PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (**http://bmjopen.bmj.com/site/about/resources/checklist.pdf**) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Association of antibiotics use in preschool age with atopic and allergic skin diseases in young adulthood: A population-based retrospective cohort study.
AUTHORS	Li, Yajia; Jing, Danrong; Huang, Yuzhou; Su, Juan; Li, Ji; Tao, Juan; Shan, Shijun; Wang, Xiaohui; Kang, Xiaojing; Wu, Bin; Xiao, Yi; Chen, Xiang; Shen, Minxue

VERSION 1 – REVIEW

REVIEWER	Lindbaek, Morten University of Oslo
REVIEW RETURNED	10-Feb-2021

GENERAL COMMENTS	General comments: This is a large and imposing study to investigate an important clinical topic; to assess the relationship between antibiotic use in childhood and subsequent atopic and allergic diseases. The data are gathered from students in 5 different regions of China and may be regarded to be representative, and the study has managed to cover a population with variation of socioeconomic status. The associations found were significant, but with moderate magnitude. The statistical analyses seem to be adequate. However, I have some important issues to be addressed before final accept: 1. As stated by the authors, the validity of reporting of URTIs and antibiotic use can be questioned to a high extent. Students at the age of 18-20 cannot be expected to remember how often they experienced URTIs or got antibiotics by the age of 7 and before. Especially will the data from 0-5 years of age be quite uncertain. Did the researchers do any efforts to meet this problem? To what extent were their parents involved in how to response to the questions? It is likely that the parents would have a more clear picture of this, especially at the early age. Were any medical records at primary care facilities available to validate the use of antibiotics, or were the antibiotics to buy over the counter without prescription? 2. The difference between URTIs on one side and antibiotic use as explanatory factors is discussed in the discussion and under limitations. However, I miss some more details in this, as the two factors have quite different pathways in leading to atopic disease. For example, a child with frequent allergic rhinitis may have been misinterpreted as having frequent URTIs. 3. Furthermore, we know from other studies that doctor seeking behavior varies a lot in populations. In this setting, both children with AD will seek doctor frequently and more likely get a URTI-diagnosis if they also have a cold. Equally, children with asthma and wheezing will more frequently get a URTI diagnosis and are

	more likely to get an antibiotic. So the topic of doctor seeking behavior should be discussed more in the paper. 4. As the authors rightly state, there may be a reverse relationship between AD and antibiotics, as the skin manifestations may have been treated with antibiotics. This could also be validated by asking the parents about this topic. 5. I miss a table showing the actual numbers of students reporting frequency of URTIs and antibiotic use, especially intravenous use. This is important to evaluate the clinical relevance of the findings in the study.

REVIEWER	Verhamme, Katia Erasmus Medical Center, Medical Informatics
REVIEW RETURNED	14-Feb-2021

GENERAL COMMENTS	With this paper, the authors aim to explore the association between the use of antibiotics and URTI in preschool age and development of allergic skin diseases My main concern relates to the information on exposure data. I believe that risk of misclassification will be extremely high if you ask first year college students about the use of antibiotics and/or URTI in their preschool age. The authors state that risk of recall bias is important - in my opinion, this recall bias is huge and why did the authors not consider checking with the parents. I am not only concerned about recall bias but also selection bias. I have the impression that people invited for the study new about the research question. It is thus likely that patients with dermatological questions - and being treated with antibiotics when they were young, will be more interested to participate which will introduce a bias. With regard to exposure, was there not a way to link the student's file to electronic health care data such as claims data? this would resolve the issue of recall bias. The authors state that there is a dose dependent association but information on dose was not captured? What was captured was the frequency of antibiotic use or the frequency of URTI but this is not the same as a dosing effect. With regard to the analysis, I do not understand why adding an interaction term would solve the issue on what came first - the skin disease or the use of antibiotics/URTI With regard to the analysis, did the authors conduct a logistic regression analysis (as for a case-control analysis) or was this a retrospective cohort study where a relative risk was calculated? As an additional limitation, the authors should refer to fact that they did not have info on all covariates to adjust for such as prematurity, smoking status (parents) . Minor comments: Please check size and font of figures (very difficult to read) Although the authors made a huge effort in writing this article in English, review by English Native person might be of interest

VERSION 1 – AUTHOR RESPONSE

Responses to reviewer 1's Comments :

1. As stated by the authors, the validity of reporting of URTIs and antibiotic use can be questioned to a high extent. Students at the age of 18-20 cannot be expected to remember how often they experienced URTIs or got antibiotics by the age of 7 and before. Especially will the data from 0-5 years of age be quite uncertain. Did the researchers do any efforts to meet this problem? To what extent were their parents involved in how to respond to the questions? The parents would likely have a more clear picture of this, especially at an early age. Were any medical records at primary care facilities available to validate the use of antibiotics, or were the antibiotics to buy over the counter without prescription?

