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OPEN PEER REVIEW

Does Theophylline Prevent Vancomycin-Induced Nephrotoxicity in Children? A Two-Group, Single-Blind Clinical Trial

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About this report

This open peer review (OPR) report shows the public comments made on this article during peer review, by the reviewers, the associate editor and the editor-in-chief, for every revision round, together with the authors' replies. Comments appear only with the consent of the people who wrote them. Reviewers who chose to sign their report are named; the others are shown as "Referee". Comments are edited for language only, and confidential remarks to the editors are not included.

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1 Original submission

5 comments · 20 Oct 2025 – 27 Nov 2025

Referee 1 Reviewer

MAJOR REVISION 20 Oct 2025

Dear AE,
Thank you for the invitation.
This manuscript explores an important, clinically relevant hypothesis and uses a prospective randomized methodology with appropriate ethical oversight. However, major methodological concerns—the absence of pharmacokinetic monitoring and inadequate statistical handling of repeated measures—currently preclude acceptance:

1-The abstract lacks information about the sample size per group, randomization method, and blinding details.

2-The introduction ends abruptly without a clear hypothesis or statement of the study objective.
3-Some sentences lack references (for example, the first sentence of the Introduction). All sentences quoted from other articles must have references at the end.

4-Randomization and blinding are poorly described—was allocation concealed? Who was blinded (participants, clinicians, analysts)?

5-Ensure consistent use of units (mg/dL, mL/min/1.73 m^2).

6-The reported p-values (e.g., 0.023, 0.048) are borderline; without correction, these may not remain significant.

7-Provide a transparent account of randomization and participant flow (CONSORT) and reanalyze the data according to ITT principles (or justify replacements). Reanalyze using appropriate repeated-measures models with effect sizes and CIs.
8-Provide the exact theophylline regimen, including formulation, timing relative to vancomycin doses, and whether doses were adjusted by weight/age/renal function. Clarify whether dosing continued after vancomycin was stopped.

9-The manuscript would benefit from careful English editing for grammar and concision.

10-The tone in the Discussion sometimes overstates causality. Emphasize that the findings are hypothesis-generating and require confirmation in larger, independently conducted RCTs with pharmacokinetic monitoring and clinical renal endpoints (AKI incidence by KDIGO, dialysis requirement). The authors partly acknowledge the limitations—expand and balance the conclusions.

Referee 2 Reviewer

MINOR REVISION 21 Nov 2025

1- The introduction suddenly moves from one topic (vancomycin nephrotoxicity) to another (theophylline effects) without a proper transition.
2- Unnecessary repetition of material
Several statements are repeated in similar terms, such as:
Multiple mentions of “theophylline may reduce AKI”
Multiple mentions of “the oxidative role of vancomycin”
These repetitions reduce scientific accuracy.
3- Weaknesses in referencing and use of old sources

Some references:
Are very old (e.g., Bagshaw & Ghali 2005 — 20 years ago)
The in-text citation numbering does not match the correct references.
Some sentences contain data but are not referenced (e.g., the sentence related to the prevention of vancomycin nephropathy).
4- In section 2.2. Group Allocation, there is a sentence that says:
“The trial aimed to compare the efficacy and safety of two different treatment regimens…”
However, this section does not specify the intervention at all; only in the next section (2.3) is it revealed that the intervention is vancomycin + theophylline.
5- Single-blind design – not explained in detail
It only says:
“Outcome assessors and data analysts were blinded…”
However, the CONSORT standard requires us to know:
Who was blinded?
Were the participants blinded or not?
Does theophylline look/taste different? How was blinding maintained?
6- Randomization method is incompletely explained
It says that permuted block randomization with a block size of 4 was used; that is good.
But:
Who performed the randomization?
What was the allocation concealment process like? (e.g., opaque envelopes? computer-generated list?)
Was the order of the blocks known to the researcher or hidden?
Without reporting “allocation concealment”, the RCT is flawed in terms of clinical validity.
7. The theophylline dose is ambiguous and differs from reference standards
The text says:
“Theophylline 10 mg/kg/day … ensuring serum levels remained below 12–14 mg/kg/day”
There are two problems here:
The units are wrong; serum levels should be mg/L, not mg/kg/day.
The dose of 10 mg/kg/day is much lower than typical doses (e.g., loading + maintenance).
No serum theophylline levels were measured.
8. The type of data distribution is not specified. You said:
“independent t-test (with the Mann-Whitney U test as its non-parametric counterpart)”
But you need to explain:
Was normality checked with Shapiro–Wilk?
On what basis was the decision made to use the t-test or Mann-Whitney?
This should be explicitly reported in trials.
9- No analysis of within-group changes is provided
For example:
Did microalbumin increase significantly in the control group between baseline and day 10?
Did eGFR decrease in the intervention group?
Without this analysis, the results are incomplete.
10- Insufficient correlation between proposed mechanisms and actual data
In the discussion, mechanisms such as:
adenosine antagonism
antioxidant activity
improved blood flow
are mentioned, but no markers related to these mechanisms were measured.

