PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Human papillomavirus prevalence and genotype distribution among young women and men in Maputo city, Mozambique
AUTHORS	Viegas, Edna; Augusto, Orvalho; Ismael, Nália; Kaliff, Mallin; Lillsunde-larsson, Gabriella; Ramqvist, Torbjorn; Nilsson, Charlotta; Falk, Kerstin; Osman, Nafissa; Jani, Ilesh; Andersson, Sören

VERSION 1 - REVIEW

REVIEWER	BERENICE ILLADES-AGUIAR UNIVERSIDAD AUTÓNOMA DE GUERRERO MEXICO
REVIEW RETURNED	27-Mar-2017

GENERAL COMMENTS	The objetives of the abstract are not clearly defined. Results: Page 12, line 15: “Participants with higher educational degree had less HPV infections (p=0.004)” should add (Table 1A). Page 12, line 18-19: “multipe HPV infections in the univariate and multivariate analysis (p=0.003 and p=0.008) respectively)” should be revised because: Table 1A line 38, multiple-infection vs negative mentions p=<0.001 not p= 0.003 Table 1B, at the end mentions multiple-infection vs negative p=0.005 not p=0.008 Page 12, line 25: “(p=0143)” should add (Table 1A). References 6, 7, 9, 10, 11, 12, 14, 15, 18, 22, 23, 26, 27 does not have publication year. The limitations of the study are not discussed. There are very few current references. This is an important study because it allows to know about the epidemiology of HPV in Mozambique. However, there should be more care in presenting the results. In addition, the references are not current and they are poorly cited.

REVIEWER	Ingeborg Zehbe Lakehead University, Thunder Bay, Ontario, Canada
REVIEW RETURNED	30-Mar-2017

GENERAL COMMENTS	This is a potentially interesting HPV study in an urban context, Maputo, in Mozambique but with serious flaws in the study design. The study seems unfocused trying to deal with aspects such as HPV prevalence, HPV and HIV, HPV in females versus males as well as HPV and genome variants at the same time with a very limited size

	of convenience samples. The variant part seems out of place unless all HR HPV types detected in the study are included but this would rather warrant another study. I hope the following comments are helpful for the authors to improve the study. Abstract: Methods: line 23, add respectively so that it is understood that urethra samples are from males. Introduction: Line 7, change ¾ to 75%; lines 31 to 49: literature regarding the more frequent types found in that population of young adults (18 to 24) should also be covered and considered in future studies; how do the findings regarding HPV types correlate with other African studies? Methods: Laboratory testing, line 33f: has the HPV test used, been approved by health authorities or alternatively by the FDA? How does it compare to other, FDA-approved tests like Hybrid Capture? Sexual partners should have been differently reported, why limit it to one or more? Distinction should have been made between males and females throughout the manuscript. Describe the methodological challenges to collect male swabs based on scientific literature. Why was the current method chosen? What prevalence have other studies found in terms of differences females/males? How was the sampling performed? Results: In the text, males and females need to be distinguished and the results need to be summarized so that the reader gets an immediate idea of the most important findings. This information cannot be easily gauged by the current format, which makes the results hard to read. There is a reason why HPV testing is done in women >25 years old in most populations because most of the earlier infections are cleared. A drawback of this study is that there is no older cohort to compare these results with in the given geographical context. It may well be that the detected HPV types would differ from those identified in the current study. References: They need to be revised and formatted according to BMJ Open. Many lack the year of publication. HPV16 variant literature is not representative with the same statements made already in the 1990ies without crediting this fact and the many groups having performed this line of research. HR HPV variants regarding other HR HPV types than HPV16 are virtually not discussed. This refers to my earlier comment that it makes not much sense to only check variants of HPV16 as the most common HPV identified was type 52. However, given the very limited group of women analyzed, especially regarding age, HPV variants do not really have a place in

	this manuscript

VERSION 1 – AUTHOR RESPONSE

Comments from Reviewer 1
Berenice Illades-Aguiar
Universidad Autónoma de Guerrero, Mexico

1. The objectives of the abstract are not clearly defined. We have modified the text in the abstract to better reflect the objectives of the study.