Response: Thanks for your advice. We mentioned in limitation that the recall bias in the measurement of exposure to antibiotics might have been introduced, which could not be ignored in most retrospective studies.
There is recall bias in the measurement of URTIs and antibiotic use, but we are not able to validate the medical records because China does not have a registry system for primary care, and a large number of patients with mild conditions also visit doctors in secondary and tertiary hospitals. While participants could obtain the information from their parents, but unfortunately, we could not evaluate the extent of recall bias. This will be in our further consideration in future studies.

2. The difference between URTIs on one side and antibiotic use as explanatory factors is discussed in the discussion and under limitations. However, I miss some more details in this, as the two factors have quite different pathways in leading to atopic disease. For example, a child with frequent allergic rhinitis may have been misinterpreted as having frequent URTIs.
URTIs
Response: Thanks for your advice. We can not ignore that there is the possibility that a child with frequent allergic rhinitis may have been misinterpreted as having frequent URTIs. The two factors may involve different mechanisms in leading to atopic diseases, but they are highly correlated in the context of China where antibiotics have been overused for the treatment of URTI. While allergic rhinitis might be misinterpreted as URTI and cause bias, other conditions, such as skin allergies, are less likely to be misclassified.

3. Furthermore, we know from other studies that doctor seeking behavior varies a lot in populations. In this setting, both children with AD will seek doctor frequently and more likely get a URTI-diagnosis if they also have a cold. Equally, children with asthma and wheezing will more frequently get a URTI diagnosis and are more likely to get an antibiotic. So the topic of doctor seeking behavior should be discussed more in the paper.
Response: Thanks for your advice. We have provided the data on Health seeking behavior on a cold or fever in Table S3, and we found there were 66.2% in participants with the atopic march and 64.7% in participants allergic skin disease showed that they/their parents would like to receive antibiotics treatment when the participants had a cold/fever in their preschool age. In those without atopic/allergic diseases, this proportion ratio was 61.5%. We did not observe a significant difference in health seeking behavior in our study, as most of the Chinese parents could pay close attention to the preschool health of children, and keep a non-exclusion attitude to antibiotics use. Keeping antibiotics at home for children was pervasive in China, as well as the parents sought medical care and use antibiotics in dealing with respiratory tract infections.

4. As the authors rightly state, there may be a reverse relationship between AD and antibiotics, as the skin manifestations may have been treated with antibiotics. This could also be validated by asking the parents about this topic.

Response: Thanks for your advice. This is a limitation of our study. The questionnaire was distributed to the students, and we did not investigate their parents. The next step in our future studies

5. I miss a table showing the actual numbers of students reporting the frequency of URTIs and antibiotic use, especially intravenous use. This is important to evaluate the clinical relevance of the findings in the study.

Response: Thanks for your advice. The actual numbers of students reporting frequency of URTIs and antibiotic use have been supplied in Table S1.

Responses to reviewer 2's Comments :

-My main concern relates to the information on exposure data.
I believe that the risk of misclassification will be extremely high if you ask first-year college students about the use of antibiotics and/or URTI in their preschool age.
The authors state that the risk of recall bias is important - in my opinion, this recall bias is huge and why did the authors not consider checking with the parents.
Response: Thanks for your advice. Owing to the feasibility, we only investigated the students. While the report of URTIs and antibiotic use may be misclassified, participants could obtain the information from their parents. But unfortunately, we are unable to evaluate the extent of recall bias. We started this in the limitation section.

-With regard to exposure, was there not a way to link the student's file to electronic health care data such as claims data? this would resolve the issue of recall bias.
Response: Thanks for your advice. We are not able to validate the medical records because of the lack of an information system for primary care in China. Besides, a large number of patients with mild conditions also visit doctors in secondary and tertiary hospitals.

-The authors state that there is a dose dependent association but information on dose was not captured? What was captured was the frequency of antibiotic use or the frequency of URTI but this is not the same as a dosing effect.
Response: Thank you for your suggestion and we removed the use of the term "dose-response relationship"

-With regard to the analysis, I do not understand why adding an interaction term would solve the issue on what came first - the skin disease or the use of antibiotics/URTI
Response: Thanks for your advice. e can not solve the issue of what came first - the skin disease or the use of antibiotics/URTI. Indeed, there is a possibility of reversed association, as we stated. The two variables are highly correlated with the use of antibiotics in the context of China where antibiotics have been overused for the treatment of URTI.

-With regard to the analysis, did the authors conduct a logistic regression analysis (as for a case-control analysis) or was this a retrospective cohort study where a relative risk was calculated?
Response: Thanks for your advice. In our manuscript, we only used the two-level Probit regression models to obtain the estimates of relative risks.

