Author’s response to reviews

Title: Nasopharyngeal carriage, spa types and antibiotic susceptibility profiles of Staphylococcus aureus from healthy children less than 5 years in Eastern Uganda

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Author’s response to reviews:

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The Associate Editor,
BMC Infectious Diseases

Dear Professor Adebayo Osagie Shittu Ph.D.,

RE: Submission of revised INFD-D-19-01209R1: “Nasopharyngeal carriage, spa types and drug susceptibility profiles of Staphylococcus aureus from healthy children under 5 years in Eastern Uganda”

We thank you for forwarding our manuscript for peer-review and finding it potentially acceptable for publication in BMC Infectious Diseases. We also thank the peer-reviewers for taking time to critically look at the manuscript. We found the comments to be very useful and we have fully addressed all of them and made the necessary corrections to manuscript. We confirm that the revised manuscript conforms to the journal style, and we hope that you will find it suitable for publication in your esteemed journal. The text that has been significantly changed is indicated in red color.

We look forward to hearing from you soon concerning an editorial decision.
Regards,

David P Kateete, Ph.D., Makerere University

POINT-BY-POINT RESPONSE TO THE REVIEWERS’ COMMENTS / CONCERNS

COMMENTS

• Line 35: Antibiotic susceptibility testing (AST) was not included as part of the experimental procedure in the study but AST results are described in the next section (Results).

Response:

Antibiotic susceptibility testing (AST) is now included in the experimental procedure (lines 34-36).

• Line 39: Disk diffusion method is not used to determine susceptibility/resistance to vancomycin.

Response:

Indeed. We thank you for pointing out this error. In light of this, susceptibility testing was repeated for all isolates, by using the minimum inhibitory concentrations (MICs) based on the BD Phoenix 100 ID/AST expert system from the Becton &amp; Dickinson Inc., see lines 34-36.

• Line 45: and clindamycin resistant, while………

Response:

The suggested revision has been effected, see line 46.

• Line 52-53: Suggestion: ….could rapidly increase if mupirocin is administered in low-income settings.

Response:

The suggested revision has been effected, see lines 52-54.

• Line 53: S. aureus strains of spa types t064, t037 (MRSA) and t645 &amp; t4353 (MSSA)………

Response:

The suggested revision has been effected, see lines 54-55.

• Line 93: Suggestion: The procedure for the isolation and identification of S. aureus is described in Figure 1.

Response:

The suggested revision has been effected, see line 107.

• Line 102: Suggestion: Furthermore, nuc gene PCR-negative isolates were further evaluated………
Response:

The suggested revision has been effected, see line 116-118.

• Line 106: Section (antibiotic susceptibility testing): definition of multidrug resistance (MDR) was not provided.

Response:

The definition of MDR is now provided, see lines 138-139.

• Line 114: Disk diffusion method is not used to determine susceptibility/resistance to vancomycin.

Response:

As clarified earlier based on your advice, we repeated the vancomycin and clindamycin AST with MICs, and updated the manuscript to this effect, see lines 34-36 & 135-138.

• Line 153: …..of children per household was 2, while the median number

Response:

Data on demographics has been removed from the revised manuscript. For details please see our response to Reviewer #2.

• Line 154: Based on reports of mothers, 90%...........

Response:

Please see response to comment above.

• Line 156: Of the children who had been sick, around 30%........

Response:

Please see response to comment above.

• Line 165: delete vancomycin

Response:

As clarified earlier, we repeated vancomycin susceptibility testing with automated MICs, in which all the isolates studied were found to be vancomycin susceptible. Hence, we have retained the phrase in light of data from the repeat assays.

• Lines 160 & 161: (Figure 1 and Tables S1 & S2).
Response:

The suggested revision has been effected, line 177.

•Lines 166, 172, 177, 183, 187, 189 & 192: (Tables S1 & S2).

Response:

The suggested revision has been effected throughout the “Results” section of the manuscript.

•Line 172-173: carried the PVL genes………..

Response:

The suggested revision has been effected, line 178.

• Line 179: On PFGE analysis, isolates of spa type t064 clustered together (Figure S1).

Response:

This sentence was rephrased to read in accordance with suggestion from Reviewer #2, see lines 189-190.