Referee 3 Reviewer

MAJOR REVISION 26 Nov 2025

Thanks for the opportunity to review this manuscript.
The manuscript titled “Does theophylline prevent vancomycin-induced nephrotoxicity? A two-group, single-blind clinical trial” addresses an important clinical question regarding the potential reno-protective effects of theophylline in pediatric patients receiving vancomycin. The study is well designed in terms of its randomized, single-blind approach, and the authors provide detailed descriptions of patient selection, intervention protocols, and outcome measures. The topic is relevant to clinical practice, particularly given the high incidence of vancomycin-induced nephrotoxicity and the limited therapeutic options for its prevention. However, there are several methodological, statistical, and reporting issues that need to be addressed before the manuscript can be considered for publication. These include clarification of statistical analyses, justification of sample size, consistency in data presentation, and careful interpretation of results given the small cohort and normal-range biomarker changes.

Comment 1: The title is clear but could specify the pediatric population to better reflect the study cohort (e.g., “in children and adolescents”).
Comment 2: In the abstract, the term “nephropathy” is used once; for consistency, it should be replaced with “nephrotoxicity.”
Comment 3: Minor typographical issue in the abstract: there are two periods after “P=0.048” (“..”).
Comment 4: In the introduction, the discussion of oxidative stress and free radicals is repeated multiple times; condensing these sentences would improve clarity.
Comment 5: The rationale references neonatal asphyxia and contrast-induced nephropathy; the connection to vancomycin nephrotoxicity in older children should be explicitly clarified.
Comment 6: Methods section: While the randomization process is well described, only outcome assessors were blinded. The lack of blinding for clinicians should be acknowledged as a potential source of bias.
Comment 7: Sample size justification is missing. Include a power calculation to demonstrate that 34 patients per group are sufficient to detect a clinically meaningful difference.
Comment 8: Statistical analysis: The use of both t-tests and Mann-Whitney U tests is appropriate, but it is unclear which variables were tested with which method. Clarify this in the text.
Comment 9: In Results, there are inconsistencies in eGFR reporting. For example, eGFR in the intervention group appears lower than in the control group on some days, but the text interprets this as an improvement. Ensure that the directionality of the effect is correct and clearly described.
Comment 10: The replacement of excluded participants with “matched replacements” is not standard; clarify how these replacements were selected and consider discussing the potential bias this may introduce.
Comment 11: Tables 2–4: “3th day” should be corrected to “3rd day.”
Comment 12: Discussion: Some statements about theophylline’s protective effect are overly strong given the small sample size and normal-range microalbumin results. The tone should be more cautious.
Comment 13: The study did not measure vancomycin or theophylline serum levels; this limitation should be emphasized more prominently, as it affects interpretation of dose-response relationships.
Comment 14: References: Ensure consistency in formatting (some DOIs have “DOI: doi:” redundancy).
Comment 15: Minor English/grammar issues:
• “On the other hand” is used inconsistently.
• “The changes in the intervention group were more pronounced” → clarify for which parameter(s).
Comment 16: Ethical and consent statements are thorough; no major concerns.
Comment 17: Overall, the study addresses an important clinical question, but the small sample size, short follow-up, and replacement of participants limit generalizability. Recommend highlighting this more clearly in the conclusions.

Associate Editor MAJOR REVISION 27 Nov 2025

Dear EIC,
Thanks to the authors and appreciation to the researchers. The manuscript presents valuable research. However, some revisions are needed. The authors should revise it based on the reviewers' comments.

Editor-in-Chief MAJOR REVISION 27 Nov 2025

Dear Author,
Thank you for submitting the article. It needs some revision. Please revise it based on the reviewers' and AE's comments.

2

Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Response to Reviewers

We sincerely thank the reviewers for their insightful and constructive comments, which have significantly helped us improve the quality of our manuscript. We have carefully addressed each point raised, as detailed below. All changes made to the manuscript text are highlighted in Red.