-As an additional limitation, the authors should refer to fact that they did not have info on all covariates to adjust for such as prematurity, smoking status (parents).
Response: Thanks for your advice. We have added in the limitation that we did not have information on all covariates to adjust for such as prematurity. Actually, we have assessed the passive smoking conditions in the previous version (as shown in item C04 of Material S1) and adjusted it in the final

results. While we have omitted information on passive smoking in the previous version and now provided it in Table 1.

-Minor comments: Please check size and font of figures (very difficult to read)
Although the authors made a huge effort in writing this article in English, review by English Native person might be of interest
Response: Thanks for your advice. We have edited the font of figures and English expression for a better review and consideration.

VERSION 2 – REVIEW

REVIEWER	Lindbaek, Morten University of Oslo
REVIEW RETURNED	05-May-2021

GENERAL COMMENTS	SEE MY COMMENTS UNDER EACH POINT, stated as ML: . One point needs revision, the health seeking behaviour, where there seems to be a moderate, but statistical significant difference. Also look at the statistical comments from referee 2. Responses to the Editor's Comments : -Please include the study design in the title. Response: Thanks for the advice, we have edited the title of the manuscript into “Association of antibiotics use in preschool age with atopic and allergic skin diseases in young adulthood: A population-based retrospective cohort study.” ML: OK -Please include a copy of the questionnaire used as a supplementary file. Response: Thanks for the advice, we have included the questionnaire used as a supplementary file. Please see Material S1. Chinese college students health survey questionnaire (English version). ML:OK Formatting Amendments (where applicable): 1. Required Supplementary format: - Please re-upload your Supplementary files in PDF format. Response: We have re-upload the supplementary files in PDF format. 2. Patient and Public Involvement: - We have implemented an additional requirement to all articles to include 'Patient and Public Involvement statement within the main text of your main document. Please refer below for more information regarding this new instruction: Authors must include a statement in the methods section of the manuscript under the sub-heading 'Patient and Public Involvement'. This should provide a brief response to the following questions:

	How was the development of the research question and outcome measures informed by patients'priorities, experience, and preferences? How did you involve patients in the design of this study? Were patients involved in the recruitment to and conduct of the study? How will the results be disseminated to study participants? For randomised controlled trials, was the burden of the intervention assessed by patients themselves? Patient advisers should also be thanked in the contributorship statement/acknowledgements. If patients and or public were not involved please state this. Patient and Public Involvement Response: In the part of 'Patient and Public Involvement, our declarations are as follows: This is a retrospective cohort study based on the data from the China College Student Skin Health Study (CCSSHS). The first-year college students from five universities were recruited and investigated. They underwent a health examination and completed a questionnaire survey, and the results will be disseminated to study participants by a medical examination report. Participants were not involved in the design and implementation of the study. ML:OK Responses to reviewer 1's Comments : 1. As stated by the authors, the validity of reporting of URTIs and antibiotic use can be questioned to a high extent. Students at the age of 18-20 cannot be expected to remember how often they experienced URTIs or got antibiotics by the age of 7 and before. Especially will the data from 0-5 years of age be quite uncertain. Did the researchers do any efforts to meet this problem? To what extent were their parents involved in how to respond to the questions? The parents would likely have a more clear picture of this, especially at an early age. Were any medical records at primary care facilities available to validate the use of antibiotics, or were the antibiotics to buy over the counter without prescription? Response: Thanks for your advice. We mentioned in limitation that the recall bias in the measurement of exposure to antibiotics might have been introduced, which could not be ignored in most retrospective studies. There is recall bias in the measurement of URTIs and antibiotic use, but we are not able to validate the medical records because China does not have a registry system for primary care, and a large number of patients with mild conditions also visit doctors in secondary and tertiary hospitals. While participants could obtain the information from their parents, but unfortunately, we could not evaluate the extent of recall bias. This will be in our further consideration in future studies. ML:OK 2. The difference between URTIs on one side and antibiotic use as explanatory factors is discussed in the discussion and under limitations. However, I miss some more details in this, as the two factors have quite different pathways in leading to atopic disease. For example, a child with frequent allergic rhinitis may have been misinterpreted as having frequent URTIs.