•Lines 185-186: but compared with to MRSA, they were not as resistant to ciprofloxacin (2%, 2/99)?? Statement should be rephrased.

Response:

As advised, the statement has been rephrased for clarity, please see lines 191-193.

•Line 189: Generally, all isolates (MSSA and MRSA) were susceptible to rifampicin

Response:

The suggested revision has been effected, please see line 201-202.

•Line 190: delete vancomycin

Response:

This concern has already been addressed (please see responses above).

•Line 193: (Table 1).

Response:

The suggested revision has been effected, please see line 205.

•Line 198: (15.6%), respectively (Table 2).
Response:

The suggested revision has been effected, please see line 210.

• Line 199: Table 2: MRSA isolates resistant to cefoxitin are also resistant to penicillin

Response:

Correct. Table 2 shows MRSA isolates with antibiotic types R1 through R6, and they all have both FOX (cefoxtine) and PEN (penicillin) resistance patterns, depicting resistance to both drugs. For clarity and consistence we have now begun all the MRSA resistance patterns with PEN- (Table 2).

• Line 208-209: Suggestion: When the genotypes of S. aureus isolates at the IMHDSS were compared with previously characterized isolates in Uganda, it was observed that the spa types detected at IMHDSS……..

Response:

The suggested revision has been effected, please see lines 219-222.

• Lines 211, 214: (Figure S2).

Response:

The suggested revisions have been effected, see lines 222, 229, etc.

• Line 214:……accounted for clinical……

Response:

The suggested revision has been effected, see line 225.

• Line 216: (Figure 2 & Table S3).

Response:

The suggested revision has been effected, see line 227.

• Line 219: (Table S3 & Figure 2)

Response:

The suggested revision has been effected, see line 232.

• Line 219: Suggestion: When the isolates from the three sites were analyzed, spa types t4353, t002 & t355 were neither associated…..
Response:

The suggested revision has been effected, see line 232-235.

•Line 222: (Table S3)
Response:

The suggested revision has been effected, line 235.

•Line 236: “Several factors could be responsible for the high MRSA carriage rate in Ugandan children e.g. previous exposure to antibiotics as one third of the children had been given ampicillin and cotrimoxazole”. This assertion is debatable; carriage by parents and overcrowding could be appropriate factors.
Response:

Of course there are many factors that could be associated with MRSA carriage, and here we only mentioned a few of them as examples. We would like to thank you for bringing our attention to carriage by parents and overcrowding, which are now added to the list of probable factors in the revised manuscript, see lines 252-255.

•Line 242: Suggestion: In this study, the proportion of MSSA isolates resistant to penicillin and tetracycline was 78-79.8%........
Response:

The suggested revision has been effected, line 260-261.

•Line 248: delete “being MDR”
Response:

“being MDR” has been deleted as suggested, lines 266-267.

•Lines 249-251: The reported coexistence between hospital-acquired MRSA and community-acquired MRSA strains in Uganda [38] and other countries in Africa [39] likely contributes to the increasingly high MDR-MRSA rates reported from Africa. This statement is speculative as it is not be a plausible reason for this trend.
Response:

The statement has been revisited so that it is not speculative. It now reads “However, it is important to note that the resistance profiles between countries would very much depend on the different practices of drug use between the countries, antibiotic stewardship and enforcement of infection control practices”, lines 267-269.

•Line 251: delete vancomycin
This concern has already been addressed (please see responses above on susceptibility testing).

- Line 270: associated with ST121.

Response:

The suggested revision has been effected, line 294.

- Line 271: staphylococci in Uganda date 1958??

Response:

Yes. Here we meant to inform that microbial typing of Staphylococcus aureus clinical isolates in Uganda was first reported in 1958, see R. S. F. Hennessey & R. A. Miles, 1958 [reference 21], also available at https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2026807/

- A limitation of the study was the use of http://spatyper.fortinbras.us/ for the identification of the spa types (a number of unidentified spa types) and lack of information on the clonal complexes based on MLST. These limitations were not mentioned in the manuscript.

Response:

These limitations are now mentioned in the revised manuscript, lines 297-299. Thank you.

- Line 278: delete “we”

Response:

The suggested revision has been effected, line 310-312.

