Author’s response to reviews

Title: Polymicrobial bloodstream infections in the neonatal intensive care unit are associated with increased mortality: A case-control study

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Author’s response to reviews: see over
Dear Editor,

We have revised the manuscript entitled ‘Polymicrobial bloodstream infections in the neonatal intensive care unit are associated with increased mortality: Case control study’ for consideration as an original article for publication in ‘BMC Infectious Diseases’. There are two tables and one figure in the manuscript.

Polymicrobial nature of microbial communities on human body surfaces has been highlighted by the human microbiome project. Isolation of more than one organism from neonatal specimens is often discarded as contamination. In our study we show the neonatal polymicrobial bloodstream infections are common and associated with increased mortality. We investigated the frequency of polymicrobial bacteremia in neonates in our institution over a 16 year period and by a case control design, we investigated the impact of polymicrobial infections on neonatal mortality and morbidity. We report that neonatal polymicrobial infections comprise nearly 14% of all bacteremia and are associated with more than three-fold increased mortality. We strongly believe that our article will increase awareness of neonatal polymicrobial infections among clinicians and lead to effective strategies against polymicrobial infections.

All co-authors have seen and agree with the contents of the manuscript. We certify that the submission is original work and is not under review by any other publication.

None of the authors have any financial interests to disclose or conflict of interest.

The responses to each of the reviewers’ comments are as follows:

We thank the reviewers for their insightful comments and have made the revisions as suggested. I have italicized our responses to the comments.

1. Reviewer: Dr. Dempsey

Major Compulsory revisions
A general comment would be to follow the Strobe criteria (STrengthening the Reporting of OBservational studies in Epidemiology) for case control studies.

We thank the reviewer for this suggestion. We have followed the STROBE criteria and have quoted the guidelines in the methods section.

I would recommend adding a section on what constitutes a polymicrobial infection.

Added as suggested
Line 77: I would remove to results/discussion section

*Changed as suggested*

**Methods:**
1. Who enters the data into the clinical microbiology database? How were potential contaminants which may have been real clinical infections dealt with?

*The clinical microbiological database is managed by the microbiologist and all cultures are recorded including those regarded as contaminants. Uncommon organisms would have to be isolated twice to regard as true infections and was determined by the investigators. We have added a sentence explaining this in the methods section.*

2. The definition of a polymicrobial infection needs to be clarified. Whilst I understand the definition as it stands, I had to read it a few times to clearly understand what it means. This is important for external generalizability of the results. A clearer definition should be provided e.g.

A polymicrobial infection was defined as either:
1. Isolation of more than one organism from a single blood culture specimen or
2. Isolation of two different organisms from two different blood culture specimens obtained from a single infection episode.

Or something to this effect.

*We have clarified the definition of polymicrobial infections as suggested.*

3. Line 108; Please add..... during the time period 2009 to 2012.

*Revised as suggested*

**Results**
1. Table 1. The denominators are confusing. 102 versus 74? What time period is this over? Is it different from 2009-2012?

*We agree and have modified the table and explained it in the table footnote*

2. Table 1A is not necessary. *Agree and deleted Table 1A*

3. Did the authors consider a subgroup analysis of those babies less than 1500 grams? And those with complex medical or surgical conditions?

*Thanks for the suggestion. In a subgroup analysis of VLBW infants, polymicrobial infections compared to monomicrobial infections, had a significantly higher mortality (Odds Ratio 6.4 [95% CI 1.3 to 30.3], longer duration of infection (OR 1.4 [95% CI 1.04 to 1.8]) and had more infants with congenital malformations (OR 17.7 [95% CI, 2.1 to 148.7). Presence of major malformations, any surgery, or need for ECMO, HFOV or iNO did not increase the risk for polymicrobial infections. Also when used in the logistic regression model for the outcome of mortality, these factors did not increase mortality significantly.*

**Discussion**
1. The discussion is generally well presented although there is some repetition which should be removed.

2. The mortality question is interesting and could be further developed.

3. A general discussion on antimicrobial use, resistant organisms and the benefits of an antimicrobial surveillance

*The discussion was revised on the suggestions above.*
**Reviewer 2: Michael Carter**

**Major compulsory revisions**

Were polymicrobial infections treated as aggressively as monomicrobial?
Potential for disregarding polymicrobial infections as contaminants/less severe infections, therefore giving shorter (perhaps less effective) courses of antibiotics.

