Author's response to reviews

Title: Bacterial vaginosis, vaginal flora patterns and vaginal hygiene practices in patients presenting with vaginal discharge syndrome in The Gambia, West Africa

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Version: Date: 27 December 2004

Author's response to reviews:

26th December 2004

The Editor
BMC Infectious Diseases

Sir,

RE: Re-submission of manuscript: "Bacterial vaginosis and vaginal flora patterns in patients presenting with vaginal discharge syndrome in The Gambia, West Africa." E Demba et al.
Thank you for giving us the opportunity to revise and resubmit our paper, and to the reviewers for their comments.

The paper has now been revised in line with the reviewers' comments. In particular, we have included data on HIV prevalence and vaginal douching, and we have modified table 1 to comply with the reviewers' major comments. We have added a section on HIV testing strategy in the Methods section and we have provided additional references for Mycoplasma culture and anaerobic identification as requested, as well as making reference to more recent BV literature, particularly on association with HIV and vaginal douching. Specific replies to each comment are included in the attached document. We hope you will now find the paper acceptable for publication in your journal.

Looking forward to hearing from you,

Yours sincerely,

Dr. Philippe Mayaud
Clinical Senior Lecturer
(on behalf of co-authors)

Responses to Reviewers' comments

Reviewer #1 (Cathy Ison):

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached):

1. A semi-quantitative analysis of the flora has been undertaken by dividing growth into confluent,
semi-confluent and scanty. While this is a perfectly valid approach, it is not clear what extra information this gives as the data as it is presented for all women. This type of data is not often available and it would be valuable to divide the analysis into those with or without BV.

We have revised table 1 as suggested and included the Nugent's categories of BV (score 7-10) versus all other flora categories (scores 0-6). This table supports the notion that isolates more closely associated with one type of flora (e.g., Lactobacilli with normal flora, or Gardnerella with BV flora) have more 'confluent' growth.

2. Nugent's gives three categories including an intermediate category, which is not recognised by Amsel's. It would be useful for the authors to comment on this difference and to describe the microflora of intermediate, which can be seen in figure 1 but is not directly referred to. There is very little discussion about this intermediate category.

The re-arranged table 1 and the figure support the notion that to the 'intermediate flora' category (by Nugent's score) corresponds an intermediate flora in terms of quantity and type of microorganisms isolated. We have added a comment at the end of the Discussion.

"Our study found that where Gram-staining led to a classification of 'intermediate flora' by Nugent's score, this was reflected in the microbiological findings, which were 'intermediate' quantitatively and qualitatively between 'normal' and 'BV' categories (table 2 and figure 1) and distinct from them. This supports the validity of the classification and could indicate that the 'intermediate' flora precedes the development or follow the resolution of frank BV."

3. The lack of association between the diagnosis of BV by Nugent's and Amsel's (figure 1) is very surprising. While it would not be expected to get a total correlation, almost 30% of patients that are Amsel's negative having a Nugent's score of 7-10 is very unexpected. What do the authors think are the reasons for this? Was there any quality control checks on reading of pH or detection of a fishy odour?

As the reviewer points out perfect correlation between the two methods would not be expected. Several evaluation studies have noted that the individual elements of the Amsel's criteria have a strongly subjective value- particularly the olfactory 'whiff test' and even the criterion of 'clue cells' on direct microscopy, which depends on the skill of the microscopist, and that these criteria could have low positive predictive value compared to the Nugent's score (Hillier & Holmes 1999). Moreover, other evaluation studies have been done in high-skilled research settings of industrialised countries, whilst our study was conducted in a clinical setting of a developing country.