- Line 284-285: delete “we”: used as a tie-breaker confirmed their identity.

Response:

The suggested revision has been effected, line 318-319.

- Line 292: Suggestion: Use “Moreover” rather than “As well”

Response:

The suggested revision has been effected, line 324.

- Lines 294-296: Suggestion: Surveys of adult populations showed a reduction in S. aureus nasal carriage rate and this is attributed to factors including improved personal hygiene, smaller families.
Response:

The suggested revision has been effected, line 326-328.

• Line 296: kindly delete “etc”

Response:

The suggested revision has been effected, line 328.

• Lastly, S. aureus isolates of spa types t037, t064 (MRSA), and t645 and t4353 (MSSA).

Response:

The suggested revision has been effected, line 329-331.

• Line 482: Suggestion: Study flow chart illustrating the procedure for the isolation and identification of S. aureus

Response:

The suggested revision has been effected, line 542.

• Line 492: S. aureus should be in italics

Response:

S. aureus is now in italics, line 543.

REVIEWER 1:

GENERAL COMMENTS

1. Are the methods appropriate and well described?
   - The study was nested on previous studies but authors will need to clarify issues on the appropriateness of S. aureus isolates used from these previous studies for the current study (that was designed for children under 5 years of age).

2. Are the data sound?
   - The data are sound but authors will need to clarify the effects of issues raised above on the data used;

Response to the two general comments above:

Thanks for these concerns. The clarification on appropriateness of using Staphylococcus aureus isolates from the previous study is that this organism (i.e. Staphylococcus aureus) is consistently ranked with the pneumococcus, Haemophilus influenza, and Moraxella catarrhalis amongst common causes of pneumonia in children under 5 years of age, especially in the developing countries. Therefore, the previous study by Rutebenderwa et al 2015 focused on only three of the four commonly implicated bacterial species (Streptococcus pneumoniae, Morexella catarrhalis, and Haemophilus influenza) in pneumonia in children but did not characterize Staphylococcus aureus. Importantly, nasopharyngeal
colonization of these bacteria is a known risk factor for subsequent infection and/or invasion. Furthermore, characterizing the population structure of colonizing strains yields information on genotypes of strains that are likely to cause infection in a given population. Therefore, to close the existing gap in colonization by potentially pathogenic bacterial species among children under 5 years in rural eastern Uganda, the current study focused on Staphylococcus aureus. This clarification has been included in the background and methods sections, see lines 78-87 & 93-105, respectively.

MINOR REVISIONS

COMMENTS

ABSTRACT:

1. Line 42: Authors to correct error highlighted in the attached revised and 'tracked' manuscript

2. Correct "34/99" as "34/45"

3. Correct "60%" as "42.2%" and "19/99" as "19/45"

4. Line 43: Replace "an" with "one"

5. Line 46: Authors to add "MSSA"

Response:

All the above suggested revisions were effected as suggested in the “Abstract” section. However, we retained “19/99” as we were referring to chloramphenicol resistance among MSSA isolates, which was 19.2% (19/99), Table S1.

INTRODUCTION

6. Line 73: Authors to correct as "Haemophilus"

Response:

The suggested revision has been effected, line 74.

METHODS

7. Line 86: Authors to address comments and issues concerning study design Authors need to be explicit about their study design. There are some disparities between the previous study (ref 10) and the current study; (i) the previous study was on 152 households (with one nasopharyngeal sample per child/household) while the current study which is said to be nested on the previous study, had 742 nasopharyngeal samples; authors need to clarify this disparity in number; (ii) The previous study (ref 10) was conducted in 2008 (and not 2011 stated by the authors), and was published in 2015; (iii) Authors should clarify how the same nasopharyngeal samples cultured for isolation of S. pneumoniae, H. influenzae & M. catarrhalis in 2008 were then sub-cultured for S. aureus isolation; Were these samples processed for S. aureus in 2008 or were they kept and then processed afresh for the present study? (iv) The other study (ref 20) by Kalyango et al., 2013 referenced by the authors was a follow-up
study nested within an ongoing cluster-randomized trial of community health workers providing healthcare in the ICCM of malaria and pneumonia (intervention) and malaria only (control) started in 2009 involving 1,276 children. Authors need to clarify the relationship of their current study with this study.

