burning breast and nipple pain “nipple and breast thrush.” The paper would be strengthened the term nipple and breast thrush were replaced with “burning breast and nipple pain (BBNP).” There are also concerns about the decision to use PCR to define “presence of candida Sp.” What is the clinical significance of the presence of candida via PCR that does not grow on culture? Does this reflect colonization? Non-viable spores? Or actual infection? Furthermore, the decision to perform PCR of nipple swabs, but not of milk samples or infant oral swabs, should be justified. If burning pain reflects ductal colonization with candida, as some have suggested, it would seem that PCR of milk, rather than of the nipple, would be the measure of interest.

	More detail should also be provided regarding cleansing of the nipple before collection of specimens, particularly before collection of the milk sample. It would be informative to see what proportion of positive milk cultures were among women with positive nipple cultures – eg is a positive milk culture (for either staph or candida) reflective of true ductal colonization, or contamination by organims present on the nipple? If Candida is the causal agent in burning breast and nipple pain, to seems plausible that women who actually grew out candida on culture would have the most intense symptoms, and the association between positive candida culture and BBNP would be stronger than between Candida PCR+ and BBNP. What proportion of women with positive cultures had BBNP? There are also concerns about the differences in the analytic approach to S. Aureus vs. Candida. The authors perform PCR for Candida species from nipple swabs, but not for Staph, and they include candida, but not staph, in the infant nose and mouth in their definition of "present." The rationale for this distinction is not clear. If Candida was transmitted from the infant's mouth to the nipple, then it would presumably be detected by PCR of the nipple swab – so why was Candida in the baby's mouth included? The authors note that when candida, staph and nipple damage were included in the time-to-event model, each was a significant predictor. Were interactions among these predictors considered? It seems likely, for example, that the combination of nipple damage and staph would be more strongly associated with pain than either condition alone. To the extent that Candida is more prevalent in the setting of eczema / dermatitis, it would also be important to test for interactions between candida and nipple damage. What was the hazard ratio for BBNP for women with candida but no nipple damage or staph present? It would also strengthen the study to report whether Candida species remained present among women whose BBNP resolved. A longitudinal repeated measures design, rather than a time-to-event analysis, would allow for investigation of this issue. In the discussion, the authors should address the finding that while candida species were present in 52% of women with BBNP, they were also present in 35% of women without BBNP – and absent in almost half of women with BBNP. Please discuss potential reasons why 35% of women had candida present but were pain-free – and why half of women with BBNP had no evidence of candida. Given this weak correlation, there are substantial concerns about the conclusions drawn by the study authors: "This large cohort study confirms that Candida spp play a role in nipple and breast pain in lactating women…" (p 9) I would argue that this large cohort study confirms that Candida spp are more common among women with burning breast and nipple pain than among women without such symptoms. Indeed, the presence of Candida in 35% of women without symptoms is a major challenge to the hypothesis that Candida causes breast pain. The authors should discuss their results in the context of criteria for establishing a causal association, such as specificity of association, temporality, biological gradient, and experimentation.

	Given the limitations of causal inference from this analysis, I would suggest revising the title to, "Are Candida and Staph Aureus associated with burning breast and nipple pain?" This paper demonstrates that BBNP is prevalent in the first 8 weeks postpartum, underscoring the need for clinicians to be knowledgeable about the differential diagnosis and options for treatment. However, this paper does not provide evidence that clinicians should presume that all BBNP is caused by Candida – in fact, only 50% of women with this syndrome had molecular evidence of Candida, and this molecular evidence may or may not be associated with a therapeutic response to antifungals. Further analysis including other clinical findings, including pink skin, evidence of vasospasm, nipple trauma, and other data to develop a more specific set of diagnostic criteria associated with candida colonization would greatly enhance the value of this paper for clinical decision-making. Finally, the statement, "'thrush in the breast' should not be dismissed as 'psychosomatic' as has been stated by some clinicians (p 9)" as well as the language in the background that suggests that clinicians who question the evidence for Candida as a causal agent in breast pain are dismissing women as "neurotic," (page 2, line 57), is potentially damaging to clinical care. In fact, pain reflects both peripheral stimulation and central neural processing. Treatment of pain requires thoughtful consideration of both physical and psychological factors. Mothers who experience pain during breastfeeding have a clinical problem, and their providers should entertain a full spectrum of potential contributing factors in order to provide adequate treatment.

