Author's response to reviews

Title: Nasal Carriage and Antimicrobial Susceptibility of Staphylococcus aureus in healthy preschool children in Ujjain, India

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Author's response to reviews: see over
To,
Editorial Office,

BMC Pediatrics

Subject: Submission of revisions for the Manuscript ID: 9319196094067711

Dear Dr. Melissa Norton,
I am enclosing herewith the following manuscript: “Nasal Carriage and antimicrobial susceptibility of *Staphylococcus aureus* in healthy preschool children in Ujjain, India”. The manuscript is coauthered by:

Ashish Pathak, Yogyata Marothi, Rama V Iyer, Binita Singh, Megha Sharma, Bo Erikson, Ragini Macaden and Cecilia Stålsby Lundborg.

We hereby submit point by point reply of the queries and suggestions of the reviewers:

**Reply to reviewer 1**

**Major compulsory revisions:**

**Comment 1:** The conclusion of the abstract and the MS should be rephrased, the fact that a relatively low carriage rate was found does not necessarily reflect on disease. Nothing regarding disease was reported and these conclusions are not based on the current study.

*Reply:* We agree with the reviewer that the nasal carriage rate does not necessarily reflect the disease and have revised the conclusions in the abstract “We found a relatively low rate of nasal carriage of *S. aureus* in children below five years when compared to children of older age group in India.” and in the manuscript “The study shows that attending pre-school or school and living in a large family was associated with nasal carriage of *S. aureus*.”

**Comment 2:** Methods: It appears that a multivariate analysis was performed, yet it is not described in the statistical analysis methods. Give detail on which variables were entered, how was this determined, etc.

*Reply:* We have now given the details of the multivariate analysis in the statistical analysis “The relationship between each variable and the outcome (nasal carriage of *S. aureus*) was explored using odds ratios (OR). Crude OR’s were calculated from two by two tables. A given variable was entered in the multiple logistic models if the bivariate analysis yielded a P value less than 0.1. In the final model all the variables were adjusted for age and sex. A complete case series analysis was used. The independent variables included were: sex (boys versus girls), age group (7 to 12 months, 13 to 24 months and 25 to 59 months versus 0 to 6 months), current
breastfeeding status (yes versus no), child attends no school, preschool, school (no school/ no preschool versus preschool, school), family size (between 5 to 10 members and more than 10 members to less than or equal to 4 members), education of the mother (up-to primary, up-to higher secondary and graduate or postgraduate versus illiterate), occupation of the breadwinner (self employed versus salaried), antibiotic use in the last 2 weeks (yes versus no), hospitalization in the last 2 weeks (yes versus no), hospital visit in the last 2 weeks (yes versus no). Chi-square tests were used to test for statistical significance (5%).”

Comment 3: Refrain from using 'Risk factors', since this is a cross sectional study, only factors associated with S. aureus carriage can be determined, or predictors of S. aureus, but the study cannot define risk factors.

Reply: We agree with the reviewer and have corrected the anomaly by using “association” in place of “risk factors” in abstract page 2 in objectives and results; on page 5, para2, page 6, para3, on page 9, para 3 the sub-heading “Risk factors for colonization” has been changed to “Factors associated with nasal carriage”, page 11, para1 and also in the title of table 1 and also in the abstract and also in table 1 page 17.

Comment 4: Page 13 4th paragraph: the sentence starting with: "Therefore, the main strength of the study..." is incorrect. The study did not identify risk factors, but rather factors associated with carriage. Furthermore, I dont see how this is the main strength, since the only predictor was attending school or preschool, which is not a novel finding.

Reply: We have removed the above-mentioned lines from the text. The text now starts as follows “This study is the first contribution to the database of nasal carriage in healthy children below five years from India”. Also as replied in comment 3 above we now have refrained using “risk factors” in our manuscript.

Comment 5: Table 1 is unnecessary, totals can be added to Table 2 and thus it will include the data from Table 1.

Reply: In response to the above comments we have omitted Table 1 and added the totals to table 2, which now becomes table 1.

Comment 6: Tables 3 +4 as are presented are not very effective. Perhaps a figure with the prevalence of different susceptibility patterns will be more useful?

Reply: As per the suggestion we have included Figure 1 of the susceptibility pattern of MSSA, Figure 2 susceptibility pattern of MRSA and a new table (Table 3), which shows the pattern of co-resistance to different groups of antibiotics among 82 MSSA isolates.

Minor essential revisions:
Comment 1: When describing the antibiotic sensitivity pattern - more relevant is the pattern of antibiotic resistance among different isolates, not just the resistance to a single antibiotic. It seems like most MRSA observed, is more the HA-MRSA type, but unclear due to the way it is presented. How many are MDR? How many only resistant to b-lactams? how many clinda-inducible...