Response:

Apologies for the discrepancies in the study design. We wish to clarify as follows;

The previous 2008 study (published in 2015) was a pilot that involved 152 children/households in the same study setting i.e. the Iganga/Mayuge Health &amp; Demographic Surveillance Site (IMHDSS) in rural Eastern Uganda, with focus on nasopharyngeal carriage of the Pneumococcus, Haemophilus influenzae and Moraxella catarrhalis, among children less than 5 years of age. This pilot study paved way to a larger study that was conducted in 2011 in the same setting i.e. IMHDSS in rural Eastern Uganda; the main study involved hundreds of households and approx. 1,300 children under 5 years of age were screened for carriage of Pneumococcus, Haemophilus influenzae and Moraxella catarrhalis. Please note that this larger cohort had several objectives among which was investigating integrated community case management of malaria and pneumonia in communities and appropriate treatment of pneumonia symptoms in children under five years; some of the outputs of this objective are publication number 20 by Kalyango et al 2013 (like you correctly observed). We wish to inform that in 2011, approx. 1,300 children in the same cohort were screened for carriage of the Pneumococcus, Haemophilus influenzae and Moraxella catarrhalis but the results are not yet published. The current study on S. aureus was conducted concurrently in 2011 on the same samples hence, nested in this larger cohort. Samples from 742 children of the ~1,300 children were randomly selected and processed in parallel, for isolation of S. aureus. Please note that in 2011, samples were simultaneously processed by two study groups: one group focused on isolating the pneumococcus, Haemophilus influenzae &amp; Moraxella catarrhalis while the second group focused isolating S. aureus. Results for the former (i.e. pneumococci, Haemophilus influenzae &amp; Moraxella catarrhalis) not yet published.

As clarified above, the previous study (ref 10) was a pilot conducted in 2008 published in 2015. By “2011”, we referred to the larger cohort that was conducted in 2011, of which the current study on S. aureus was part. As mentioned above, the pneumococcus, Haemophilus influenzae and Moraxella catarrhalis were also isolated in 2011 but the results not yet published for reasons beyond our control.

We have reworked the methods section to clarify this (see lines 93-105).

8. Line 91: Authors to address comments
Authors should clarify how the same nasopharyngeal samples (n=152) cultured for isolation of S. pneumoniae, H. influenzae &amp; M. catarrhalis in 2008 (ref 10) were then sub-cultured for S. aureus isolation; Were these samples processed for S. aureus in 2008 or were they kept and then processed afresh for the present study? There is need to clarify the difference between 152 samples in the 2008 study and the 742 samples in the current study. Did the authors recruit more children afresh to make up the number to 742?

Response:

We have responded to this query in our response to the concern above. Briefly, the previous 2008 study (published in 2015) was a pilot that paved way to a larger study that was conducted in 2011 in the same setting i.e. IMHDSS in rural Eastern Uganda; it involved hundreds of households and approx. 1,300
children under 5 years of age were screened for carriage of Pneumococcus, Haemophilus influenzae and Moraxella catarrhalis. In 2011, approx. 1,300 children were screened for carriage of the Pneumococcus, Haemophilus influenzae and Moraxella catarrhalis but the results are not yet published. The current study on S. aureus was conducted concurrently in 2011 hence, nested in this larger cohort, in which samples from 742 children of the ~1,300 children were processed for isolation of S. aureus. We did not recruit more children, but we conducted a nested study within a larger study in 2011.

RESULTS

9. Line 149: Authors to address issues/comments.
   • The demographic characteristics of children for the current study (n=742) should be clearly presented in a separate table and not as previously described. Although the two previous studies referenced are from the same IMHDSS, the number of children recruited and the study period for each study were different, study ref 10 had 152 in 2008 while study ref 20 had 1,276 from 2009.

Response:

As clarified already, the current study on S. aureus was nested in a study that was conducted in 2011, which, among other objectives, investigated the carriage and antibiotic susceptibility patterns of Pneumococci, Haemophilus influenzae and Moraxella catarrhalis. S. aureus was not included in this study and the Ethics Committee that approved our study on S. aureus waived the requirement to obtain informed consent / assent from the participants as we were dealing secondary data and not interfacing with the participants. As such, we have removed aspects of demographic data from the revised manuscript and focused our investigations to secondary data i.e. S. aureus characteristics, given that the pneumococcal data in the main study is not yet published.