Whilst staff participating in the study were properly trained and supervised for this study and quality checks were conducted (as this had been already indicated in the Diagnosis of bacterial vaginosis paragraph of the Methods section of the original manuscript ["Ten percent random quality control checks were carried out on the microscopist by an experienced BV microscopist, for Nugent's score and wet preparation readings for 'clue cells'.] and the fact that the same people were used throughout, ensuring internal consistency of the findings, the findings of such discrepancies between the Amsel's criteria and the Nugent's score suggest that the clinical methods may not be reliably applied in some developing country routine clinical settings as it is not easily amenable for quality checks. We inserted a comment in the Discussion:

"The Amsel's method can be highly subjective with regards the description of the discharge and the olfactory component ('whiff' test); the wet mount microscopy depends on the experience of the microscopist and can represent a further subjective element, particularly when performed under pressing clinical conditions [ref- Hillier 1999??]. Despite regular supervision and the use of the same clinician and microscopist throughout the study (which insured internal consistency of our results), the discrepancies we found between the Amsel's criteria and the accepted gold standard diagnosis (Nugent's methods) in this study appear to preclude the use of this method under routine clinical conditions in our setting."

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

4. Identification of strict anaerobes was performed using Gram staining and susceptibility to a range of antibiotics. It would be useful for others if this could be referenced or details giving for the identification.
5. Mycoplasma culture should be referenced.


6. The high percentage of clue cells in wet preparations compared to that in the Gram stains is also unexpected and would in part explain the poor correlation between Nugent's and Amsel's diagnosis of BV. Do the authors have any opinions on this discrepancy?

See response to #3 above.

7. In the discussion, group B streptococci are described as highly pathogenic. This can be true but they are also associated with vaginal flora in normal women. It is probably more accurate to say 'can be highly pathogenic'.

This is corrected.

Reviewer #2 (Marleen Temmerman):

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached):
None.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct):

1. The literature review and references list is outdates: the authors include only 2 references of > the year 2000, while only in 2004 over 20 papers have been published on bacterial vaginosis. The reference list surely needs to be updated.

There were actually only 7 papers on BV in Africa (most relevant to this paper) published after 2000 and 2 have been published by our group. Nonetheless we accept this criticism and we have included more recent papers on BV in Africa and vaginal douching.

2. BV has been associated with HIV acquisition and shedding. In this paper, the authors do not mention HIV status, while the HIV prevalence might be high in their population. Please comment.

This is a very interesting comment. We initially thought of writing a separate paper on the risk factors of BV in this study population, but we have realised that some data on HIV, and vaginal hygiene practices, and relation to detailed vaginal flora categories would be a useful addition to this paper. We have therefore added a section on HIV testing methodology (in Methods) on HIV prevalence (in Results) and tested for associations (See Statistical Methods and Results). We have also added a new table (table 4) to summarise some of the results.

Interestingly, contrary to previous findings, we did not find any associations between HIV and vaginal douching, menstrual hygiene practices and female genital cutting on the one hand; nor between HIV and BV or vaginal microflora organisms (with the exception of two anaerobic isolates, Bacteroides spp and Prevotella spp, which were significantly more common among HIV positive women); and finally no associations were found between vaginal hygiene practices and BV. The main reason is that some of the vaginal hygiene practices were so widely reported by the women, that associations between them and other variables would be hard to find (added to the Discussion). (see also responses to comment #3 below).

The modified sections are as follows:
Methods/Study population and sample collection:
"A blood sample was collected for syphilis and HIV serologies as routinely performed at the GUM clinic. Serological diagnosis of HIV infection was done according to a strategy described elsewhere [Schim, AIDS 2002]. In brief, sera were screened by the ICEHIV-1.O.2 (Murex Diagnostics Ltd, Dartford, UK) and reactive samples were retested by type-specific ELISAs: Wellcozyme HIV recombinant -1 (Murex) for HIV-1, and ICEHIV-2 test (Murex) for HIV-2. Samples clearly positive in one type were assigned the corresponding serological status; samples positive in both ELISAs were further tested by a synthetic peptide-based strip method, Pepti-Lav 1-2 (Sanofi Diagnostics Pasteur, Marne laCoquette, France)."

Methods/Statistical Methods:
"Chi-square and Fisher's Exact (for small numbers) tests were used to examine associations between categorical variables such as: isolation of lactobacilli, particularly H2O2-producing strains and G vaginalis, anaerobic isolates, Mycoplasma hominis, as well as N gonorrhoeae, C trachomatis, Candida spp or T vaginalis; HIV and each of the vaginal flora micro-organisms mentioned above, and with BV (Nugent's score 7-10); HIV and vaginal hygiene variables; and between BV and vaginal hygiene variables."

