PEER REVIEW HISTORY

This paper was submitted to the JECH but declined for publication following peer review. The authors addressed the reviewers' comments and submitted the revised paper to BMJ Open. The paper was subsequently accepted for publication at BMJ Open.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Evaluation of swabbing methods for estimating the prevalence of bacterial carriage in the upper respiratory tract: a cross sectional study
AUTHORS	Coughtrie, Abigail; Whittaker, Robert; Begum, Nelupha; Anderson, Rebecca; Tuck, Andy; Faust, Saul; Jefferies, Johanna; Yuen, Ho Ming; Roderick, Paul; Mullee, Mark; Moore, Michael; Clarke, Stuart

VERSION 1 - REVIEW

REVIEWER	Elisabeth AM Sanders Department of Pediatric Immunology and Infectious diseases, University Medical Center Utrecht, the Netherlands
REVIEW RETURNED	09-Jun-2014

GENERAL COMMENTS	The subject is timely and relevant since bacterial carriage is the focus of efficacy and effectiveness in vaccine studies like pneumococcal conjugate and meningococcal vaccine studies as well as the recent research into the respiratory microbiome and relation to respiratory symptoms and disease. In case subjects at home may provide swabs and washings from the nose and mouth that are as reliable to establish carriage as health care professional assisted swabs and washings, this would mean a high benefit in costs and feasibility for large cohort studies to evaluate interventions. A large study in over 10.000 subject was undertaken to compare self-swabbing with health care professional swabbing. Of these, only 15% of all invited subjects actually participated and only 6% participated in the health care professional swabbing. This is much lower than the initially estimated response rates (25% self-swabbing and 25% response rate for HCP swabbing). This resulted not only in low numbers of participant divided over the various age groups, but also less children and more elderly participating in the health care professional group. The authors present the study as s a pilot study and not designed to have the power to detect non-inferiority of estimating carriage rates by HCP-administered versus self-administered swabs, which would be a main question. This leaves us with an unanswered main question and no conclusion. The lower age of the self-swabbers compared to the groups that visited the health care center might explain the higher carriage rates in the self-swab group. The large non-response bias needs to be addressed to answer the basis question whether this method of self- swabbing is reliable can be used.

	Abstract Bacterial carriage in the upper respiratory tract is usually asymptomatic but may proceed towards respiratory or invasive infections. Disease is always preceded by colonization. Setting: patients; would that be better subjects instead of patients? Conclusion: Since no non-inferiority testing was not possible and nose swabs did not significantly differ between the self -swabbing and health care professional, the conclusion may not be justified. Strengths and limitations of this study There have been several large studies indeed that have reported on NP swabs and several species like S. pneumoniae, S. aureus and N. meningitidis. But none that I know that have compared self-swabbing with health care professional swabbing. Apart from response rates, this would be the main topic for me. The question is first whether self- swabbing is equal or better that assisted by a HCP. This was not answered. Introduction Ref 13 O'Brien, K.L. and H. Nohynek, Report from a WHO Working Group: standard method for detecting upper respiratory carriage of Streptococcus pneumoniae. Pediatr Infect Dis J, 2003. 22(2): p. e1-11. This reference can better be updated with Satske et al, vaccine 2014; 32 (165-179) Page 8, lines 5-8 their GP practice where nasopharyngeal (NPS) and whole mouth (WMS) swabs were taken by a registered HCP. The self-swabbing group involved participants being sent a self-swabbing pack containing nose (NS) and whole mouth (WMS) swabs by Danvers International (London, UK). I miss all information on the type of swabs, and transport materials. Also the time to get to the laboratory before further analysis. Methods: page 8, lines 19-21 The study questionnaire requested the following details pertinent to bacterial carriage: participant age, recent use of antibiotics, recent RTI and vaccination status. What is meant by recent use? One month, 3 months? And what is recent RTI? One week, 4 weeks? And how was the patient motivated to participate? This is relevant I think. Page 14, lines 19-28 Overall, total costs per participant were over a third lower in the self-swabbing group at £41.21 ($67.92) versus the HCP group at £69.66 ($114.82) (Table 1). NHS service support costs made up a large proportion of the difference between the two study groups, representing 56.7% (£39.52/person) of costs in the HCP group but only 6.8% (£ 2.81/person) of costs in the self-swabbing

	group. How was this calculated? All swabs sent but not used included, as stated in the legend of table 1? DISCUSSION We compared multiple swabbing sites in order to assess the most effective way of sampling the human respiratory tract flora in the hope to provide information for implementation of a standardised swabbing method. This question was not answered. Furthermore, low participation rates were observed, even in the self swabbing group, in particular in children with only half the general practices with 25% participation rate after the invitation. Page 16, lines 10-12 lower participation rates within this group have most probably resulted in reduced carriage rates within NPS. Carriage rates can be high in small groups, so there must be another explanation like few children participating and more elderly (see Table 1). Table 1: point missing in % of vaccination up-to date. (860 %)

