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:

All the suggestions were fully effected as required by the Associate Editor. The revised text in light of each editorial comment is presented in blue font. Where necessary specific clarification is provided immediately for certain comments.

We look forward to hearing from you soon regarding an editorial decision.

EDITOR’S COMMENTS:
-Line 30-31: suggestion: and identify S. aureus lineages that cause infection in Uganda
-Line 34-35: suggestion: Antibiotic susceptibility testing based on minimum inhibitory concentrations (MICs) was determined by the BD PhoenixTM system.
-Line 39: suggestion: …yielding a carriage rate of 6.1% (45/742). In this study, all the isolates were susceptible to rifampicin, vancomycin and linezolid. Moreover, all MRSA were susceptible to vancomycin, linezolid and clindamycin.
-Lines 40-44: The information that MRSA in the study were more resistant to non beta-lactam antibiotics in comparison with MSSA does not provide the values for the MSSA resistant to those antibiotics so it is difficult to compare the values and the assertion. Moreover, it will be nice to know if this assertion was based on statistical analysis.
-Lines 44-46: long sentence; suggestion: Furthermore, 42 MRSA (93.3%) were multi-drug resistant (MDR) and one exhibited high level resistance to mupirocin. Overall, 61 MSSA (61.6%) were MDR, including three mupirocin and clindamycin resistant isolates.
-If one of the objectives of the study is to identify S. aureus lineages that cause infection in Uganda, this information is not provided in the abstract.
- Line 47: suggestion: delete “but only three MSSA were pan-susceptible to antibiotics” – I do not think it is important.
- Line 51: suggestion: …carriage rate in healthy children in Eastern Uganda
- Line 57: suggestion: “carriage” instead of “Staphylococcus carriage”
- Line 85-86: suggestion: to identify S. aureus lineages that can cause infection in community……..
- Line 98: suggestion: (less than 5 years)
- Line 102: I am confused with the term “follow-up study” is this the main 2011 study or another?
  RESPONSE: Yes, this is the main 2011 study. Hence, for consistence we have used ‘main 2011 study’
- Line 112-116: long sentence
  RESPONSE: The sentence has been broken-up into two shorter sentences
- Line 126: Mueller Hinton agar
- Lines 124-139: suggestion: If the results on antibiotic susceptibility testing presented in this manuscript was clarified and based on the BD PhoenixTM system, then it should be indicated that the antibiotic susceptibility testing was performed based on minimum inhibitory concentrations (MICs) using the BD PhoenixTM system.
  RESPONSE: Correct. Antibiotic susceptibility testing for all the isolates was repeated and clarified based on the BD PhoenixTM system. As advised we have updated the text to this effect.
- Line 145: suggestion: To obtain spa types, sequences were submitted to an online……
- Lines 146-147: I think it is important to indicate the reason/objective for comparison of previously described spa types with the current study.
  RESPONSE: The objective was to identify S. aureus lineages that are likely to cause infection in Uganda. We have updated the text to this effect.
- Line 153: Criteria for selection of isolates for PFGE not mentioned. I suggest that the borders of the Figure S1 should be trimmed.
  RESPONSE: PFGE typing was done on isolates with frequent spa types (e.g. t064, t037, etc.), aiming to determine whether they would have the same profiles. However, funding limitations curtailed this as we were offered to analyze only those reported numbers.
  The borders of Figure S1 have been trimmed and the revised version submitted.
- Lines 176-181: Suggestion: In this study, all the isolates were susceptible to rifampicin, vancomycin and linezolid, while the MRSA were susceptible to vancomycin, linezolid and clindamycin.
  The information that MRSA in the study were more resistant to non-beta-lactam antibiotics in comparison with MSSA does not provide the values for the MSSA resistant to those antibiotics so it is difficult to compare the values and the assertion. Moreover, it will be nice to know if this assertion was based on statistical analysis.
  RESPONSE: The suggestion has been effected. Statistical values are now provided and we have updated the text accordingly.
- Lines 183-185: suggestion: the statement on comparing PVL+ MSSA and MRSA+ PVL should be deleted, and replaced with the number of PVL+ MSSA and MRSA.
- Line 190: (Table 1).
It is very important to know the reason some S. aureus isolates were not genotyped by spa typing. The number and frequency of the spa types might change if these isolates were genotyped. If there was a limitation in typing all the isolates, it was not indicated in the manuscript. 

RESPONSE: Yes, there was a limitation (funding) in typing all the isolates. The available support for typing of isolates was an offer, which funded the typing of the reported numbers. Of course the number and frequency of the spa types could change if these isolates were genotyped. We have mentioned this as a limitation in the text.

I suggest that the calculation for relative frequency should be defined.

The percentage and proportion of MSSA isolates resistant to the following antibiotics are as follows: penicillin (78.8%; 78/99), tetracycline (79.8%; 79/99), cotrimoxazole (27.3%; 27/99).

RESPONSE: Because t645 is a frequently occurring spa lineage in Uganda and we were able to associate it with MSSA. The frequencies and hence relative frequencies of other spa types that exclusively occurred in MRSA i.e. t078, t3662, t318, t1456, t10394, t1476, t2168, t213 were too low (i.e. ≤2 occurrences) to draw meaningful conclusion. We have clarified the text to this effect.

Please delete “Generally, all isolates (MSSA & MRSA) were susceptible to rifampicin, vancomycin and linezolid but only three MSSA (R16, R180 & R716) were pan-susceptible to antibiotics”

Spa type t645 exclusively occurred in MSSA (Table 1)? However, there were other spa types that were not identified with the MRSA isolates. Please clarify.

RESPONSE: Because t645 is a frequently occurring spa lineage in Uganda and we were able to associate it with MSSA. The frequencies and hence relative frequencies of other spa types that exclusively occurred in MRSA i.e. t078, t3662, t318, t1456, t10394, t1476, t2168, t213 were too low (i.e. ≤2 occurrences) to draw meaningful conclusion. We have clarified the text to this effect.

The word “antibiotype” should not be broken; # should be replaced with Number (No.); R2: CIP. As indicated above, the number of isolates not genotyped will affect the frequency indicated in Table 2.

RESPONSE: We have already responded to the second part of the comment in our response to related comments above. The other suggestions have been effected.

This was intentional. Throughout the manuscript we used smaller font size below the Tables to draw attention to particular details that we feel is necessary to understand what we are communicating, and we may not have provided it at that particular section of the manuscript. We hope this is acceptable.

GEN: gentamicin

were previously reported from Mulago Hospital in Kampala, but slightly different from rural Western Uganda.

I am confused with the information provided. I guess it is indicating the number of isolates from previous studies, however, it is not clear.

RESPONSE: This information was requested by Reviewer #2, who requested for information on the number of isolates from the other sites. We have reworked it for clarity.

As indicated by one of the reviewers, the population is different.

RESPONSE: We have added this fact as a caution.
-Line 266: clarification: “compared to the current rate (91%)?  
RESPONSE: We meant compared to the 91% rate reported in this study. The text has been updated to this effect.

-Line 269: delete “very much”  
-Line 272: gentamicin  
-Line 276: S. aureus