Reply: We have now included a table (table 3), which shows the pattern of co-resistance to different groups of antibiotics among 82 MSSA isolates. The table also gives information on resistance pattern to beta-lactams. The information on clindamycin-inducible strains is now added in the result section on page 10, para 1 as “Among the erythromycin resistant strains of MSSA 15% were clindamycin inducible” and page 10 para 2 “Inducible resistance to clindamycin was 35% in MRSA isolates.”

Comment 2: The fact that MRSA was not definitely determined using mecA, is a major disadvantage. Can this be tested and added?

Reply: We are unable perform mecA gene to definitely determine the MRSA status due to financial constraints. If the funding becomes available we will follow-up this study with molecular study of S. aureus. We have discussed this disadvantage in the manuscript in the section on limitations of the study page14 para1 as “Also, we did not confirm MRSA status by doing mecA gene due to financial constrains.”

Comment 3: Page 13 3rd paragraph: "In MRSA isolates..." is unclear. Needs to be rephrased.

Reply: We have rephrased the paragraph to “In MRSA isolates resistance was seen to antibiotics that are important for empirically treating severe infections. These antibiotics include doxycycline (44%), levofloxacin (31%) and clindamycin (5%). Resistance to levofloxacin and clindamycin is a cause of concern because of their therapeutic value in treating serious S. aureus infections in high-risk patients.”

Discretionary revisions:

1) The very low prevalence rate, especially in the very young, is surprising. Would be interesting to see the prevalence among the very young - <1 month, or <3 months and then up to 6 month. Is it still so low?

Reply: We thank the reviewer for the suggestion. In this study children from age 1 month to 59 months were included. So, we do not have the data for the very young. We would like to look at the prevalence of S. aureus in the very young when we have the funds for the same.
Reply to reviewer 2

Comment: GENERAL
This is a nice straightforward cross-sectional study on S. aureus nasal carriage and antibiotic resistance in a large cohort of young children under-5 in India. The main finding is the rather low prevalence of SA nasal carriage in this cohort, which is not really explained by the authors.

Reply: We thank the reviewer for considering our manuscript as “nice straightforward”. We have explained in the introduction of the manuscript that one of the reasons of doing the study was lack of studies in the age group below five years; therefore the purpose of the study was to explore the prevalence in this age group in Ujjain, India. This being a cross-sectional study we did not want to speculate the reasons for rather low prevalence of SA nasal carriage in this study. Also there are no studies from India in the similar age group to compare our results. Thus, we feel that the study contributes to the database of SA in this age group from India.

MAJOR COMPULSORY REVISIONS

Comment 1: for the message of their study, this article could easily be condensed into a 'brief report'. Mainly is the discussion, the authors spend too much effort into the antimicrobial resistances found, without having typed the respective SA isolates (for instance SCC MEC typing, or PFGE).

Reply: We are unable to confirm resistance in MRSA by performing mecA gene due to financial constraints. We have discussed this disadvantage in the manuscript in the section on limitations of the study page14 para 1 as “Also, we did not confirm MRSA status by doing mecA gene due to financial constrains.”

Comment 2: Furthermore, the authors conclude that 'targeted screening based on risk factors, isolation and decolonization could be effective in preventing further spread of resistance in the community' (page 13). However, using their 'risk factors' the identification of a child carrying MRSA will only go up from 1% (6%*16%) up to 4% (6%*16%*4), while (as can be read from table 1) 26.8% of children had been prescribed antibiotics in the last 2 weeks, which in my opinion is a 'massive' percentage. In my opinion, to prevent spread of resistance in the community, focus should be on 'prudent antibiotic use' and 'simple hygienic measures' much more than on trying to identify children carrying resistance SA strains. This should be discussed by the authors.

Reply: We agree with the reviewer and have made our conclusions more in line with the results. We have also refrained to use the term “risk factors” and use “factors associated” in the manuscript.

We thank the reviewer for stressing that “prudent antibiotic use” and “simple hygienic measures” are better then trying to identify children carrying resistance SA strains. We have now incorporated this in the manuscript. (page 13 para1) “In MRSA isolates resistance was seen to antibiotics that are important for empirically treating severe infections. These antibiotics include doxycycline (44%), levofloxacin (31%) and clindamycin (5%). Resistance to levofloxacin and clindamycin is a cause of
Concern because of their therapeutic value in treating serious S. aureus infections in high-risk patients. However, it is important to note that the present results are of carriage state and not clinical infections.”