REVIEWER	Anne von Gottberg National Institute for Communicable Diseases, Johannesburg, South Africa
REVIEW RETURNED	23-Jul-2014

GENERAL COMMENTS	Abstract The number of participants randomised to self-swabbing or HCP-led swabbing is not clear – the numerator is the respondents, but the denominator is not given here – and later on not clearly in the results. Methods: The number of invited individuals (not really patients? as described in the abstract) are 10,448 in the abstract but 5220 in the sample size description? This should be reconciled. Any patient deemed unfit: it would be good to have a full list of the exclusion criteria, and a total number of the individuals that were excluded in this step. How were the numbers 202 (<5 years) and 320 (=>5 years) arrived at? The swabbing appointments that were offered: what days, how many options, after hours, on Saturdays? And for how long was the option open to go for the appointment? Were any reminders sent to both groups? How often? How far away were these clinics? Or places for the appointments? The reader needs to know how convenient or not the appointment may have been? We need more details about the swabs: cotton-wool swabs? Flocked swabs? Did they slot into a transport medium? Or were they just dry swabs that just slotted back into plastic tube? Did the self-swabbing group and the healthcare provider group all use the same swabs, from all sites? What was the average time from taking of the swab and arriving at the laboratory (for the self-swabbing group)? Or any indicators of turn-around-time? How many swabs were lost in the post? . Did equal numbers of nasal and whole mouth swabs get returned from

	the self-swabbing group? For the HCP group? What was the definition of “recent use of antibiotics”? one month ago? Three months ago? One week ago? Only antibiotics? Or all antimicrobials? Definition of recent RTI? How was RTI defined for the self-swabbing group? Were there differences in the questionnaire for the two groups? For vaccination status: was this really thought to be accurate for adults? As I read the manuscript, this was analysed for both children and adults, but I am not sure how useful the data would have been for adults. It is mentioned in the limitations, and I agree that it is a significant limitation: vaccination history could have been specific for the vaccine that may have impacted on specific carriage (pneumo and Hi) and specifically analysed in the age groups most likely to have been affected by vaccination. The statement: “participants were invited to undertake swabbing between May-August 2012” is not very helpful. Is this the time period when 1st invites went out to all GP practices? With 1st or 2nd reminders? When did the first swabs arrive back by post? And the last? The first HCP performed swab performed was when? And the last? Did they all trickle in evenly over a similar period? Or was there a final push? How much time were both groups given to respond (I asked this earlier)? Genus and species should be written out in full when appearing the first time. Results Throughout is would be useful to give both n/N for % (e.g. participation rates; swab positivity rates, co-carriage rates) especially if these data are not available in tables or figures. Table 1: for vaccination status HCP swabbing the % is incorrect for up-to-date Table 4 is cited before Tables 2-3? The significant findings by age group in table 4 are mentioned, but not those by RTI or recent use of antibiotics, nor vaccination history? Discussion The first sentence would be improved by simply stating the most important findings from your study: there have been many studies looking at carriage of multiple bacterial species and not much is made of this one being population-based – no carriage incidences are given etc. And depending on how you define large population-based study across all age groups – I can already think of other studies. Your study is worthwhile and should be published – it is unnecessary to make such a statement as the first sentence. Do not use the first paragraph to repeat the methods, rather highlight your most important findings. Lack of isolation of meningococcus may also have had something to do with turn-around-times for the swabs, transport media etc. part of the reason for providing more details in the methods. The herd effect (not really immunity) statement should have a reference. I think the lack of any effect with vaccination status may also be due to misclassification? For future studies a careful hypothesis should be generated about what you may expect to see, in which age groups, with which vaccines, and which organisms, then the questions can be more carefully tailored for these analyses, which may only be relevant in certain age groups.

	The final statement seems a little out of place – age groups were slightly problematic, as response rate differed by age, and determining at-risk population for what? And we have just agreed that vaccination/vaccine was a weak point in this manuscript, and antibiotics were not discussed in detail at all?