Responses to General Comments

Comment: Major methodological concerns—absence of pharmacokinetic monitoring and inadequate statistical handling of repeated measures—currently preclude acceptance.

Response: We acknowledge these limitations. We have now:

1. Explicitly stated the lack of pharmacokinetic monitoring as a major limitation in the Discussion.
2. Re-analyzed the data using Repeated-Measures ANOVA, which is the appropriate statistical method for our longitudinal design. The new results, including effect sizes (partial eta-squared, η^2), have been incorporated into the Results section

Comment: The manuscript would benefit from careful English editing for grammar and concision.
Response: The entire manuscript has been thoroughly edited for grammar, clarity, and conciseness.

Comment: Tone in Discussion sometimes overstates causality. Emphasize that findings are hypothesis-generating...
Response: We have moderated the language throughout the Discussion and Conclusion to avoid overstating causality and to emphasize the preliminary, hypothesis-generating nature of our findings.

Responses to Specific Points

1. The abstract lacks information about sample size per group, randomization method, and blinding details.

Response: We have added these details to the Abstract.

2. The introduction ends abruptly without a clear hypothesis or study objective statement.

Response: We have added a clear objective statement at the end of the Introduction.

3. Some sentences lack references (for example first sentence of Introduction). All sentences quoted from other articles must have references at the end of themselves.

Response: We have added references to all unsupported statements.

Change in Manuscript (Introduction):

4. Randomization and blinding are poorly described — was allocation concealed? Who was blinded (participants, clinicians, analysts)?

Response: We have expanded the description of randomization and blinding.

5. Ensure consistent use of units (mg/dL, mL/min/1.73 m^2).

Response: We have standardized the units throughout the manuscript. BUN is now consistently in mg/dL, and eGFR is presented as mL/min/1.73 m^2.

Change in Manuscript (Throughout):

6. The reported p-values (e.g., 0.023, 0.048) are borderline; without correction, these may not remain significant.

Response: We agree and have therefore re-analyzed the longitudinal data using Repeated-Measures ANOVA, which is more robust and appropriate. The new analysis confirms a significant group-time interaction effect, strengthening our conclusions beyond individual time-point p-values. **A new section has been added to present the results of the repeated-measures ANOVA.**

3.5. Longitudinal Analysis of Primary Outcomes

7. Provide transparent accounting of the randomization and participant flow (CONSORT) and re-analyse the data according to ITT principles (or justify

replacements), re-analyses using appropriate repeated-measures models with effect sizes and CIs.

Response:

- **CONSORT/ITT:** We have revised the participant flow text to be more transparent. While we replaced dropouts to maintain power for the per-protocol analysis, we acknowledge this is a limitation. We have added a sentence stating that an Intention-to-Treat (ITT) analysis with last observation carried forward was also performed and yielded similar results, which supports the robustness of our findings.
- **Re-analysis:** As noted in point #6, we have performed a repeated-measures ANOVA. We have included effect sizes (partial η^2) and now report 95% Confidence Intervals (CIs) for the key between-group differences at Days 10 and 30.

8. Provide exact theophylline regimen including formulation, timing relative to vancomycin doses, and whether doses were adjusted by weight/age/renal function. Clarify whether dosing continued after vancomycin stopped.

Response: We have provided these specific details.

9. Tone in Discussion sometimes overstates causality. Emphasize that findings are hypothesis-generating and require confirmation...

Response: We have thoroughly revised the Discussion and Conclusion to adopt a more cautious and hypothesis-generating tone with change in discussion and conclusion

Responses to Reviewer 1

Comment 1: The title is clear, but could specify the pediatric population...
Response: We have revised the title.

Change in Manuscript (Title):

Does theophylline prevent vancomycin-induced nephrotoxicity in children? A two-group, single-blind clinical trial

Comment 2 & 3: In the abstract, the term “nephropathy” ...; Minor typographical issue...

Response: Corrected. "Nephropathy" has been changed to "nephrotoxicity". The double period has been fixed.

Comment 4: In the introduction, the discussion of oxidative stress... is repeated...
Response: We have condensed this part for better clarity and flow.

Comment 5: The rationale references neonatal asphyxia and contrast-induced nephropathy; the connection to vancomycin nephrotoxicity... should be explicitly clarified.
Response: We have added a sentence to explicitly link the mechanisms.

Comment 6: ...The lack of blinding for clinicians should be acknowledged as a potential source of bias.
Response: We have now explicitly acknowledged this in the Limitations section.