Results:
"A serum sample was obtained from 210 (93%) women and 27/210 (12.8%) of them were HIV infected, (19 with HIV-1, 7 with HIV-2, and 1 dually with HIV1 and HIV-2) [...]"

Discussion:
"An association between BV and HIV has been reported in several studies [3, 4, 30], possibly influenced by vaginal hygiene practices [18, 20, 31]. However, as in our study, not all studies reporting on douching, BV and HIV have found associations between these factors [19]. The relationship between HIV, risk for BV or other STIs is complex, and could be contributed to by high risk sexual behaviour. Our study population consisted only of symptomatic women attending a GUM clinic, thus high-risk behaviours may have blurred any possible association. To our knowledge, this study is one of the first to report on female genital cutting in relation to HIV and vaginal flora. There was no significant impact of female circumcision on vaginal flora or HIV serostatus."

The Abstract was also modified.

3. Table 3 reports p values between lactobacilli and genital micro-organisms whereby 3 columns are compared. Do the p values refer to a chi square for trends? or do the authors compare the last column with the first one? please explain the relation between the fischer exact test and the columns.

The Fisher's Exact test is an exact version of the X2 test which is used because of small numbers in some of the categories. In this case it is testing whether there are any differences in the prevalence of the micro-organisms across the Lactobacilli categories. It is not testing for 'trend' and it is not comparing the first and last columns (as suggested by the reviewer). Clarification for these notions has been made in footnotes of relevant tables and in Statistical Methods section:

"Chi-square and Fisher's Exact (for small numbers) tests were used to examine associations between categorical variables such as [...]"

4. On page 14 the authors state that the reasons for the higher BV rates in African women are not known. There might be indeed no evidence based data, but many authors have reported an association between bv and genital douching. The authors should at least refer to some of these papers and discuss the (lack of) evidence.

We have now made reference to these papers, particularly those reporting the practices in Africa and we have included mention of a useful review on vaginal douching and its impact on women's health (Martino & Vermund, 2002). We have found no associations between BV categories and the many vaginal hygiene practice variables recorded in our study (as well as the presence of genital cutting) - perhaps owing to the high level of reporting of these practices by our study population. We have included a rather large section in the Introduction, Results and Discussion section (and relevant sections in the Abstract) and we have slightly modified the Title of the paper to reflect these additional analyses.

Title:
"Bacterial vaginosis, vaginal flora patterns and vaginal hygiene practices in patients presenting with vaginal discharge syndrome in The Gambia, West Africa"
Introduction:
"In particular, it is important to know whether vaginal flora changes may enhance HIV acquisition as suggested [5], and to unravel some of the factors that influence such changes, as these could be perhaps modified. Behavioural factors such as vaginal douching or menstrual hygiene practices have been suggested as important factors that might influence vaginal flora composition [18], but little data is available from African populations[19], [20]." [...] We report here on the vaginal micro-flora patterns and vaginal hygiene practices found in these patients and associations with their HIV serostatus.

Methods:
"A standardised questionnaire elicited socio-demographic characteristics, reproductive and sexual health history including vaginal douching practices [...]"

Results:
Inserted section titled: "Associations between vaginal flora, vaginal hygiene practices and HIV."

Discussion:
"Lifestyle practices such as vaginal douching have also been associated with an increased prevalence of BV [15, 18, 19], although, the direction of causality is uncertain, since most studies have been of cross-sectional nature and many potentially confounding factors such as educational, socio-economical and behavioural factors have not always been entirely controlled for. We did not find any association between BV or vaginal micro-organisms and vaginal hygiene practices such as douching before or after sex, the nature of douching compounds used, or source of the water, or with menstrual sanitary protection. This finding perhaps owes to the fact that a very large proportion of women reported these practices, thus any relatively small association with BV would be hard to find with our sample size. On the other hand, additional possible explanations for the high prevalence or incidence of BV in African populations have to be sought - the role of hormonal factors should also be explored. [...] To our knowledge, this study is one of the first to report on female genital cutting in relation to HIV and vaginal flora. There was no significant impact of female circumcision on vaginal flora or HIV serostatus.

We hope these changes will find the approval of reviewers and editors.