*We agree with the reviewer about the concern for inadequate treatment leading to mortality differences. During 2009 to 2012 period, when neonatal risk factors and outcomes were assessed, we could not discern any differences in the treatment regimens from the data collected. Polymicrobial infections were treated adequately for all the organisms isolated. Duration of therapy was consistent with our written neonatal guidelines. No changes in the treatment guidelines were implemented during the study period 2009-2012.*

Were the polymicrobial infections more likely to have organisms resistant to common antibiotics (e.g. penicillin resistant Enterococcus faecalis)?

*We agree that differences in antibiotic susceptibility patterns between cases and controls could affect mortality. We did not find any differences in the type of organisms or antibiotic susceptibility patterns between cases and controls during the period 2009-2012.*

Use of multiple logistic regression analysis for mortality, at least including presence or absence of surgery into the equation (as a significant risk factor), and arguably also including CVC presence or absence (although I note that p=0.20 for the association with polymicrobial infections, so this may or may not be appropriate)

*Yes, we agree with the comment. We have reanalyzed including ‘any surgery’ in the model for logistic regression analysis and the revised mortality odds ratio is represented in the Table2.*

Why were umbilical catheters not included as a CVC? It would make sense to include them as a CVC? Please justify non-inclusion.

*Our neonatal policy is to replace the umbilical venous catheters in the first few days of life with percutaneously inserted central catheters (PICC) and hence most umbilical lines lasted only a few days. Also, the mean age of infection is > 30 days in both cases and controls and hence we chose not to include umbilical venous catheters.*

**Minor revisions**

Abstract

• Line 30: "neonatal unit" (not plural)

*The data is from both level 2 and level 3 neonatal units and hence the plurality*

Main article

• Throughout paper species names not be "Candida species", but should ideally be "Candida spp." etc. to be consistent (see lines 115 and 116 for inconsistencies here). There are lots of occurrences of this error.
• "gram" should be Gram when referring to Gram staining (proper noun)
• Line 129: "Bell's" classification (proper noun)
• Line 142: "Student's t-test" (proper noun)
• Line 158: Candida spp.

We agree and have corrected the above five suggestions.

• Line 193: Remove "busy" – it sounds like an excuse for poor care, but I am sure that you are providing exemplary care!

Removed ‘busy’

• Lines 202 to 203 almost an exact repetition of 194-195, perhaps rephrase?

Rephrased as suggested

• Lines 221: extracorporeal membranous oxygenation could be abbreviated to "ECMO" as previously in the manuscript

Revised as ECMO

• Line 225: give percentages to one decimal (as previously in paper)

Changed as suggested

• Line 227: "Staphylococcus aureus", "Escherichia coli"

Expanded as suggested

• Generally TPN is actually PN (as the neonates are often also partly enterally fed) hence it may be more accurate and appropriate to use "PN" not "TPN"

We agree and revised as suggested

Table 1
Spelling and formatting errors: "Escherichia coli", "Klebsiella oxytoca", "Bacillus spp."

We apologize and corrected the mentioned errors.

Table 2
Give numbers AND percentages (e.g. I doubt 41.18% in born in both groups until see the data). Give percentages to one decimal. Don't use standard error about a mean, use 95% confidence intervals – much easier to interpret for the reader.
Use regression analysis adjusted (at least) for surgery (see note above).

We have revised Table 2 as suggested.

Table A1
Give numbers AND percentages. Give percentages to one decimal. Don't use standard error about a mean, use 95% confidence intervals – much easier to interpret for the reader.

Table A1 deleted based on other reviewers’ comments

Discretionary revisions
Perhaps the discussion could mention the potential future use of molecular techniques for the identification of causes of sepsis (and therefore the likely increase in identification of multiple causes of sepsis).

We agree with the reviewer on the increased identification of sepsis by molecular methods. We have included a couple of sentences in the discussion section as suggested.