REVIEWER	Lesley McGee Microbiologist Centers for Disease Control and Prevention USA
REVIEW RETURNED	25-Jul-2014

GENERAL COMMENTS	The paper requires some revision and I would need to review again before considering for publication. **Comments** If you have any further comments for the authors please enter them below. The authors describe a cross sectional study comparing 4 swabbing methods with the aim of identifying a single specimen collection method for estimating the prevalence of bacterial carriage in the upper respiratory tract. Overall comments: The authors suggest that there are few papers in the literature describing carriage rates of multiple bacterial species (Discussion page 15, 6-8). I don't agree with this statement. Over the past 2 decades there have certainly been more than a "few" studies looking at the associations between various pathogens in the upper respiratory tract as well as studies that have looked at the impact of vaccination on multiple URT pathogens in a single study. I do agree that the majority of these have focused on children since this is the age group where carriage rates to most pathogens studied are higher and therefore an ideal target population to study. The age specific changes in carrier status and co-carriage associations of different pathogens reported here are the same as those observed in other previously published studies. The study was limited to culture detection for 6 bacterial species. The 4 methods included a nose self-swab, mouth self-swab, nasopharyngeal HCP-swab and mouth HCP-swab. The author's conclusions are that a self-taken nose swab would provide sufficient sensitivity for the estimation of prevalence for all 6 organisms tested, even though Neisseria meningitidis was not isolates and sensitivity for Moraxella catarrhalis detection was lower for nose versus mouth swabs? Specific comments: Title: Would recommend adding in "Comparison" or "Evaluation" of swabbing methods… to the title for clarity. Abstract: Page 2, line 6. Would suggest: "Bacterial carriage in the upper respiratory tract can lead to respiratory…" Page 2, line 39. What does "… more deprived practice locations" mean? Do you mean GP practices in areas with lower socioeconomic status? Introduction:

	Page 6, line 20. I think “socioeconomic status” is a better term that “deprivation”? Methods: Page 7, lines 23-34. This section is confusing. You state that “We invited 2,020 children aged 0-4 years and 3,200 older children and adults to participate”? But in the Abstract and on page 8, lines 41, says a total of 10,448 patients were invited to participate? Page 8, lines 10-12. I don’t agree that the authors suggest that mouth swabs can be used as a proxy for throat swabs. These are different anatomical sites with very different flora present in each of these. Just present the data how it is? It’s a mouth swab and not a throat swab, and deal with the limitations of this for the various pathogens which might be more readily isolated from the throat versus the mouth. Page 8, line 28, delete “..and recent…” Page 8, line 52. The methods section lacks detail. No information is given as to what type of swabs were used in the study? Also what transport medium was used? S.pneumoniae recovery works best if swabs are placed directly into STGG medium which can serve both as a transport and storage medium (WHO recommendations). So authors should indicate that this procedure for transport is not optimal for pneumococcal recovery. Page 9, line 5. What volume of STGG was plated. Some details should be provided. Results Page 22. Table 1. Please correct (860) to (86.0) in HCP swabbing column. Discussion Page 17, lines 21-24. Please clarify the last sentence of the discussion. It’s not clear how this study enabled the “determination of at-risk populations”? At risk for what? Carriage? The study didn’t have any antibiotic susceptibility component so how does this study guide antibiotic strategies?

VERSION 1 – AUTHOR RESPONSE

Reviewer 1 – Elisabeth AM Sanders

1. The subject is timely and relevant since bacterial carriage is the focus of efficacy and effectiveness in vaccine studies like pneumococcal conjugate and meningococcal vaccine studies as well as the recent research into the respiratory microbiome and relation to respiratory symptoms and disease. In case subjects at home may provide swabs and washings from the nose and mouth that are as reliable to establish carriage as health care professional assisted swabs and washings, this would mean a high benefit in costs and feasibility for large cohort studies to evaluate interventions. A large study in over 10.000 subject was undertaken to compare self-swabbing with health care professional swabbing. Of these, only 15% of all invited subjects actually participated and only 6% participated in the health care professional swabbing. This is much lower than the initially estimated response rates (25% self-swabbing and 25% response rate for HCP swabbing). This resulted not only in low numbers of participant divided over the various age groups, but also less children and more elderly participating in the health care professional group. The authors present the study as a pilot study and not designed to have the power to detect non-inferiority of estimating carriage rates by HCP-administered versus self-administered swabs, which would be a main question. This leaves us with an unanswered main question and no conclusion.