REVIEWER	Jenny Murase, MD Asst. Clinical Professor, UCSF Department of Dermatology Director of Phototherapy, Palo Alto Foundation Medical Group
REVIEW RETURNED	24-Dec-2012

THE STUDY	1. I appreciated that this paper took into consideration both staph aureus and candida because often prior studies took one or the other into consideration but not both. This is definitely a strength of the paper. I would list this as a strength in the "strength/weakness" section of the paper. 2. I would remove the comments about the psychosomatics because I feel it is in some ways accusatory: examples include PDF file page 2 lines 53-60 in Background ("Health professionals...neurotic women") as well as page 9 lines 25-28 ("and thrush....stated by some clinicians.") 3. Reliable fungal culture of breastmilk is not possible because it requires special processing with iron to overcome the effect of lactoferrin. (Morrill JF, Pappagianis D, Heinig MJ, et al. Detecting Candida albicans in human milk. J Clin Microbiol. 2003; 41: 475-78.) It is unclear to me if the authors were aware of this and incorporated this into their results. I do not think that they did. If not, this needs to be listed as a study limitation. It also needs to be discussed in the result section that lists the low milk candida yield. If

	so, it needs to be addressed within the body of the paper. This has been previously cited as the major reason that candida is often not detected on culture, so I would focus on this fact instead of stating that some physicians feel "it is all in a patient's head." I like the fact that PCR was used in this study (at least for the nipple skin; it does not appear to have been used in breast milk according to the figure); it could be that the addition of lactoferrin and the use of PCR can increase the sensitivity even further and this should be addressed. 4. Is it possible to signficantly revise the Background session of this piece? It needs to be more crisp and readable. 5. Page 14 line 29 % sign is missing. Also it would be good to have a PCR vs. non PCR candida detection rate listed in this summary section because the PCR rate is much higher. 6. Table 3 needs to specify if this is PCR or non-PCR candida detection rate.

VERSION 1 – AUTHOR RESPONSE

Reviewer: Alison Stuebe, MD, MSc
Assistant Professor
University of North Carolina at Chapel Hill

I have no competing interests to declare.

See notes to author below -- the instruments used to define "nipple thrush" are not well described, and this classification appears to have been developed post hoc. The conclusions drawn are not supported by the data, and other studies casting doubt on the association between candida and breast pain are either not discussed (Hale et al) or not referenced -- eg "Andrews, J. I., D. K. Fleener, et al. (2007). "The yeast connection: is Candida linked to breastfeeding associated pain?" Am J Obstet Gynecol 197(4): 424 e421-424."
Response: Our responses to instruments and classification are below. Regarding other studies casting doubt on the association between candida and breast pain, we had cited five papers in the first paragraph of the Background (p. 2, refs 7-11). We agree that this is brief, however this paper does not set out to be a comprehensive literature review. We have added a comment about the Hale et al study in the Discussion (see below). The study by Andrews et al (2007) actually supports an association between candida and pain – from their Abstract: "RESULTS: Six of the 20 symptomatic women had breast milk cultures positive for yeast, compared with 6 of 78 controls (30% vs 7.7%, P = .015). Among the 12 women from whom yeast was isolated, 11 grew Candida albicans. Incidence of Staphylococcus aureus isolation did not differ significantly between groups (5 of 20 vs 15 of 78, P > .05)."

The authors sought to determine the association between breast pain and both candida and s aureus. However, their definition of breast pain is not clearly described or justified, and their techniques for assessing presence of candida and s aureus differ, without justification. The previous evidence is not adequately discussed. In addition, the authors use inflammatory language to suggest that clinicians who do not routinely diagnose and treat thrush are providing inappropriate care.
Response: These topics are all addressed below.

Policies regarding treatment of symptomatic women are not discussed.
Response: The topic of treatment is not within the scope of this paper. The aim of this paper is to

explore the associations between Candida and S. aureus and maternal symptoms. Readers can refer to several papers, cited in the Background section, which discuss treatment (Montgomery 2000; Brent 2001; Moorhead et al 2011; Hoddinott et al 2008; Heller et al 2012).

