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A Decade-long Evaluation of Neonatal Septicaemic *Escherichia coli*: Clonal Lineages, Genomes and New Delhi Metallo- β -Lactamase Variants

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Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

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February 13, 2023

Dr. Sulagna Basu
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India

Re: Spectrum05215-22 (**A Decade-long Evaluation of Neonatal Septicaemic *Escherichia coli*: Clonal Lineages, Genomes and New Delhi Metallo- β -Lactamase Variants**)

Dear Dr. Sulagna Basu:

Thank you for submitting your manuscript to Microbiology Spectrum. When submitting the revised version of your paper, please provide (1) point-by-point responses to the issues raised by the reviewers as file type "Response to Reviewers," not in your cover letter, and (2) a PDF file that indicates the changes from the original submission (by highlighting or underlining the changes) as file type "Marked Up Manuscript - For Review Only". Please use this link to submit your revised manuscript - we strongly recommend that you submit your paper within the next 60 days or reach out to me. Detailed instructions on submitting your revised paper are below.

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Sincerely,

Krisztina Papp-Wallace

Editor, Microbiology Spectrum

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American Society for Microbiology
1752 N St., NW
Washington, DC 20036
E-mail: spectrum@asmusa.org

Reviewer comments:

Reviewer #1 (Comments for the Author):

The fact that a long-term study of ExPECs was conducted on newborns admitted to the NICU is valuable information. Although the study was conducted in a single region, the content of the study was substantial, and I believe it will contribute to future research activities. On the other hand, I cannot find any evidence that the understanding of the transition of resistant strains itself will change the current practice of antimicrobial chemotherapy. In order to understand that the emergence of resistant strains is a critical situation, it is necessary to analyze the relationship between detected bacteria or isolates and outcomes (e.g., death, prolonged hospital stay, etc.). I have attached a comment on how. Please refer to the reference.

Executive Summary.

There is no information on the source of the isolates considered in this study (e.g., information about the population, geographic region, etc.).

L93 "This versatility is observed in its genome and also in its function or potential to cause disease" A reference to this statement is needed.

L106 What is "[WHO]"?

L171 "Whole genome sequencing (WGS)" WGS is spelled out in the sentence. The description of the method is redundant. It is too long compared to other items and should be shortened or moved to a supplement file.

Result.

L272 "Seventy E. coli were identified from the blood of septicaemic neonates (2009 to 2019)."

The number of cases, patient background, and outcomes (treatment outcomes) are unknown. Also, whether the results are similar to other parts of India or unique to this facility needs to be added to the discussion.

L231 "Isolates were resistant to second (52/80, 65%) and third-generation cephalosporins (67/80, 84%)" In the Methods section, the study on cephalosporins I am aware that the study regarding cephalosporins is not explicitly mentioned in the Methods section.

L267 "Transmissibility of metallo- β -lactamases and plasmid profile:" Is a colon necessary?

Discussion

L434 "under-5 mortality" The phrase "under-five" is also mentioned.

L480-489 "Several studies have shown that commensal E. coli are " The sentence is redundant due to the extensive use of literature citations. I think that you should state shortly and precisely what you want to assert from the results of this review. Overall, we believe that the authors over-explain the contents of the cited references.

Table 1. Although the table is organized in an easy-to-read manner, please consider creating a table that includes previous reports and results from other regions to determine if the results are appropriately compared or discussed.

Table 2. It is unclear what the criteria are for the type of rules.

The description of the discussion items is polite and clear, but is it possible to separate the items to make them more readable?

The reviewer has studied several reports of genetic testing of bacterial isolates, and it is not particularly rare to find such a heterogeneous population of isolates in a single institution's study?

Reviewer #2 (Comments for the Author):

Would be interesting to know if clinical outcomes changed after 2013 when transition from NDM-1 to NDM -5/ -7.

Staff Comments:

Preparing Revision Guidelines

To submit your modified manuscript, log onto the eJP submission site at <https://spectrum.msubmit.net/cgi-bin/main.plex>. Go to Author Tasks and click the appropriate manuscript title to begin the revision process. The information that you entered when you first submitted the paper will be displayed. Please update the information as necessary. Here are a few examples of required updates that authors must address:

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- Each figure must be uploaded as a separate file, and any multipanel figures must be assembled into one file.
- Manuscript: A .DOC version of the revised manuscript
- Figures: Editable, high-resolution, individual figure files are required at revision, TIFF or EPS files are preferred

For complete guidelines on revision requirements, please see the journal Submission and Review Process requirements at <https://journals.asm.org/journal/Spectrum/submission-review-process>. **Submissions of a paper that does not conform to Microbiology Spectrum guidelines will delay acceptance of your manuscript. "**

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Thank you for submitting your paper to Microbiology Spectrum.

Reply to Editor's comments

Reviewer #1

The fact that a long-term study of ExPECs was conducted on newborns admitted to the NICU is valuable information. Although the study was conducted in a single region, the content of the study was substantial, and I believe it will contribute to future research activities. On the other hand, I cannot find any evidence that the understanding of the transition of resistant strains itself will change the current practice of antimicrobial chemotherapy. In order to understand that the emergence of resistant strains is a critical situation, it is necessary to analyze the relationship between detected bacteria or isolates and outcomes (e.g., death, prolonged hospital stay, etc.). I have attached a comment on how. Please refer to the reference.