Author comment: We appreciate the reviewers' positive comments and understand the limitations of our study which we discuss in our manuscript. Although the study does not have the power to detect non-inferiority of estimating carriage rates by HCP-administered versus self-administered swabs, we are able to say that self-swabbing is as effective as HCP swabbing with respect to the six organisms which we detected.

2. The lower age of the self-swabbers compared to the groups that visited the health care center might explain the higher carriage rates in the self-swab group. The large non-response bias needs to be addressed to answer the basis question whether this method of self- swabbing is reliable can be used.

Author comment: We agree that the higher rates of participation by the young within the self-swabbing group may have resulted in higher carriage rates observed in this group. However, very few of the age groups differed significantly in the carriage rates of the different organisms between self-swabbing and HCP swabbing (taking into account the 95% confidence intervals).

3. Bacterial carriage in the upper respiratory tract is usually asymptomatic but may proceed towards respiratory or invasive infections. Disease is always preceded by colonization.

Author comment: The first sentence of the abstract has been amended. Within the introduction (lines 3-5) it is stated that colonisation is the first stage of disease.

4. Setting: patients; would that be better subjects instead of patients?

Author comment: Agree that the term 'patients' is not appropriate - this has been changed to 'individuals' throughout.

5. Conclusion: Since no non-inferiority testing was not possible and nose swabs did not significantly differ between the self -swabbing and health care professional, the conclusion may not be justified.

Author comment: The conclusion has been modified to take into account the ‘non-inferiority testing’ of this pilot study. Non-inferiority allows us to say that self-swabbing is as effective as HCP swabbing.

6. There have been several large studies indeed that have reported on NP swabs and several species like S. pneumoniae, S. aureus and N. meningitidis. But none that I know that have compared self-swabbing with health care professional swabbing. Apart from response rates, this would be the main topic for me. The question is first whether self- swabbing is equal or better that assisted by a HCP. This was not answered.

Author comment: The comparison of self-swabbing and healthcare professional swabbing has been included in the strengths and limitations section. This has also been addressed in the results (lines 167-177) and discussion (lines 259-264).

7. Ref 13 O'Brien, K.L. and H. Nohynek, Report from a WHO Working Group: standard method for detecting upper respiratory carriage of Streptococcus pneumoniae. Pediatr Infect Dis J, 2003. 22(2): p. e1-11.This reference can better be updated with Satske et al, vaccine 2014; 32 (165-179).

Author comment: This reference has been updated to Satzke et al. (2014).

8. Page 8, lines 5-8 - their GP practice where nasopharyngeal (NPS) and whole mouth (WMS) swabs were taken by a registered HCP. The self-swabbing group involved participants being sent a self-swabbing pack containing nose (NS) and whole mouth (WMS) swabs by Danvers International (London, UK). I miss all information on the type of swabs, and transport materials. Also the time to get to the laboratory before further analysis.

Author comment: Information on type of swabs, transport media and timings for return of swabs are now given (lines 68-73).

9. Page 8, lines 19-21 - The study questionnaire requested the following details pertinent to bacterial carriage: participant age, recent use of antibiotics, recent RTI and vaccination status. What is meant by recent use? One month, 3 months? And what is recent RTI? One week, 4 weeks?

Author comment: Recent has now been defined for both RTI and antibiotics use (lines 78-79).

10. And how was the patient motivated to participate? This is relevant I think.

Author comment: No cash or prize incentive was given to participants as this might create additional participation bias. Information sheets aimed to generate interest and motivate individuals to participate. This has been added into the methods section (line 75).

11. Page 14, lines 19-28 - Overall, total costs per participant were over a third lower in the self-swabbing group at £41.21 ($67.92) versus the HCP group at £69.66 ($114.82) (Table 1). NHS service support costs made up a large proportion of the difference between the two study groups, representing 56.7%(£39.52/person) of costs in the HCP group but only 6.8% (£2.81/person) of costs in the self-swabbing group. How was this calculated? All swabs sent but not used included, as stated in the legend of table 1?

Author comments: The method for calculation of study costs has now been included in the methods section (lines 131-133).

12. We compared multiple swabbing sites in order to assess the most effective way of sampling the human respiratory tract flora in the hope to provide information for implementation of a standardised swabbing method. This question was not answered.