Figure 1 Could you identify why polymicrobial episodes seem to have decreased since 2010? Is this statistically significant? Could you use some time series statistics on this?

We did not note any significant changes in the number of admissions of VLBW and ELBW infants. A dedicated vascular access team was formed in June 2009 followed by better implementation of catheter care bundles and we think this strategy along with increased compliance in hand hygiene may have contributed to the decrease in infections from 2010. We did a time series regression analysis using the software Stata and did not see significant trending of data. However, the number of infections and polymicrobial infections are different if analyzed in the 2 time epochs, 1998-2009 and 2010-2012 (p < 0.01) using the Student’s t test.

Reviewer 3: Matthew Laundy

Major Compulsory Revisions
1. Title: Title of paper implies causation which the text quite rightly states that there can be no conclusions of causality. Association only

We agree and changed the title as suggested.

2. Causality or association – the main concern I have about this paper is that the controls are not matched with the cases appropriately. While the gestational age, birth weight, age at infection etc are matched, surgery is not. What this paper says to me as it stands is that surgery is a risk factor for mortality, an observation long recognized. The effect of polymicrobial infections independent of surgery has not been assessed. Either a subset analysis of those polymicrobial patients without other surgery should be analyzed or the controls matched to surgery. The numbers of patients with polymicrobial infections without other surgery will be small so subset analysis may not be possible.

We have revised our logistic regression model for the outcome of mortality, adjusting for ‘Any surgery’ and did not observe any significant differences in mortality. We have presented the revised odds ratio for mortality in Table 2.

Independently analyzing mortality and surgery, in our data set we did not see an increase in mortality after any surgery (OR 0.69 [95% CI, 0.3 to 1.5])

In addition, we analyzed the subset of all observations with and without ‘any surgery’ (80 observations of those had surgery and 55 did not have surgery). Analysis revealed that there were no significant differences in risk factors or mortality in a logistic regression analysis model with or without surgery from our neonatal dataset.

3. Blood Culture Duration – how this was calculated is important. How often were blood cultures taken to assess the first negative? Daily etc.? Was this consistent?
Yes, we have a consistent policy of repeating blood cultures every 24 hrs in all patients whose blood culture has grown an organism, which is continued till 2 blood cultures are negative. So we think our estimation of infection duration is fairly accurate.

Minor Essential Revisions
1. Incorrect reference: In line 65 a neonatal review is ascribed to reference 13. I do not have access to the full reference but I do not believe Weinstein et al is referring to neonates but to adult patients.

We apologize for this oversight and have corrected the reference.

Minor Discretionary Revisions
1. Type of blood culture should be stated. Does the unit use a single pediatric bottle or a separate aerobic and anaerobic bottle? I note no anaerobes in the organism list.

Our neonatal units use only one pediatric blood culture bottle which are aerobic cultures. Anaerobic cultures are not performed routinely for evaluation of neonatal sepsis.

2. What was the other type of surgery cardiac, GIT? May influence type of organisms grown.

The ‘other surgery’ included any other surgery such as cardiac, thoracic, abdomen (NEC surgery is enumerated separately) or those involving the joints or extremities.

3. The paper mentions that lines were at time of infectious episode but not when surgery was in relation to infectious episode.

The retrospective nature of this study makes it hard to tease out the timing of the surgery in relation to the infectious episode. However, we believe most of the infectious episodes were during or after the surgery.

4. You should comment on the reduction in infections between 2009 and 2012, the time period over which case control analysis took place. Why did this occur? A change in patient mix or unit policy. Is it possible that it may have affected your analysis?

There were significant decrease in the number of infections and polymicrobial infections between the time epochs, 1998-2009 and 2010-2012 (p < 0.01). We did not note any significant changes in the number of admissions of VLBW and ELBW infants to our neonatal units. A dedicated vascular access team was formed in June 2009 followed by better implementation of catheter care bundles in June 2009 and this strategy along with better compliance with hand hygiene may have contributed to the decrease in infections.

5. I am unsure as to the purpose of the additional table?

We agree and have deleted the additional table.

Please do not hesitate to contact us if you have any questions regarding this manuscript. We are looking forward to hearing from you. Thank you for your consideration.

Yours truly,
Mohan Pammi MD