2. Results: Page 12, line 15:
"Participants with higher educational degree had less HPV infections (p=0.004)" should add (Table 1A). We have redone the analysis and re-structured the results sections. Text related to factors associated with HPV infection is now on page 14.

3. Results: Page 12, line 18-19:
"multipe HPV infections in the univariate and multivariate analysis (p=0.003 and p=0.008) respectively)" should be revised because:
Table 1A line 38, multiple-infection vs negative mentions p=<0.001 not p= 0.003
Table 1B, at the end mentions multiple-infection vs negative p=0.005 not p=0.008
Analyses on Mono-infections and Multiple-infections are now presented in Table 4 and all p-values have been revised and updated in the text accordingly (page 17).

4. Results: Page 12, line 25:
"(p=0143)" should add (Table 1A). The tables in the manuscript have been revised and the respective text updated accordingly.

5. References 6, 7, 9, 10, 11, 12, 14, 15, 18, 22, 23, 26, 27 does not have publication year. All references have been revised to adhere to BMJ open requirements. All references have now their year of publication.

6. The limitations of the study are not discussed. We have further elaborated on the limitations of our study. These have been addressed throughout the discussion section of the manuscript and a paragraph was added specifically to discuss about these limitations (pages 23 and 24).

7. There are very few current references. Several of the more recent references were lacking the year of publication. These have been updated. We have also included a few additional recent publications.

8. This is an important study because it allows to know about the epidemiology of HPV in Mozambique. However, there should be more care in presenting the results. In addition, the references are not current and they are poorly cited. We have revised all references to adhere with BMJ Open requirements. Several of the more recent references were lacking the year of publication and have now been corrected. Additional recent references have been added and cited.

Comments from Reviewer 2
Ingeborg Zehbe
Lakehead University, Thunder Bay, Ontario, Canada

1. This is a potentially interesting HPV study in an urban context, Maputo, in Mozambique but with serious flaws in the study design. The study seems unfocused trying to deal with aspects such as HPV prevalence, HPV and HIV, HPV in females versus males as well as HPV and genome variants at

the same time with a very limited size of convenience samples. The variant part seems out of place unless all HR HPV types detected in the study are included but this would rather warrant another study. I hope the following comments are helpful for the authors to improve the study. We have re-structured the results text to allow it to be more focused on HPV prevalence and genotypes distribution. We removed the HPV16 genotype variants from this report and we described with more detail the HPV genotype distribution in males and females. We have kept the HIV-HPV discussion since HIV is highly prevalent in our context and information on HIV-HPV co-infection is of great importance to the country.

2. Abstract: Methods: line 23, add respectively so that it is understood that urethra samples are from males. We have added the word "respectively" as suggested.

3. Introduction: Line 7, change ¾ to 75%; We have changed from ¾ to 75% as suggested.

4. Introduction; Lines 31 to 49: literature regarding the more frequent types found in that population of young adults (18 to 24) should also be covered and considered in future studies; how do the findings regarding HPV types correlate with other African studies? We added information to contextualize regarding the epidemiology of HPV infection and age distribution worldwide and in Mozambique. We also incorporated information related to introduction of the HPV vaccination worldwide and in Africa, to allow the readers to have a better understanding of the global context of HPV vaccination (page 5).

5. Methods: Laboratory testing, line 33f: has the HPV test used, been approved by health authorities or alternatively by the FDA? How does it compare to other, FDA-approved tests like Hybrid Capture? We added information on the performance of the Clart® Human Papillomavirus 2 and references of studies that compared this assay to the FDA-approved tests like Hybrid Capture (page 7).