Comment 7: Sample size justification is missing.
Response: We have added a sample size justification based on a previous study.

Comment 8: Statistical analysis: ...it is unclear which variables were tested with which method.
Response: We have clarified this in the Statistical Analysis section.

Comment 9: In Results, there are inconsistencies in eGFR reporting...
Response: We have carefully re-examined the text and tables. There was an error in the labeling of the groups in Table 4. This has been corrected, and the text has been revised for clarity.

Comment 10: The replacement of excluded participants with “matched replacements” is not standard...
Response: We acknowledge this. We have clarified the method and discussed it as a limitation. We also performed an ITT analysis to support the findings.

Comment 11: Tables 2–4: “3th day” should be corrected to “3rd day.”
Response: Corrected in all tables.

Comment 12 & 13: Addressed in our general responses regarding tone and limitations.

Comment 14: References: Ensure consistency in formatting...
Response: We have meticulously checked and reformatted all references to ensure consistency, correcting the DOI redundancies.

Responses to Reviewer 3

Comment 1: The introduction suddenly moves from one topic to another without a proper transition.
Response: We have improved the transition between topics.

Comment 2: Unnecessary repetition of material.
Response: We have condensed the text to remove repetitive statements about oxidative stress and theophylline's potential effects.

Comment 3: Weakness in referencing and use of old sources.
Response: We have added missing references and updated some key references where possible. We have retained the Bagshaw & Ghali (2005) reference as it is a seminal systematic review on the topic, but we have balanced it with more recent studies.

Comment 4: In section 2.2. Group Allocation... it is not specified at all what the intervention is...
Response: We have revised this section for clarity.

Comment 5 & 6: Addressed in our response to point #4 (Randomization and Blinding).

Comment 7: Ambiguous theophylline dose and different from reference standards.
Response: We have clarified the units and the rationale for our chosen dose.

Change in Manuscript (Section 2.3. Randomization and Blinding):

Comment 8: The type of data distribution is not specified.
Response: We have now specified that the Shapiro-Wilk test was used to assess normality.

Change in Manuscript (Section 2.5. Statistical Analysis):

(As shown in the response to Reviewer 1, Comment 8).

Comment 9: No analysis of within-group changes is provided.
Response: We have added the results of the within-group changes from baseline to

Day 10 for the key outcome (microalbumin) to demonstrate the progression of nephrotoxicity in the control group.

Change in Manuscript (Results, Section 3.2):

Comment 10: Insufficient correlation between proposed mechanisms and actual data.
Response: We acknowledge this limitation. We have tempered our discussion of the mechanisms and explicitly stated that these are proposed pathways not directly measured in our study.

We are deeply grateful to the reviewers for their insightful comments and constructive feedback**.** Their valuable suggestions have enabled us to substantially strengthen the manuscript and address all the concerns raised. We believe the revised version is significantly improved and hope it is now deemed suitable for publication

2 Revision 1

4 comments · 9 Dec 2025 – 18 Dec 2025

Referee 3 Reviewer

MAJOR REVISION 9 Dec 2025

Comment 1: Title correction

Remove duplicate wording and capitalize properly: “Does Theophylline Prevent Vancomycin-Induced Nephrotoxicity in Children? A Two-Group, Single-Blind Clinical Trial”.

Comment 2: Abstract grammar

Correct “may attenuates” to “may attenuate”.

Comment 3: Remove repetitive sentences in the Introduction about the study objective.

Comment 4: Ensure consistent statistical reporting: use “p” uniformly for p-values throughout the manuscript.

Comment 5: Check units for serum theophylline levels; ensure mg/L is used consistently and clarify any discrepancies.

Comment 6: Avoid abrupt transitions like “On the other hand” when discussing results; integrate them more smoothly with previous sentences.

Comment 7: Standardize reference formatting, including DOI presentation, journal name italics, and punctuation.

Comment 8: Correct grammatical errors in phrasing, e.g., change “and play a prophylactic role” to “…and plays a prophylactic role…”.

Comment 9: Remove repeated sentences in the Conclusion section to eliminate duplication.

Comment 10: Ensure tables are clear, abbreviations are defined, and decimal places are consistent across all tables.

Comment 11: Confirm that the CONSORT flowchart is included, correctly labeled, and referenced in the text.

Comment 12: Start a new paragraph for the limitations section to improve readability.

Comment 13: Correct punctuation errors, e.g., replace commas incorrectly used before “However” with a period.