1. Executive Summary

There is no information on the source of the isolates considered in this study (e.g., information about the population, geographic region, etc.).

Answer: Isolates were collected primarily from the blood of septicaemic neonates admitted to a tertiary care hospital (IPGME&R and SSKM hospital of Kolkata, India). This tertiary care hospital in Kolkata, West Bengal caters to a population in Kolkata and also patients from different districts (at a distance of 100 km radius from Kolkata) of West Bengal.

2. L93 "This versatility is observed in its genome and also in its function or potential to cause disease" A reference to this statement is needed.

Answer: Authors have included the reference in reference number 9.

3. L106 What is "[WHO]"?

Answer:

Authors have expanded the term "WHO" as World Health Organization on **Page No. 6 & Line No. 104 in the Basu_S_Marked Up Manuscript.**

4. L171 "Whole genome sequencing (WGS)" WGS is spelled out in the sentence. The description of the method is redundant. It is too long compared to other items and should be shortened or moved to a supplement file.

Answer:

This is the sub-heading for the following section, hence it is not spelled out.

The authors have shortened this section in the revised manuscript (**Basu_S_Marked-Up Manuscript**) and moved part of it to the **Supplementary file** under the "Method for whole genome sequence (WGS) analysis" subheading.

5. Result.

L272 "Seventy *E. coli* were identified from the blood of septicaemic neonates (2009 to 2019)." The number of cases, patient background, and outcomes (treatment outcomes) are unknown. Also, whether the results are similar to other parts of India or unique to this facility needs to be added to the discussion.

Answer: A separate table (**Table 4**) depicting the association of outcome related to clinical and bacterial factors of neonates have been presented in the manuscript.

Two supplementary tables (**Supplementary Table S2A, S2B**) highlighting studies on *E. coli* causing neonatal sepsis from different parts of India and across the globe have been incorporated. Authors have compared data from these studies wherever applicable.

6. L231 "Isolates were resistant to second (52/80, 65%) and third-generation cephalosporins (67/80, 84%)" In the Methods section, the study on cephalosporins I am aware that the study regarding cephalosporins is not explicitly mentioned in the Methods section.

Answer: Antibiotic susceptibility pattern was determined for cephalosporins (cefoxitin, cefuroxime as 2nd gen and cefotaxime, ceftriaxone as 3rd gen cephalosporins) as a part of the antibiotic susceptibility test [disk diffusion data of cefoxitin, cefotaxime (2009-2017) and cefuroxime, ceftriaxone in VITEK®2 AST 280 (2018-2019)]. This has been clarified in the foot note of **Table 1 (Page No. 32 & Line No. 759)**.

7. L267 "Transmissibility of metallo- β -lactamases and plasmid profile:" Is a colon necessary?

Answer: Authors have now omitted the colon.

8. Discussion

L434 "under-5 mortality" The phrase "under-five" is also mentioned.

Answer: Authors have rephrased the term "under-5 mortality" by "under-five mortality" on **Page No. 20 & Line No 444-445**.

9. L480-489 "Several studies have shown that commensal *E. coli* are " The sentence is redundant due to the extensive use of literature citations. I think that you should state shortly and precisely what you want to assert from the results of this review. Overall, we believe that the authors over-explain the contents of the cited references.

Answer: Authors have modified the part of the discussion, taking into consideration comments from the reviewer.

10. Table 1. Although the table is organized in an easy-to-read manner, please consider creating a table that includes previous reports and results from other regions to determine if the results are appropriately compared or discussed.

Answer: Authors have included supplementary Table S2A and S2B in supplementary file representing list of published literature from different regions of India (S2A) and other countries (S2B) focusing on neonatal sepsis.

11. Table 2. It is unclear what the criteria are for the type of rules.

Answer: Authors have now modified Table 2.

12. The description of the discussion items is polite and clear, but is it possible to separate the items to make them more readable?

Answer: Authors have now modified the discussion.

13. The reviewer has studied several reports of genetic testing of bacterial isolates, and it is not particularly rare to find such a heterogeneous population of isolates in a single institution's study?

Answer: Yes, the authors agree to this comment. However, since this unit had a heterogeneous population of isolates, hence it is mentioned in the study.

14. Reviewer #2:

Would be interesting to know if clinical outcomes changed after 2013 when transition from NDM-1 to NDM -5/ -7.

Answer: A descriptive table with patient data and outcomes has been incorporated (**Table 4**) in the main manuscript. The possible relationship between mortality and sepsis due to a *bla*_{NDM}⁺ isolate was tested (**Supplementary Table S3B**) but not found to be statistically significant. It was noted that mortality increased from 2013 onwards (before 2013-39%, after 2013-57%). This has been incorporated on **Page No. 19 & Line No 432-433**.

Note:

New sequence types have been obtained for two study isolates which have been included in Table 1, Table 2, Figure 1 and Supplementary Figure 1.

May 27, 2023

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Re: Spectrum05215-22R1 (**A Decade-long Evaluation of Neonatal Septicaemic *Escherichia coli*: Clonal Lineages, Genomes and New Delhi Metallo- β -Lactamase Variants**)

Dear Dr. Sulagna Basu:

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Sincerely,

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