Comment 3: Another issue is the antibiotics tested. I cannot understand why the SA isolates were tested for 6 different quinolones, 3 3rd generation cephalosporines, 2 aminoglycosides and 2 glycopeptides. Also, I do not understand why MRSA isolates are tested for amoxicillin, amoxicillin-clavulanic acid, cefixime, ceftriaxone and cefoperaxone. I imagine that in a resource-constrained setting, resources could be used more effectively.

Reply: We have now described in the methods section that the selection of antibiotics was based on the common antibiotics prescribed, whoever we could have been more conservative in our approach. In response to this comment we have now present the most relevant antibiotics and in our further studies we will consider carefully which antibiotics to test, although the cost of doing the disc diffusion is minimal as compared to MIC or mecA gene determination.

“Susceptibility testing was done and results are presented for the following most important antibiotics: co-trimoxazole, ampicillin, co-amoxiclav, ciprofloxacin, levofloxacin, ceftriaxone, erythromycin, clindamycin, doxycycline, chloramphenicol, tetracycline, gentamicin, amikacin, linezolid, teicoplanin and vancomycin.”

Comment 4: In the whole article, no clear distinction is made between carriage and infection. For a clear discussion, this distinction should be made very clear.

Reply: We have made changes in the introduction (page 5, para 2) as follows “The present study is done to understand the epidemiology of nasal carriage of S. aureus in view of paucity of studies in healthy children below five years of age in India.” and also in the discussion (page 14, para 1) “In MRSA isolates resistance was seen to antibiotics that are important for empirically treating severe infections. These antibiotics include doxycycline (44%), levofloxacin (31%) and clindamycin (5%). Resistance to levofloxacin and clindamycin is a cause of concern because of their therapeutic value in treating serious S. aureus infections in high-risk patients. However, it is important to note that the present results are of carriage state and not clinical infections,” to make it clear to the readers that the article deals with carriage and not clinical infection.

MINOR ESSENTIAL REVISIONS

Comment 1: page 4, 3rd paragraph: also MSSA can cause complicated infections; in that respect MRSA is not different from MSSA.

Reply: We agree with the reviewer and have made it the following change in the para “The individuals colonized with S. aureus (both CA-MRSA or community acquired methicillin-sensitive S. aureus) tend to have a complicated clinical course from a disease originating from their endogenous S. aureus [3]. The complicated clinical course results from increasing resistance in S. aureus isolates and also because it can cause deep-seated infections and sepsis [3, 4].”
Comment 2: page 6, 1st paragraph: This was a cross-sectional study, with data-collection from Nov 2007-Feb 2009!
Reply: We have revised the sentence to “This was a prospective study conducted during 15 months from November 2007 to February 2009.”

Comment 3: page 11, paragraph 2: there are data that in resource-limited settings, children and adults are more prone to be colonised with gram-negative bacteria (E. coli, Klebsiella etc.) instead of SA, which could contribute to the low SA carriage rate found. Please discuss.
Reply: This was the study of nasal carriage of S. aureus in children below 5 years of age. In our review of literature E. coli and other gram-negative bacilli were not implicated as important bacteria in nasal carriage in children. We are thus, unable to state that this could be important reason for relatively low rate of nasal carriage of S. aureus.

Comment 4: page 12, paragraph 2: the relation between hygiene and day-care absence and SA carriage is not explained. please remove.
Reply: We have removed the above-mentioned reference from the page12 para 2. However, since the Swedish study highlights the importance of hygiene, especially hand washing we have included it in the discussion of measure to curb further spread of resistant strains in the community in discussion section on page para as “Antibiotic use is one of the most important determinants of antibiotic resistance (24). A high antibiotic use rate is reflected by the fact that 26% of children enrolled in the study had received an antibiotic in the previous two weeks. Antibiotic stewardship programmes that promote judicious use of antibiotic are urgently needed and could prove to be more cost effective than targeted screening based on risk factors, isolation of the carriers and decolonization (28). Simple hygiene measures like hand washing are effective in preventing spread of resistant organisms in the community. The importance of hygiene is exemplified in an intervention program in Swedish day care centres, which introduced alcohol-based hand washing for children. This intervention significantly reduced (by 12% points) children’s absence from the day care [19].”

Comment 5: table 2: 'Child attends' numbers are mixed up between Positive and Negative!
Reply: Thank you for identifying the anomaly! We have now changed it in the revised table number 1.
We have corrected some mistakes previously made in the manuscript, like on page 12, para 3 “Hospitalization in the recent past was not a factor significantly associated with nasal carriage in our study. However, hospitalization has been demonstrated by other studies [3, 16, 21-23] as a significant risk factor. Similarly, hospital visit is considered a risk factor [3] for acquiring S. aureus carriage however, in our study hospital visit was not identified as a statistically significant factor associated with nasal carriage. “

Thanking you in anticipation!

Ashish Pathak MBBS, DNB (pediatrics), doctoral student