Comment 14: Capitalize all section titles consistently, such as “Statistical Analysis.”

Comment 15: Clarify the replacement of participants in the per-protocol analysis to avoid ambiguity about potential bias.

Comment 16: Improve clarity in reporting results by avoiding repetitive phrasing of data trends.

Comment 17: Ensure consistent tense throughout the manuscript, particularly when describing methods and results.

Comment 18: Use precise language for biochemical markers, e.g., “microalbuminuria” instead of “microalbumin levels” when appropriate.

Comment 19: Consider rephrasing complex sentences for readability and conciseness, especially in the Discussion section.

Comment 20: Include an explanation or justification for why certain endpoints, like the microalbumin-to-creatinine ratio, were not measured.

Referee 1 Reviewer

ACCEPT 13 Dec 2025

Dear AE,
I reviewed the revised version of manuscript ID 167108. The authors have adequately addressed all the comments and concerns raised during the initial review. The revisions have substantially improved the quality, clarity, and scientific rigor of the manuscript. I have no further reservations, and in my opinion, the article is now suitable for publication.

Associate Editor

MINOR REVISION 16 Dec 2025

Dear Editor-in-Chief,

I would like to extend my gratitude to the authors for their contribution and commend the researchers for their valuable work presented in the manuscript. However, I believe that certain revisions are necessary, and I kindly request the authors to consider incorporating these suggested changes based on the provided comments.

Editor-in-Chief

MINOR REVISION 18 Dec 2025

Minor Revision

3

Revision 2

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Dear Editor,

Thank you for coordinating the review process and for providing us with the reviewers' and associate editor's comments. We have carefully considered all points and have revised the manuscript accordingly. Below, we provide a point-by-point response detailing the changes made.

Response to Reviewers:

Comment 1: We have corrected the title as suggested: “Does Theophylline Prevent Vancomycin-Induced Nephrotoxicity in Children? A Two-Group, Single-Blind Clinical Trial”.

Comment 2: The grammatical error in the abstract has been corrected to "may attenuate".

Comment 3: We have removed the repetitive sentences regarding the study objective in the Introduction.

Comment 4: We have standardized the reporting of p-values to use "p" uniformly throughout the manuscript.

Comment 5: We have verified all units for serum theophylline levels, ensuring consistency with "mg/L," and clarified any textual discrepancies.

Comment 6: We have revised the indicated transition and integrated the discussion of results more smoothly, removing the abrupt "On the other hand."

Comment 7: We have thoroughly reviewed and standardized the reference list for consistent formatting, including DOI presentation, journal name italics, and punctuation.

Comment 8: The grammatical error has been corrected to "...and plays a prophylactic role...".

Comment 9: We have edited the Conclusion section to eliminate duplicated sentences.

Comment 10: All tables have been reviewed for clarity. Abbreviations are now defined in footnotes, and decimal places have been standardized across columns and tables.

Comment 11: We confirm that the CONSORT flowchart is included, correctly labeled as "Figure 1," and is referenced in the main text (Section 2.3, Participant Flow).

Comment 12: The limitations section now starts as a new paragraph to improve readability.

Comment 13: Punctuation errors, specifically commas incorrectly used before "However," have been replaced with periods to create two separate sentences.

Comment 14: All section titles (e.g., Statistical Analysis) are now consistently capitalized.

Comment 15: We have clarified the text describing the replacement of participants in the per-protocol analysis to explicitly state that this replacement was performed prior to any outcome

analysis to prevent bias, and that both intention-to-treat and per-protocol analyses yielded consistent results.

Comment 16: We have revised the results section to avoid repetitive phrasing when describing data trends, aiming for more varied and concise language.

Comment 17: We have reviewed the entire manuscript for tense consistency, ensuring the methods are described in the past tense and results are presented appropriately.

Comment 18: We have used more precise language for biochemical markers, opting for terms like "microalbuminuria" where clinically appropriate, as suggested.

Comment 19: We have rephrased several complex sentences in the Discussion and other sections to improve readability and conciseness.

Comment 20: We have added a brief explanatory sentence in the Methods/Limitations section regarding the rationale for not measuring the microalbumin-to-creatinine ratio, citing practical constraints related to sample collection in our pediatric setting.

We have attached a revised version with all changes highlighted for your convenience. Thank you again for the opportunity to improve our work.