This paper addresses an important clinical problem – maternal pain during breastfeeding – and draws on a rich longitudinal study design that includes assessment of maternal symptoms and collection of biological specimens to explore the association between microorganisms and pain in the first 8 weeks postpartum.

The authors note in their introduction that the differential diagnosis for breast pain is broad, and includes infection, dermatitis, and vasospasm. However, a key weakness of the paper is the failure to explain how these diagnoses were considered in the analysis. Symptoms of burning pain are commonly associated with vasospasm, but there is no discussion of how mothers with vasospasm or with visible nipple damage were classified in the study. Were these women included in the "nipple thrush" group if they had burning pain or evidence of dermatitis?
Response: It is not possible to make a clinical diagnosis for women in the study, because they were a cohort of women who agreed to complete questionnaires and have samples collected: they were not a clinical cohort seen by a medical practitioner. We agree this is a limitation. On the other hand, the strength is that all women completed the questionnaires at each time point in their homes; thus, we have a more complete picture of women's experiences, than in previous studies which relied on women seeking help for their breastfeeding problems.
Vasospasm – We asked if women had noticed if their nipple/s blanched or turned white for more than 5 mins (at each time point). Positive response were - week 1: 1/336; week 2: 4/336; week 3 7/326; week 4 10/323. If we excluded women with vasospasm from our case definition of burning nipple and breast pain (weeks 2 to 8) – 48 women had this definition; compared to 50 without vasospasm. The analysis with Candida and S. aureus changes very little. (No women took nifedipine during the 8 week study period.) We added this to the text (p. 9) and Table 4 (old Table 3):
"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."
Visible nipple damage – We included this in the time-to-event analysis, nipple damage was associated with the case definition. We've added a new sentence to the revised section on Clinical implications (p. 11).
"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."
Dermatitis - Dermatitis is not synonymous with nipple pain or damage, it is often synonymous with eczema. Eczema of the nipple and areola is described as either "an erythematous eruption with vesicles, crusting and erosions" or "a dry, erythematous, lichenified and scaling dermatitis" Barankin and Gross (2004). Barankin and Gross (2004) described this condition in less than 2% of their patients (17/1016). No-one in the CASTLE study had a clinical diagnosis of nipple dermatitis, and no-one used a topical steroid during the study period.

The authors further indicate that they had planned to use previously derived criteria of shiny, flaky nipple and pink color as diagnostic criteria for thrush, but found that the prevalence of shiny and flaky nipples was low, whereas pink color was very common (N=140), so they changed their case definition to "burning nipple pain and breast pain" in the absence of mastitis. However, they report that 111 women reported such pain in weeks 2 to 8. Thus the decision to ignore pink color but include burning pain seems arbitrary. Similarly, the authors chose to exclude burning pain in week 1 from their diagnostic criteria because it was "very common" (N=146) (page 7, line 18-19). Again, this appears to be arbitrary. Please justify these decisions based on the hypothesized underlying biology.
Response: Pain: We have now presented the case definition for weeks 1-8, as well as weeks 2-8, and

both are shown in Table 4 (old Table 3). It is likely that most pain at week 1 is related to attachment difficulties, and micro-organisms would be only playing a small role at this time. New sentence in Methods p. 6):
"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."
Pink colour: We had not expected our research assistants to record that 140 of the 346 women (40%) had "pink" nipples. The research assistants were keen to document the appearance of the nipples. We have no way of knowing if a lactation consultant or doctor would have found the same findings. Nipple appearance was documented up to week 4 (as week 8 was a telephone interview). There were 23 women who had pink nipple at the same visit as burning nipple and radiating breast pain, 52% had candida (nipple/milk/baby) compared to 38% of other women ($p = 0.164$). The numbers are too small to confirm an association. We haven't included this in the paper, as we feel readers may become confused with too many alternative definitions.

Moreover, from the survival curve for time to first positive candida culture, it appears that no women at 7 days postpartum had a positive candida culture, despite the high prevalence of BBNP. Would the inclusion of symptoms at one week change the statistical significance of the observed association between candida sp and burning breast and nipple pain?
Response: We have included the week 1 symptoms in the one version of the case definition (see Table 4, old Table 3). The p-values are only slightly changed.
Thank you for pointing out this problem with our previous survival curves. Our original survival curves did not include data from hospital. We have recalculated them starting from the day prior to birth to enable use of hospital data (as some women had the hospital visit on the day of birth). We've uploaded the new survival curves, with data according to the day postpartum which is more accurate.