6. Methods: Sexual partners should have been differently reported, why limit it to one or more? Distinction should have been made between males and females throughout the manuscript. This is one of the limitations of our study and is now discussed in pages 23 and 24. The study questionnaires only included three categories for number of sexual partners: none, one and more than one. Therefore, no additional information on the number of sexual partners is life or in the past six months is available.

7. Methods: Describe the methodological challenges to collect male swabs based on scientific literature. Why was the current method chosen? What prevalence have other studies found in terms of differences females/males? How was the sampling performed? This comment has been addressed on page 20.

8. Results: In the text, males and females need to be distinguished and the results need to be summarized so that the reader gets an immediate idea of the most important findings. This information cannot be easily gauged by the current format, which makes the results hard to read. We have re-structured the results text and distinguished the females from male reports throughout the analyses. The results section is now presented as follows: first the baseline demographics of the study participants including gender differences; second a description of HPV prevalence and genotypes distribution by gender; third the description of vaccine-associated HPV genotypes also by gender; fourth the risk factors associated with HPV infection and lastly the comparison between mono and multiple-infections.

9. There is a reason why HPV testing is done in women >25 years old in most populations because most of the earlier infections are cleared. A drawback of this study is that there is no older cohort to compare these results with in the given geographical context. It may well be that the detected HPV types would differ from those identified in the current study. This is one of our study limitation and has

now been discussed in pages 23 and 24.

10. References: They need to be revised and formatted according to BMJ Open. Many lack the year of publication. HPV16 variant literature is not representative with the same statements made already in the 1990ies without crediting this fact and the many groups having performed this line of research. HR HPV variants regarding other HR HPV types than HPV16 are virtually not discussed. This refers to my earlier comment that it makes not much sense to only check variants of HPV16 as the most common HPV identified was type 52. However, given the very limited group of women analyzed, especially regarding age, HPV variants do not really have a place in this manuscript. All references have been revised to adhere to BMJ open requirements. We removed the HPV16 genotype variants from this report.

Comments from the Editor

1. Your manuscript reporting should have adhered to the STROBE guidelines (http://www.equator-network.org/reporting-guidelines/strobe/) for the reporting of observational studies. This is so your methodology could be fully evaluated. We have re-structure the manuscript according to the reviewers suggestions and STROBE guidelines.

2. The 'Strengths and Limitations' section of your study should have discussed at least one limitation of the study referring to its methodological aspects. We have added "The absence of an older cohort (aged 25 years) for comparison of circulating HPV genotypes was a limitation of this study" in the Strengths and Limitations section. We have further elaborated on the limitations of our study. These have been addressed throughout the discussion section of the manuscript and a paragraph was added specifically to discuss about these limitations (pages 23 and 24).

3. The research question, outcome measures and clinical relevance of the study should be clearly defined throughout the text. We removed the HPV16 genotype variants from this report. The objectives of this study have been clearly stated now and include: (1) to determine the prevalence and distribution of HPV infections in sexually active young adults; and (2) to determine the suitability of the current HPV vaccines in the context of the Mozambican epidemic. The study outcomes have been revised and re-structured to facilitate reader´s understanding. We have now incorporated more information on gender differences and the manuscript tables have been updated accordingly.

Additional changes made to the manuscript

• The title has been modified to "Human papillomavirus prevalence and genotype distribution among young women and men in Maputo city, Mozambique" since we excluded the HPV16 variant data from the report.
• Information on where the study samples were analyzed was added in the laboratory testing section of the Methods (page 7).
• The statistical analysis section has been updated to accommodate the additional statistical analysis by gender (page 8).
• The tables have been modified to accommodate the re-structures results sections and the analysis by gender.

VERSION 2 – REVIEW

REVIEWER	BERENICE ILLADES AGUIAR UNIVERSIDAD AUTONOMA DE GUERRERO MEXICO
REVIEW RETURNED	30-Apr-2017

GENERAL COMMENTS	The manuscript is acceptable to publish.