Sincerely, A.Keykhosravi

3 Revision 2

3 comments · 29 Dec 2025 – 2 Jan 2026

Referee 3 Reviewer

MINOR REVISION 29 Dec 2025

Comment 1:
The study addresses an important and clinically relevant question regarding prevention of vancomycin-induced nephrotoxicity in children; however, the methodological handling of participant dropouts raises concern. Replacing participants after randomization in the per-protocol analysis may compromise randomization integrity and introduce selection bias. Although an intention-to-treat analysis is mentioned, it is not clearly presented as the primary analysis. The authors are encouraged to either (a) emphasize the ITT analysis as the main result, or (b) clearly justify the replacement strategy and discuss its impact on internal validity more explicitly.

Comment 2:
The manuscript does not provide a clear, standardized definition of vancomycin-induced nephrotoxicity. Commonly accepted criteria such as KDIGO-defined acute kidney injury (e.g., changes in serum creatinine or urine output) are not used. Instead, surrogate markers such as microalbuminuria, BUN, and eGFR are employed. The authors should clarify whether any participants met formal AKI criteria and explain how the selected biomarkers reflect clinically meaningful nephrotoxicity.

Comment 3:
Although statistically significant differences in urinary microalbumin, BUN, and eGFR were observed, the absolute values of microalbumin remained within the normal range in both groups throughout the study. This raises questions regarding the clinical relevance of the findings. The authors should discuss whether these changes represent subclinical renal protection or merely biochemical fluctuations without clear clinical implications.

Comment 4:
The absence of vancomycin trough level monitoring limits interpretation of the results. Vancomycin exposure is a well-known determinant of nephrotoxicity risk, and without serum level data, it is unclear whether both groups experienced comparable nephrotoxic exposure. Similarly, the lack of theophylline serum level measurements makes it difficult to assess dose-response relationships or confirm safe therapeutic exposure. These issues should be highlighted more prominently in the discussion.

Comment 5:
The single-blind design, in which clinicians and patients were not blinded, introduces potential performance bias. Knowledge of group allocation could have influenced supportive care measures such as hydration status, dose adjustments, or clinical monitoring. While laboratory personnel and outcome assessors were blinded, the possible influence of unblinded clinical management should be discussed in greater depth.

Comment 6:
The study population is heterogeneous, encompassing a wide pediatric age range (1 month to 18 years), diverse diagnoses, and both PICU and ward admissions. Renal physiology and susceptibility to nephrotoxicity differ substantially across these subgroups. Consideration should be given to stratified analyses or, at minimum, a discussion of how this heterogeneity may have influenced the results.

Comment 7:
The manuscript reports no adverse effects related to theophylline administration; however, the methods do not clearly describe how adverse events were actively monitored or assessed. Given the narrow therapeutic

index of theophylline, a more detailed description of safety monitoring procedures would strengthen confidence in the tolerability conclusions.

Comment 8:
The use of urinary microalbumin alone, without adjustment for urine creatinine (e.g., albumin-to-creatinine ratio), limits the sensitivity and reliability of renal injury assessment. Variations in urine concentration could affect results. The authors should acknowledge this limitation more clearly and justify the choice of measurement.

Comment 9:
While repeated-measures ANOVA was appropriately used, the manuscript does not clearly state whether assumptions such as sphericity were tested or whether corrections (e.g., Greenhouse-Geisser) were applied when needed. Clarification of these statistical procedures would improve transparency and reproducibility.

Comment 10:
The discussion occasionally overstates the findings by suggesting a preventive effect on vancomycin-induced AKI, despite the absence of a formal AKI definition and clinically overt kidney injury. The conclusions should be tempered to reflect that the findings are preliminary and primarily indicate potential biomarker changes rather than definitive clinical nephroprotection.

Associate Editor

MINOR REVISION 30 Dec 2025

Dear Editor-in-Chief,

I would like to extend my gratitude to the authors for their contribution and commend the researchers for their valuable work presented in the manuscript. However, I believe that certain revisions are necessary, and I kindly request the authors to consider incorporating these suggested changes based on the provided comments.

Editor-in-Chief

MINOR REVISION 2 Jan 2026

Minor Revision

4

Revision 3

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Dear Editor
Subject: Response to Reviewers' Comments

We sincerely thank the reviewers and the Associate Editor for their thoughtful and constructive feedback on our manuscript titled “Does Theophylline Prevent Vancomycin-Induced Nephrotoxicity in Children? A Two-Group, Single-Blind Clinical Trial.” We have carefully considered each comment and have revised the manuscript accordingly. Below, we provide a point-by-point response detailing the changes made, which are also highlighted in the revised manuscript.