• Line 149: These information should be presented in a table

Response:

As we analyzed secondary data, the current study was restricted to isolate (S. aureus) population structure / characteristics (due to ethical concerns); demographic data has been removed. Please see details in our response above to a related query.

• Line 165: Authors to add "to"

Response:

The suggested revision has been effected, line 176.

• Line 168: Correct "34/99" as "34/45"; Correct "60%" as "42.2%" and "19/99" as "19/45"

Response:

Thanks for pointing out these errors, which we have been rectified. Please note that we retained the “60%” as it referred to 27 of 45 MRSA isolates (Table S1), line 179.

• Line 190: Authors to add "MSSA"
Response:

The suggested revision has been effected, line 202.

DISCUSSION

• Line 234: Authors to add "other"

Response:

The suggested revision has been effected, line 247.

• Line 271: Authors to add "back to"

Response:

The suggested revision has been effected, line 283.

• Line 273: Authors to rephrase statement: 1992 cannot be said to be relatively recent to 2019 (27 years!!!). Authors can re-phrase this statement

Response:

The statement has been rephrased, see lines 285-286.

• Line 316: Authors to address issues/comments: This study referenced by the authors was conducted on surgical units involving 25 patients, 36 HCWs and 39 items in surgical units. This study did not involve children hence the bacteria isolates will be inappropriate for the current study designed for children < 5 years of age.

Response:

Our aim was to describe the population structure of S. aureus and/or MRSA in Uganda, without drawing inferences on epidemiological linkages. Otherwise we realize that it is generally inappropriate to relate isolates from children to those obtained from adults. However, there are very few studies in Uganda that have been conducted on this topic. We therefore restricted our comparisons to bacterial isolates only, and we generally avoided making any inference from our findings to the study subjects from which the isolates originated, and discussed this limitation, lines 307-310. We hope that our findings will advance this nascent field of S. aureus and/or MRSA research in Uganda and beyond, and that other researchers will put these limitations in consideration.

• From the reference section, ref 29 is Kizito et al., 2007 and not Kateete et al., 2013. Authors should correct this error

Response:

This has been corrected as advised (now reference 15), thank you.

Line 317: Authors to address issues/comments:
•The studies (ref 9 & 27) referenced by the authors used the same subjects who were 314 patients with SSI in general surgery, orthopaedic and obstetrics & gynaecology units. S. aureus isolates from these studies will not be appropriate for the current study as they were not from children. Authors should clarify this.

Response:

Please see response to similar concern above (“line 316”): the same response suffices for this comment. Thank you.

•This study as earlier stated was conducted in 2008 on 152 children 2-59 months old. This is appropriate for the current study on children < 5 years but the number is far less than 742 in the current study. The author will need to clarify issues here.

Response:

We have adequately addressed this concern; please see our responses above to similar concerns.

REVIEWER 2:

GENERAL COMMENT:
This is a very good, well written article describing the rates of S. aureus nasopharyngeal carriage amongst healthy children under the age of 5 years. The paper is worthy of publication should the authors address my comments below:

ABSTRACT
•Line 42: Since you are referring to MRSA being more resistant to non-beta lactams than MSSA please use the denominator for MRSA (45 not 99).

Response:

Many thanks for identifying this error. We have corrected and updated accordingly, in the Abstract and throughout the main manuscript.

METHODS:

•There is no mention on the SCCmec typing, how and what protocol was used to type the MRSA strains.

Response:

SCCmec typing and the protocol that we used are now described, see lines 149-150

•What was the statistical test used assess the significant difference between relative frequencies (i.e. how you generated the p-values)?
Response:

We used the Chi Square Test to determine the relationship between the relative frequencies of spa types, SCCmec types, and setting (i.e. IMHDSS, Mulago Hospital). A p-value of \( <0.05 \) was considered to be significant. This information is now provided in Methods, lines 164-167.

RESULTS:

•I understand that this is a nested study, but since the authors are comparing the genotypes between all the sites, it would be good if you recall the numbers of participants/isolates from the other two sites.