Author comment: This sentence has been removed, as it was misleading, instead we highlight the effectiveness of self-swabbing (lines 238-242).

13. Furthermore, low participation rates were observed, even in the self-swabbing group, in particular in children with only half the general practices with 25% participation rate after the invitation.

Author comment: Non-response bias is highly likely and may lead to biased estimates of carriage if factors associated with participation are also associated with carriage. Unfortunately non-response bias will always be a problem, as certain types of individuals are more likely to respond, but this would also be true for HCP swabbing. All carriage studies of this type have low participation rates. We had limited data on factors that are associated with non-response such as age and deprivation.

14. Page 16, lines 10-12 - lower participation rates within this group have most probably resulted in reduced carriage rates within NPS. Carriage rates can be high in small groups, so there must be another explanation like few children participating and more elderly (see Table 1).

Author comment: Fewer children and more elderly individuals participating has been included as a reason for lower carriage rates in NPS versus NS. However, this only goes to highlight the benefits of self-swabbing, as a more even spread of age groups can be recruited.

15. Table 1: point missing in % of vaccination up-to date. (860 %)

Author comment: This has been corrected to 86.0%.

Reviewer 2: Anne von Gottberg

1. The number of participants randomised to self-swabbing or HCP-led swabbing is not clear – the numerator is the respondents, but the denominator is not given here – and later on not clearly in the results.

Author comment: Numbers of individuals invited in each study group and age group have now been included. Denominator values (N) are given throughout the results for clarity.

2. The number of invited individuals (not really patients? as described in the abstract) are 10,448 in the abstract but 5220 in the sample size description? This should be reconciled.

Author comment: All occurrences of ‘patients’ have been replaced by ‘individuals’ for clarity. 5,220 is the approximate number of invitees within each study group – this has now been clarified by the addition of ‘within each swabbing group’ (line 45). The total anticipated number of invitees was thus 10,440. The discrepancy between the number anticipated (10,440) and actual (10,448) invitees is due to practices inviting more participants to compensate for any excluded participants.

3. Any patient deemed unfit: it would be good to have a full list of the exclusion criteria, and a total number of the individuals that were excluded in this step.

Author comment: A full list of exclusion criteria is not available as this was done at each GP’s

discretion. However numbers of participants excluded are expected to be low as the aim was to recruit as many individuals as possible. Number of exclusions during analysis has been included (lines 155-158).

4. How were the numbers 202 (<5 years) and 320 (=>5 years) arrived at?

Author comment: These numbers are based on being about to precisely measure carriage rates (i.e. 95% confidence intervals that are acceptable for detecting differences in carriage between these age groups). We estimated that by inviting 2,020 children (202 from each GP practice) aged 0-4 years and 3,200 older children and adults (320 from each GP practice) to participate, this would result in 505 children and 800 older children and adult responders within each swabbing group, accounting for predicted lower carriage rates in older children and adults. A predicted carriage rate of 30% in 505 participating children would enable the determination of true carriage to within ±4.0% (95% confidence). A predicted carriage rate of 20% in 800 participating older children and adults would enable the determination of true carriage to within ±2.8% (95% confidence).

5. The swabbing appointments that were offered: what days, how many options, after hours, on Saturdays? And for how long was the option open to go for the appointment? Were any reminders sent to both groups? How often? How far away were these clinics? Or places for the appointments? The reader needs to know how convenient or not the appointment may have been?

Author comment: Details of swabbing appointments have now been included (lines 65-66, 68).

6. We need more details about the swabs: cotton-wool swabs? Flocked swabs? Did they slot into a transport medium? Or were they just dry swabs that just slotted back into plastic tube? Did the self-swabbing group and the healthcare provider group all use the same swabs, from all sites?

Author comment: Details of swabs have now been included (lines 68-70).

7. What was the average time from taking of the swab and arriving at the laboratory (for the self-swabbing group)? Or any indicators of turn-around-time? How many swabs were lost in the post? . Did equal numbers of nasal and whole mouth swabs get returned from the self-swabbing group? For the HCP group?

Author comment: Details of average time for return of swab have now been included (lines 71-73). We do not have information on how many swabs were lost in the post, but we would expect this to be small. Equal numbers of nasal swabs and WMS were returned for both groups.

8. What was the definition of “recent use of antibiotics”? one month ago? Three months ago? One week ago? Only antibiotics? Or all antimicrobials? Definition of recent RTI? How was RTI defined for the self-swabbing group?