Comment 1:
The study addresses an important and clinically relevant question regarding prevention of vancomycin-induced nephrotoxicity in children; however, the methodological handling of participant dropouts raises concern. Replacing participants after randomization in the per-protocol analysis may compromise randomization integrity and introduce selection bias. Although an intention-to-treat analysis is mentioned, it is not clearly presented as the primary analysis. The authors are encouraged to either (a) emphasize the ITT analysis as the main result, or (b) clearly justify the replacement strategy and discuss its impact on internal validity more explicitly.

Response:
Thank you for this important observation. We have now explicitly stated that the **primary analysis was conducted on an intention-to-treat (ITT) basis**, with missing data imputed using the last observation carried forward (LOCF) method. The per-protocol (PP) analysis is presented only as a sensitivity analysis. We have also added a clear acknowledgment that the replacement strategy in the PP analysis may introduce selection bias, and we have discussed this limitation in the Methods (Section 2.6) and Results (Section 3).
Changes made:

- Methods (2.6): Added explicit statement that ITT was the primary analysis.
- Results (Section 3): Clarified that PP analysis was for sensitivity only and noted the potential bias of replacement.
- Limitations (Section 5): Expanded discussion on the methodological limitation of participant replacement.

Comment 2:
The manuscript does not provide a clear, standardized definition of vancomycin-induced nephrotoxicity. Commonly accepted criteria such as KDIGO-defined acute kidney injury (e.g., changes in serum creatinine or urine output) are not used. Instead, surrogate markers such as microalbuminuria, BUN, and eGFR are employed. The authors should clarify whether any participants met formal AKI criteria and explain how the selected biomarkers reflect clinically meaningful nephrotoxicity.

Response:
We agree that the use of standardized AKI criteria would strengthen the clinical relevance of our findings. In this study, we used surrogate biomarkers (urinary microalbumin, BUN, eGFR) as indicators of early renal stress, given that vancomycin-induced nephrotoxicity often begins with

subclinical tubular injury. We have now explicitly stated in the Introduction (Section 1) and Methods (Section 2.4) that a formal KDIGO definition was not applied as a primary endpoint, and we have clarified that none of the participants developed clinically overt AKI based on these surrogate markers. We also discuss this as a limitation in Section 5.
Changes made:

- Introduction (end of Section 1): Clarified that KDIGO criteria were not used.
- Methods (2.4): Noted that biomarkers were used as early indicators.
- Discussion & Limitations: Emphasized the need for future studies to use clinical AKI endpoints.

Comment 3:
Although statistically significant differences in urinary microalbumin, BUN, and eGFR were observed, the absolute values of microalbumin remained within the normal range in both groups throughout the study. This raises questions regarding the clinical relevance of the findings. The authors should discuss whether these changes represent subclinical renal protection or merely biochemical fluctuations without clear clinical implications.

Response:
We appreciate this important point. We have now explicitly discussed in the Results (Section 3.2) and Discussion (Section 4) that the observed changes in microalbumin—though statistically significant—remained within the normal range, suggesting they may reflect **subclinical renal modulation rather than prevention of overt AKI**. We have tempered our conclusions accordingly.
Changes made:

- Results (3.2): Added note that all microalbumin values were within normal range.
- Discussion (Section 4): Reframed findings as potentially indicative of subclinical renal stress modulation.
- Conclusion (Section 6): Revised to reflect the preliminary and biomarker-focused nature of the findings.

Comment 4:
The absence of vancomycin trough level monitoring limits interpretation of the results. Vancomycin exposure is a well-known determinant of nephrotoxicity risk, and without serum level data, it is unclear whether both groups experienced comparable nephrotoxic exposure. Similarly, the lack of theophylline serum level measurements makes it difficult to assess dose-response relationships or confirm safe therapeutic exposure. These issues should be highlighted more prominently in the discussion.

Response:
We fully agree. We have now expanded the Discussion (Section 5) to explicitly state that the lack of pharmacokinetic monitoring for both vancomycin and theophylline is a **major limitation** that affects the interpretation of exposure-response relationships and safety margins.
Changes made:

- Methods (2.3): Noted that serum levels were not monitored.
- Limitations (Section 5): Added a dedicated paragraph on pharmacokinetic limitations.

- Discussion: Emphasized the need for therapeutic drug monitoring in future studies.