The rationale for the use of a survival curve in a repeated measure study such as this one is also unclear. Presumably, some women had pain that resolved, or no longer had Candida or Staph present in follow-up. A graph demonstrating this fluctuation over time would be more informative than a survival curve.
Response: We wanted to show the first occurrence of either Candida or S. aureus as predictors of burning nipple and breast pain, regardless of whether the pain resolved. It is hard to imagine a graph showing Candida/S. aureus and pain as all of these fluctuate at each time point.

A strength of this study was recruitment of a prospective sample and the use of home visits to collect data and biological specimens. However, this approach may lead to detection of subclinical symptoms that were not distressing to the mother and did not interfere with breastfeeding. Was a threshold used for frequency and intensity of burning pain, or was any endorsement of burning pain considered symptomatic? And were women asked to consider multiple descriptors of pain character, or were they simply asked, "Is the pain burning?" The questions used to classify women as having burning nipple and breast pain should be included in the paper to clarify this issue.
Response: We have added the actual questions asked in the Methods section (p. 6, see below). From our clinical experience of women developing mild burning nipple and breast pain following a course of antibiotics for the infant, we don't believe that pain associated with Candida has to be severe. Therefore we have not used a pain threshold.
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").

It is also unclear how women with burning pain were managed. Were they treated for presumed candida? If so, was the presence of candida on PCR / culture predictive of response to therapy? If not, what are the human subjects implications of identifying pathology and not offering therapy?
Response: These women were not in a clinical cohort, they were managed in the community by community nurses, lactation consultants and/or medical practitioners. The Participant Information statement made it clear that women would not be receiving the results of their microbiological samples, unless they specifically requested the results. Women could request results following the completion of the study at 8 weeks postpartum; and some women did so. If women wanted to know their results earlier, they could request to withdraw from the study. However, no woman requested to do this. The PCR tests were done in a block, at the completion of data collection, so this information was not available to women. We will be sending women a summary of the findings, once the results are published. This protocol was approved by the relevant Human Ethics Committees.

Given the weaknesses of the case definition, there are significant concerns with terming the syndrome of burning breast and nipple pain "nipple and breast thrush." The paper would be strengthened the term nipple and breast thrush were replaced with "burning breast and nipple pain (BBNP)."
Response: We disagree here. Since pain described as burning is common, and has a number of aetiologies we do not want clinicians to conclude that all burning pain (nipple and/or breast) is related to Candida. We feel it is important to examine if Candida is a valid aetiological agent, as at present some people believe Candida plays no role in breastfeeding pain.

There are also concerns about the decision to use PCR to define "presence of candida Sp." What is the clinical significance of the presence of candida via PCR that does not grow on culture? Does this reflect colonization? Non-viable spores? Or actual infection? Furthermore, the decision to perform PCR of nipple swabs, but not of milk samples or infant oral swabs, should be justified. If burning pain reflects ductal colonization with candida, as some have suggested, it would seem that PCR of milk, rather than of the nipple, would be the measure of interest.
Response: PCR vs culture: We knew from earlier studies that a low proportion of women with clinical symptoms of nipple thrush were positive for Candida using standard microbiological techniques (Amir et al 1996, Thomassen et al 1998). From work conducted in women with vulvovaginal symptoms, molecular microbiological techniques detected Candida in women who were negative using standard microbiology (for example, Weissenbacher et al. 2009). As molecular techniques are time-consuming and expensive we had to be selective and initially only planned to look at nipple specimens (as described in published protocol). This was already a large number: 360 women, 2 nipples at each time point, six time-points per woman – is over 4000 samples. We've added this explanation in the Methods section (p.5):
"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"

We agree that presence of an organism may reflect colonisation rather than infection. However, 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 (Fidel 2007). 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 (Fidel 2007). This has been added to the Discussion, p. 10/11.
“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”

Nipple vs milk: Although people have speculated about ductal colonisation with Candida, this is unknown. We do know that Candidal hyphae burrow into epithelium and therefore may enter ductal epithelium. The presence of Candida in milk may be from ductal epithelium or from contamination from nipple. Hale et al (2009) could not identify Candida in milk from women their study; possible explanations are that the women had other causes of their 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. We know from mastitis studies, that bacteria are not always isolated even in cases of obvious infection; possible reasons are that milk was not collected from the area of infection, or that the infection is a cellulitis rather than adenitis. We have added a discussion of Hale’s study to the Discussion (p. 9).
“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.”