Author comment: Recent has now been defined for both RTI and antibiotics use (lines 78-79). Only antibiotics were included in the questionnaire. Types of RTI were listed in the questionnaire and these have now been included (line 79).

9. Were there differences in the questionnaire for the two groups?

Author comment: No, there is no difference between the questionnaires for each group. This has been included in the text (line 77).

10. For vaccination status: was this really thought to be accurate for adults? As I read the manuscript,

this was analysed for both children and adults, but I am not sure how useful the data would have been for adults. It is mentioned in the limitations, and I agree that it is a significant limitation: vaccination history could have been specific for the vaccine that may have impacted on specific carriage (pneumo and Hi) and specifically analysed in the age groups most likely to have been affected by vaccination.

Author comment: The reason for only asking “Are you up-to-date on your vaccinations?” was due to concerns that asking for detailed vaccination history would result in a very long questionnaire that might deter individuals from participating. We agree that specific vaccines (e.g. PCV, Hib and MenC) may have been more useful, which is why this pilot study will be useful for setting up further studies.

11. The statement: “participants were invited to undertake swabbing between May-August 2012” is not very helpful. Is this the time period when 1st invites went out to all GP practices? With 1st or 2nd reminders? When did the first swabs arrive back by post? And the last? The first HCP performed swab performed was when? And the last? Did they all trickle in evenly over a similar period? Or was there a final push? How much time were both groups given to respond (I asked this earlier)?

Author comment: More detailed timings of sample receipt and invitation processes have been included (lines 94-97). No reminders were given.

12. Genus and species should be written out in full when appearing the first time.

Author comment: This has been amended.

13. Throughout is would be useful to give both n/N for % (e.g. participation rates; swab positivity rates, co-carriage rates) especially if these data are not available in tables or figures.

Author comment: The values of n and N have been included throughout, for data that is not tabulated.

14. Table 1: for vaccination status HCP swabbing the % is incorrect for up-to-date.

Author comment: This has been amended to 86.0%.

15. Table 4 is cited before Tables 2-3?

Author comment: Table 4 has been removed as this did not add significant impact within the manuscript.

16. The significant findings by age group in table 4 are mentioned, but not those by RTI or recent use of antibiotics, nor vaccination history?

Author comment: Table 4 has been removed as it did not add significant impact to the manuscript. Significant findings in carriage as a result of RTI/no RTI (lines 208-212) and antibiotics use/no antibiotics use (lines 212-214) are described. There were no significant differences in carriage observed with vaccination status and this has been mentioned (lines 214-215).

17. The first sentence would be improved by simply stating the most important findings from your study: there have been many studies looking at carriage of multiple bacterial species and not much is made of this one being population-based – no carriage incidences are given etc. And depending on how you define large population-based study across all age groups – I can already think of other studies. Your study is worthwhile and should be published – it is unnecessary to make such a statement as the first sentence. Do not use the first paragraph to repeat the methods, rather highlight your most important findings.

Author comment: The first paragraph has been removed and replaced with a paragraph highlighting the key findings (lines 238-242).

18. Lack of isolation of meningococcus may also have had something to do with turn-around-times for the swabs, transport media etc. part of the reason for providing more details in the methods.

Author comment: This is a good point and has been included in the text (lines 285-286).

19. The herd effect (not really immunity) statement should have a reference. I think the lack of any effect with vaccination status may also be due to misclassification? For future studies a careful hypothesis should be generated about what you may expect to see, in which age groups, with which vaccines, and which organisms, then the questions can be more carefully tailored for these analyses, which may only be relevant in certain age groups.

Author comment: A relevant reference has been included which related herd immunity and respiratory vaccines (Ramsay ME et al., 2003). The need for more detailed immunisation information has been highlighted (lines 290-293).

20. The final statement seems a little out of place – age groups were slightly problematic, as response rate differed by age, and determining at-risk population for what? And we have just agreed that vaccination/vaccine was a weak point in this manuscript, and antibiotics were not discussed in detail at all?

Author comment: We agree with your comment, the final sentence has been removed.

Reviewer 3: Lesley McGee

1. The authors suggest that there are few papers in the literature describing carriage rates of multiple bacterial species (Discussion page 15, 6-8). I don't agree with this statement. Over the past 2 decades there have certainly been more than a "few" studies looking at the associations between various pathogens in the upper respiratory tract as well as studies that have looked at the impact of vaccination on multiple URT pathogens in a single study. I do agree that the majority of these have focused on children since this is the age group where carriage rates to most pathogens studied are higher and therefore an ideal target population to study. The age specific changes in carrier status and co-carriage associations of different pathogens reported here are the same as those observed in other previously published studies.