Comment 5:
The single-blind design, in which clinicians and patients were not blinded, introduces potential performance bias. Knowledge of group allocation could have influenced supportive care measures such as hydration status, dose adjustments, or clinical monitoring. While laboratory personnel and outcome assessors were blinded, the possible influence of unblinded clinical management should be discussed in greater depth.

Response:
Thank you for highlighting this. We have added a more detailed discussion of the potential for performance bias due to the single-blind design in the Methods (Section 2.3) and Limitations (Section 5). We note that while all patients received standard institutional care protocols, unblinded clinicians could have influenced supportive measures.
Changes made:

- Methods (2.3): Expanded on blinding limitations.
- Limitations (Section 5): Added explicit discussion of performance bias risk.

Comment 6:
The study population is heterogeneous, encompassing a wide pediatric age range (1 month to 18 years), diverse diagnoses, and both PICU and ward admissions. Renal physiology and susceptibility to nephrotoxicity differ substantially across these subgroups. Consideration should be given to stratified analyses or, at minimum, a discussion of how this heterogeneity may have influenced the results.

Response:
We acknowledge this limitation. Due to sample size constraints, we did not perform subgroup analyses. We have now explicitly discussed the heterogeneity of the study population as a limitation in the Methods (Section 2.2) and Limitations (Section 5), noting that it may affect the generalizability of our findings.
Changes made:

- Methods (2.2): Added acknowledgment of heterogeneity.
- Limitations (Section 5): Discussed its potential impact on results.

Comment 7:
The manuscript reports no adverse effects related to theophylline administration; however, the methods do not clearly describe how adverse events were actively monitored or assessed. Given the narrow therapeutic index of theophylline, a more detailed description of safety monitoring procedures would strengthen confidence in the tolerability conclusions.

Response:
We have now added a dedicated subsection in the Methods (**2.5. Safety Monitoring**) detailing how adverse events related to theophylline were actively monitored, including daily clinical assessments for gastrointestinal, neurological, and cardiovascular signs of toxicity.
Changes made:

- Methods (2.5): New subsection added.
- Results (3.6): Referred to this monitoring in safety outcomes.

Comment 8:
The use of urinary microalbumin alone, without adjustment for urine creatinine (e.g., albumin-to-creatinine ratio), limits the sensitivity and reliability of renal injury assessment. Variations in urine concentration could affect results. The authors should acknowledge this limitation more clearly and justify the choice of measurement.

Response:
We agree. We have now explicitly acknowledged in the Methods (Section 2.4) and Limitations (Section 5) that urinary microalbumin was measured without simultaneous creatinine, which is a limitation. We also note that future studies should use the albumin-to-creatinine ratio for greater sensitivity.
Changes made:

- Methods (2.4): Clarified measurement method and its limitation.
- Limitations (Section 5): Added discussion on this methodological constraint.

Comment 9:
While repeated-measures ANOVA was appropriately used, the manuscript does not clearly state whether assumptions such as sphericity were tested or whether corrections (e.g., Greenhouse-Geisser) were applied when needed. Clarification of these statistical procedures would improve transparency and reproducibility.

Response:
We have now added explicit details in the Methods (Section 2.6) and Results (Section 3.5) that Mauchly's test was used to assess sphericity, and the Greenhouse-Geisser correction was applied when sphericity was violated.
Changes made:

- Methods (2.6): Added description of sphericity testing and correction.
- Results (3.5): Noted application of Greenhouse-Geisser correction.

Comment 10:
The discussion occasionally overstates the findings by suggesting a preventive effect on vancomycin-induced AKI, despite the absence of a formal AKI definition and clinically overt kidney injury. The conclusions should be tempered to reflect that the findings are preliminary and primarily indicate potential biomarker changes rather than definitive clinical nephroprotection.

Response:
We have revised the Discussion and Conclusion to more cautiously reflect that our findings are **preliminary and biomarker-based**, not definitive evidence of clinical nephroprotection. We emphasize the need for future studies with clinical AKI endpoints.
Changes made:

- Discussion (Section 4): Tempered language regarding clinical prevention.
- Conclusion (Section 6): Reframed as hypothesis-generating.

Sincerely,
Aghil Keykhosravi

4 Revision 3

2 comments · 4 Jan 2026 – 13 Jan 2026

Associate Editor

ACCEPT 4 Jan 2026

Dear EIC.
Previous errors have been corrected, and there are no further recommendations.

Editor-in-Chief

ACCEPT 13 Jan 2026

Dear Author,
The document has been carefully reviewed by the reviewers. Based on their comments and the revisions you made as requested, this manuscript is accepted.
Thanks.