More detail should also be provided regarding cleansing of the nipple before collection of specimens, particularly before collection of the milk sample. It would be informative to see what proportion of positive milk cultures were among women with positive nipple cultures – eg is a positive milk culture (for either staph or candida) reflective of true ductal colonization, or contamination by organims present on the nipple.
Response: Cleansing: The process of cleansing is described in the published study protocol (Amir et al 2011, p. 5 and 6). We have added a shorter version to the Methods section (p. 5).
“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."
Pos milk vs pos nipple. We have looked at this using data at each visit, to compare positive findings for nipple and milk at that visit. There were a substantial number of milk samples which were positive (for staph or candida), even if nipple swab was negative, which implies that this is likely to be a "true" finding, rather than contamination. 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. Here are the 2 by 2 tables:
milk | nipple_micro candida
candida | 0 1 | Total
----------+----------+----------
0 | 2,101 17 | 2,118

1 | 10 12 | 22
----------+----------+----------
Total | 2,111 29 | 2,140

milk | nipple staph_au
staph_au | 0 1 | Total
----------+----------+----------
0 | 1,533 182 | 1,715
1 | 89 336 | 425
----------+----------+----------
Total | 1,622 518 | 2,140

Added to text in Results: “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.” P. 8.

If Candida is the causal agent in burning breast and nipple pain, to seems plausible that women who actually grew out candida on culture would have the most intense symptoms, and the association between positive candida culture and BBNP would be stronger than between Candida PCR+ and BBNP. What proportion of women with positive cultures had BBNP?
Response: We have included standard nipple culture swabs in Table 4 (old Table 3). The number of positive swabs is small (n = 21), but the relationship is stronger (p = 0.004, or smaller p-value if we looked at weeks 2-8).

There are also concerns about the differences in the analytic approach to S. Aureus vs. Candida. The authors perform PCR for Candida species from nipple swabs, but not for Staph, and they include candida, but not staph, in the infant nose and mouth in their definition of “present.” The rationale for this distinction is not clear. If Candida was transmitted from the infant’s mouth to the nipple, then it would presumably be detected by PCR of the nipple swab – so why was Candida in the baby’s mouth included?
Response: Difference in microbiological techniques: Our previous experience with S. aureus in cracked nipples (Amir et al. BMC Pregnancy Childbirth 2004) and case-control study of mastitis (CAMEO, Amir et al. BMC Family Practice 2006) had isolated high rates of S. aureus in women and babies (69%, 124/181, infants had nasal S. aureus in CAMEO), and we felt molecular techniques were not necessary to increase detection. We felt we were more likely to miss Candida, and therefore planned to use molecular techniques. We plan to use molecular techniques to investigate the S. aureus organisms isolated and describe transmission dynamics, and will be seeking further funding for this.
Difference in including infant’s mouth/nose: We agree that this should be consistent, so we have now presented the results with S. aureus in nipple/breast milk or infant, similar to Candida.

The authors note that when candida, staph and nipple damage were included in the time-to-event model, each was a significant predictor. Were interactions among these predictors considered? It seems likely, for example, that the combination of nipple damage and staph would be more strongly associated with pain than either condition alone. To the extent that Candida is more prevalent in the setting of eczema / dermatitis, it would also be important to test for interactions between candida and nipple damage. What was the hazard ratio for BBNP for women with candida but no nipple damage or staph present?
Response: Interactions among candida, staph and nipple damage.
We realised we had not explained this very clearly. Now we have added a new table (Table 5) and

expanded the explanation in the text.
“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 also a strong 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. “

It would also strengthen the study to report whether Candida species remained present among women whose BBNP resolved. A longitudinal repeated measures design, rather than a time-to-event analysis, would allow for investigation of this issue.
Response: We have micro data for four weeks postpartum, as there were few women with this case definition before week 3, there would be insufficient cases which had resolved by week 4.