Author comment: We agree that there have been many carriage studies but to our knowledge this study is the first to determine carriage rates of this number of species simultaneously comparing self-swabbing and HCP swabbing. This sentence has been removed along with the rest of the paragraph and has been replaced by the key findings from the study. We agree that it is more important to state the key findings here than to state what other studies may have reported.

2. The study was limited to culture detection for 6 bacterial species. The 4 methods included a nose self-swab, mouth self-swab, nasopharyngeal HCP-swab and mouth HCP-swab. The author's conclusions are that a self-taken nose swab would provide sufficient sensitivity for the estimation of prevalence for all 6 organisms tested, even though Neisseria meningitidis was not isolates and sensitivity for Moraxella catarrhalis detection was lower for nose versus mouth swabs?

Author comment: The conclusions have been amended to indicate that nose swabs are sensitive for detecting S. pneumoniae, H. influenzae, S. aureus and P. aeruginosa but whole mouth swabs are better for S. aureus. Finally other swab types need to be considered for N. meningitidis.

3. Title: Would recommend adding in “Comparison” or “Evaluation” of swabbing methods… to the title for clarity.

Author comment: ‘Evaluation’ has been added to the title.

4. Page 2, line 6. Would suggest: “Bacterial carriage in the upper respiratory tract can lead to respiratory…”

Author comment: The sentence has been modified.

5. Page 2, line 39. What does “… more deprived practice locations” mean? Do you mean GP practices in areas with lower socioeconomic status?

Author comment: IMD Score has been clearly defined with the seven features of deprivation. A sentence explaining what more/less deprived means has also been included.

6. Page 6, line 20. I think “socioeconomic status” is a better term that “deprivation”?

Author comment: Deprivation has been used because this is what was actually measured. IMD score measures more than socioeconomic status, as this includes health, housing, crime and living environment in addition to income, education and employment. The IMD score than now been defined clearly in the text.

7. Page 7, lines 23-34. This section is confusing. You state that “We invited 2,020 children aged 0-4 years and 3,200 older children and adults to participate”? But in the Abstract and on page 8, lines 41, says a total of 10,448 patients were invited to participate?

Author comment: 5,220 is the approximate number of invitees within each study group – this has now been clarified by the addition of ‘within each swabbing group’ (line 45). The total anticipated number of invitees was thus 10,440. The discrepancy between the number anticipated (10,440) and actual (10,448) invitees is due to practices inviting more participants to compensate for any excluded participants.

8. Page 8, lines 10-12. I don’t agree that the authors suggest that mouth swabs can be used as a proxy for throat swabs. These are different anatomical sites with very different flora present in each of these. Just present the data how it is? It’s a mouth swab and not a throat swab, and deal with the limitations of this for the various pathogens which might be more readily isolated from the throat versus the mouth.

Author comment: This sentence has been removed.

9. Page 8, line 28, delete “..and recent…”

Author comment: This sentence had a missing word; the word has now been included.

10. Page 8, line 52. The methods section lacks detail. No information is given as to what type of swabs were used in the study? Also what transport medium was used? S.pneumoniae recovery works best if swabs are placed directly into STGG medium which can serve both as a transport and

storage medium (WHO recommendations). So authors should indicate that this procedure for transport is not optimal for pneumococcal recovery.

Author comment: Details of swab types, transport media and storage media have been included (lines 68-70).

11. Page 9, line 5. What volume of STGG was plated. Some details should be provided.

Author comment: Details of STGG volumes plated out as well as media types have now been included (lines 102-106).

12. Page 22. Table 1. Please correct (860) to (86.0) in HCP swabbing column.

Author comment: This has been corrected.

13. Page 17, lines 21-24. Please clarify the last sentence of the discussion. It's not clear how this study enabled the "determination of at-risk populations"? At risk for what? Carriage? The study didn't have any antibiotic susceptibility component so how does this study guide antibiotic strategies?

Author comment: This sentence has been removed, as it was misleading.

VERSION 2 – REVIEW

REVIEWER	Lesley McGee Centers for Disease Control and Prevention USA
REVIEW RETURNED	16-Sep-2014

GENERAL COMMENTS	All authors comments addressed adequately.