Response:

The number of isolates from the other two sites are now provided in the results section, see lines 222-225.

•Figure 1: the font within the boxes looks unclear.

Response:

Thanks for this concern. We agree that the quality of the image in the system-generated pdf file is somewhat poor however, we request that you download and take a look at the original TIFF image which has a higher resolution and clearer font than the figure embedded in the pdf file. It is the original TIFF file that will be eventually published, if accepted. However, should you find the original TIFF image to be of poor quality, we will revise the image and provide another.

•Figure 2: the dotted circles around the bars are distorting the figure, I recommend that the authors indicate the bars (of interest) with a symbol on top of each bar.

Response:

Thank you for the suggestion. Accordingly, the dotted circles have been removed from all the figures, and bars of interest are indicated with a symbol. Legends to figures have been modified accordingly.

•The authors should double check whether the y-axis is actually reflecting RF. I think it is reflecting numbers.

Response:

Correct, the y-axis indeed referred to numbers, not relative frequencies. We have modified accordingly. Thank you.

•Line 168: use the denominator for MRSA (n=45) not for MSSA (n=99).

Response:

Correct, we have used the denominator for MRSA (n=45), thank you (see line 178-179).
•Line 179: in Figure S1 (the PFGE) at least 3 isolates with spa t064 did not cluster with the others. So nothing makes this observation worth mentioning. Perhaps a general description of the number of clusters observed and the diversity of the collection.

Response:
We have revisited the statement as suggested, see lines 189-190. Thank you.

•Line 192: italicize the word 'Spa'.

Response:
Spa is now italicized, see line 204-205.

•Line 212: How do you define predominant? was there a cutoff for this?

Response:
This was based on the frequency of genotypes of isolates from the three sites. We however realized that the word “predominant” could be misleading hence we have replaced it with “frequent”, line 227.

•Lines 214 and 215: How did you defined clinical vs. colonizing strains? It would be interesting to split the data based on these definitions (clinical vs. carriage).

Response:
At best, there is no clear demarcation between clinical and colonizing, and the distinction between the two (i.e. clinical vs. colonizing) can be misleading given the fact that colonization precedes infection. We therefore revisited the phrase for clarity, lines 225-229. Thank you.

•Line 222: complete the sentence 'at Mulago Hospital'.

Response:
The sentence has been completed, see line 235. Thank you.

DISCUSSION
•Lines 229 and 230: Not only when the immune system is weakened, S. aureus could also be invasive if it enters a sterile anatomical site through skin infections or breach of a body barriers?

Response:
Indeed. The statement has been reviewed to reflect this fact, lines 240-243. Thank you.

•Lines 249-251: The concept here is vague please explain more clearly. Also the authors should emphasize that the resistance profile between countries would very much depend on the different practices of drug use between the countries.
Correct. The first Reviewer above had a similar concern and we reviewed the text accordingly, see lines 281-283

•Line 255: Mupirocin is not only used to treat impetigo?? rephrase the sentence to perhaps include examples of other infections.

Response:

We have rephrased the statement to include other infections treated with mupirocin, see lines 267-269

•Line 259 and 260: How do we know that MRSA decolonization is not common in Africa? any studies showed that?

Response:

Mupirocin use is not common in African countries other than South Africa and northern African countries. For instance, this drug has just been added to the list of essential drugs by the Uganda’s Ministry of Health hence, its importation into the country is relatively recent. We have rephrased the sentence for clarity, see lines 273.

•Just a personal opinion: Since t037 is a "dominant" strain in Uganda, and it was linked to SCCmec type I which is an unusual observation, since this strain is associated with the ST239 SCCmec III the Brazilian/ Hungarian clone. I think the authors should comment on this observation.

Response:

This is a valid point. Although spa type t037 has predominantly been associated with SCCmec type III by several investigators, others have associated it with SCCmec types I & IVc, see Mohammadi et al in Iran, also found here https://www.sciencedirect.com/science/article/pii/S120197121401457X

Generally, PCR genotyping approaches are prone to errors in interpretation by the investigator, and the molecular marker itself can yield results that may depict homoplasmy events and/or convergent evolution. This has been discussed as a limitation, see lines 301-305. Thank you.