In the discussion, the authors should address the finding that while candida species were present in 52% of women with BBNP, they were also present in 35% of women without BBNP – and absent in almost half of women with BBNP. Please discuss potential reasons why 35% of women had candida present but were pain-free – and why half of women with BBNP had no evidence of candida.
Response: We have added a comment to the Discussion, under limitations (p. 11-12):
“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 (Fidel 2007).”

Given this weak correlation, there are substantial concerns about the conclusions drawn by the study authors: “This large cohort study confirms that Candida spp play a role in nipple and breast pain in lactating women…” (p 9)
Response: We have changed the wording to say "study provides evidence that . . " (p. 10).

I would argue that this large cohort study confirms that Candida spp are more common among women with burning breast and nipple pain than among women without such symptoms. Indeed, the presence of Candida in 35% of women without symptoms is a major challenge to the hypothesis that Candida causes breast pain. The authors should discuss their results in the context of criteria for establishing a causal association, such as specificity of association, temporality, biological gradient, and experimentation.
Response: We have explained above that we believe that Candida may be colonising or commensal organisms in some women. We have shown that Candida is associated with burning nipple/breast pain in our cohort of women using two different analyses. In a similar manner to S. aureus, the presence of either organism does not necessarily imply infection – S. aureus was present in the nipple/milk of healthy women, and is not always isolated in women with an obvious infection (CAMEO study). We’ve added a couple of sentences about experimentation (p. 13).
“Animal models, as have been used in vulvovaginal candidiasis,30may be required to fully understand

the pathogenesis of this condition. Future researchers may consider the RCTs for treatment or clearance of Candida."
Given the limitations of causal inference from this analysis, I would suggest revising the title to, "Are Candida and Staph Aureus associated with burning breast and nipple pain?"
Response: We have changed the title to "Does Candida and/or Staphylococcus play a role in nipple and breast pain in lactation?"

This paper demonstrates that BBNP is prevalent in the first 8 weeks postpartum, underscoring the need for clinicians to be knowledgeable about the differential diagnosis and options for treatment. However, this paper does not provide evidence that clinicians should presume that all BBNP is caused by Candida – in fact, only 50% of women with this syndrome had molecular evidence of Candida, and this molecular evidence may or may not be associated with a therapeutic response to antifungals.
Response: We agree with the reviewer. We are arguing that not all burning pain is caused by Candida; what we have shown is that women with burning pain were more likely to have Candida than other women, and therefore clinicians should consider this in their differential diagnosis of nipple and breast pain, as we have said in the Conclusion:"a diagnosis of Candida spp. infection should not be made without considering differential diagnoses" (p. 13).

Further analysis including other clinical findings, including pink skin, evidence of vasospasm, nipple trauma, and other data to develop a more specific set of diagnostic criteria associated with candida colonization would greatly enhance the value of this paper for clinical decision-making.
Response: As mentioned above, the presence of Candida does not necessarily imply symptoms. More complete clinical assessments (e.g assessment of infant anatomy or breastfeeding) would be necessary to develop a set of diagnostic criteria. A new section on Clinical implications has been added on p. 11.

Finally, the statement, "'thrush in the breast' should not be dismissed as 'psychosomatic' as has been stated by some clinicians (p 9)" as well as the language in the background that suggests that clinicians who question the evidence for Candida as a causal agent in breast pain are dismissing women as "neurotic," (page 2, line 57), is potentially damaging to clinical care.
Response: We have deleted these sections (sentence on p. 2 and phrase on p. 9).

In fact, pain reflects both peripheral stimulation and central neural processing. Treatment of pain requires thoughtful consideration of both physical and psychological factors. Mothers who experience pain during breastfeeding have a clinical problem, and their providers should entertain a full spectrum of potential contributing factors in order to provide adequate treatment.
Response: We agree with the reviewer. We were trying to make the point that this is a controversial issue.

Reviewer: Jenny Murase, MD
Asst. Clinical Professor, UCSF Department of Dermatology
Director of Phototherapy, Palo Alto Foundation Medical Group

1. I appreciated that this paper took into consideration both staph aureus and candida because often prior studies took one or the other into consideration but not both. This is definitely a strength of the paper. I would list this as a strength in the "strength/weakness" section of the paper.
Response: A sentence has been added to the Strength/weakness section (p. 9).
"It is the first prospective longitudinal study to examine the roles of both S. aureus and Candida spp. in one cohort of breastfeeding women and their infants."

2. I would remove the comments about the psychosomatics because I feel it is in some ways

accusatory: examples include PDF file page 2 lines 53-60 in Background ("Health professionals...neurotic women") as well as page 9 lines 25-28 ("and thrush....stated by some clinicians.")
Response: We have removed both these comments in response to the reviewer above.

3. Reliable fungal culture of breastmilk is not possible because it requires special processing with iron to overcome the effect of lactoferrin. (Morrill JF, Pappagianis D, Heinig MJ, et al. Detecting Candida albicans in human milk. J Clin Microbiol. 2003; 41: 475-78.) It is unclear to me if the authors were aware of this and incorporated this into their results. I do not think that they did. If not, this needs to be listed as a study limitation. It also needs to be discussed in the result section that lists the low milk candida yield. If so, it needs to be addressed within the body of the paper. This has been previously cited as the major reason that candida is often not detected on culture, so I would focus on this fact instead of stating that some physicians feel "it is all in a patient's head." I like the fact that PCR was used in this study (at least for the nipple skin; it does not appear to have been used in breast milk according to the figure); it could be that the addition of lactoferrin and the use of PCR can increase the sensitivity even further and this should be addressed.
Response: Yes, we aware of Jimi Francis Morrill's work, but were unable to discuss all the previous literature in the Background section of this paper. We preferred to use standard laboratory techniques for the breast milk, as the use of additional iron has only been used by these researchers, and has not been tested elsewhere. Since that study, Hale et al (2009) had used other techniques to identify Candida in the breast milk of women with pain. That study did not support the hypothesis that Candida was present in breast milk. We felt using molecular techniques for nipple swabs was likely to be more helpful. We have added a sentence to the Discussion (p. 10);
"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

4. Is it possible to signficantly revise the Background session of this piece? It needs to be more crisp and readable.
Response: We have deleted the sentence about psychosomatic pain. The current word count of the Background is 436 words. This is less than 20% of the total word count (n = 2733), which appears to be appropriate.
Para 1 – Introduces a controversial topic.
Para 2 – Explains the difference between "nipple/breast thrush" and mastitis as this is misunderstood by many people.
Para 3 – Details the differential diagnoses of nipple/breast pain – again important background information for readers.
Para 4 – Provides the rationale for this study.

5. Page 14 line 29 % sign is missing.
Response: Thank you. Added to Table 2.
Also it would be good to have a PCR vs. non PCR candida detection rate listed in this summary section because the PCR rate is much higher.
Response: This is the 2 by 2 table showing Candida detection via PCR and by culture. We have added this as a new Table 3.

Any micro | Any molec candida
candida | 0 1 | Total
----------+----------+----------
0 | 1,817 169 | 1,986
1 | 41 88 | 129
----------+----------+----------

Total | 1,858 257 | 2,115

6. Table 3 needs to specify if this is PCR or non-PCR candida detection rate.
Response: This is Candida identified by either method. Added to Table title and heading (now Table 4).

VERSION 2 – REVIEW

REVIEWER	Jenny Murase, MD Asst. Clinical Professor, UCSF Department of Dermatology Director of Phototherapy, Palo Alto Foundation Medical Group
REVIEW RETURNED	07-Feb-2013

THE STUDY	1. Results abstract: do not (NOT don't) 2. Second line of Background missing space: may diagnose 3. 4th paragraph of Results missing space: women were 4. In the limitations section: You noted a limitation in your study in the discussion, but did not reiterate in the limitation section. Please rephrase this and use it in the limitations section: " 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" 5. Conclusion: This large cohort study provides evidence that Candida spp. play a role in nipple and breast pain in lactating women; (SEMICOLON) however, burning nipple pain is (SPACE) common in breastfeeding women, and a diagnosis of Candida spp. infection should not be made without considering differential diagnoses. 6. PCR and RCTs abbreviations need to be spelled out the first time of use.