	URTIs Response: Thanks for your advice. We can not ignore that there is the possibility that a child with frequent allergic rhinitis may have been misinterpreted as having frequent URTIs. The two factors may involve different mechanisms in leading to atopic diseases, but they are highly correlated in the context of China where antibiotics have been overused for the treatment of URTI. While allergic rhinitis might be misinterpreted as URTI and cause bias, other conditions, such as skin allergies, are less likely to be misclassified. ML:OK 3. Furthermore, we know from other studies that doctor seeking behavior varies a lot in populations. In this setting, both children with AD will seek doctor frequently and more likely get a URTI-diagnosis if they also have a cold. Equally, children with asthma and wheezing will more frequently get a URTI diagnosis and are more likely to get an antibiotic. So the topic of doctor seeking behavior should be discussed more in the paper. Response: Thanks for your advice. We have provided the data on Health seeking behavior on a cold or fever in Table S3, and we found there were 66.2% in participants with the atopic march and 64.7% in participants allergic skin disease showed that they/their parents would like to span style="font-family:Calibri; font-style:italic">receive antibiotics treatment when the participants had a cold/fever in their preschool age. In those without atopic/allergic diseases, this proportion ratio was 61.5%. We did not observe a significant difference in health seeking behavior in our study, as most of the Chinese parents could pay close attention to the preschool health of children, and keep a non-exclusion attitude to antibiotics use. Keeping antibiotics at home for children was pervasive in China, as well as the parents sought medical care and use antibiotics in dealing with respiratory tract infections. ML: This is a new result that should be described also in the results section, and then discussed as has been done. With such a large population, the difference between atopic march of 66.2% and 61.5% in those without seems to be significant. By this there is a difference in doctor seeking behavior in the study, statistically significant, but still not so large. 4. As the authors rightly state, there may be a reverse relationship between AD and antibiotics, as the skin manifestations may have been treated with antibiotics. This could also be validated by asking the parents about this topic. Response: Thanks for your advice. This is a limitation of our study. The questionnaire was distributed to the students, and we did not investigate their parents. The next step in our future studies ML: OK 5. I miss a table showing the actual numbers of students reporting the frequency of URTIs and antibiotic use, especially intravenous use. This is important to evaluate the clinical relevance of the findings in the study.

	Response: Thanks for your advice. The actual numbers of students reporting frequency of URTIs and antibiotic use have been supplied in Table S1. ML:OK Responses to reviewer 2's Comments : -My main concern relates to the information on exposure data. I believe that the risk of misclassification will be extremely high if you ask first-year college students about the use of antibiotics and/or URTI in their preschool age. The authors state that the risk of recall bias is important - in my opinion, this recall bias is huge and why did the authors not consider checking with the parents. Response: Thanks for your advice. Owing to the feasibility, we only investigated the students. While the report of URTIs and antibiotic use may be misclassified, participants could obtain the information from their parents. But unfortunately, we are unable to evaluate the extent of recall bias. We started this in the limitation section. ML:OK -With regard to exposure, was there not a way to link the student's file to electronic health care data such as claims data? this would resolve the issue of recall bias. Response: Thanks for your advice. We are not able to validate the medical records because of the lack of an information system for primary care in China. Besides, a large number of patients with mild conditions also visit doctors in secondary and tertiary hospitals. ML:OK -The authors state that there is a dose dependent association but information on dose was not captured? What was captured was the frequency of antibiotic use or the frequency of URTI but this is not the same as a dosing effect. Response: Thank you for your suggestion and we removed the use of the term “dose-response relationship” ML:OK -With regard to the analysis, I do not understand why adding an interaction term would solve the issue on what came first - the skin disease or the use of antibiotics/URTI Response: Thanks for your advice. We cannot solve the issue of what came first - the skin disease or the use of antibiotics/URTI. Indeed, there is a possibility of reversed association, as we stated. The two variables are highly correlated with the use of antibiotics in the context of China where antibiotics have been overused for the treatment of URTI. ML: SHOULD BE DISCUSSED WITH STATISTICIAN -With regard to the analysis, did the authors conduct a logistic regression analysis (as for a case-control analysis) or was this a retrospective cohort study where a relative risk was calculated?

	Response: Thanks for your advice. In our manuscript, we only used the two-level Probit regression models to obtain the estimates of relative risks. ML: NEED FOR STATISTICAL ADVICE? -As an additional limitation, the authors should refer to fact that they did not have info on all covariates to adjust for such as prematurity, smoking status (parents). Response: Thanks for your advice. We have added in the limitation that we did not have information on all covariates to adjust for such as prematurity. Actually, we have assessed the passive smoking conditions in the previous version (as shown in item C04 of Material S1) and adjusted it in the final results. While we have omitted information on passive smoking in the previous version and now provided it in Table 1. ML: I COULD NOT FIND THIS UNDER LIMITATIONS? -Minor comments: Please check size and font of figures (very difficult to read) Although the authors made a huge effort in writing this article in English, review by English Native person might be of interest Response: Thanks for your advice. We have edited the font of figures and English expression for a better review and consideration. ML:OK

REVIEWER	Verhamme, Katia Erasmus Medical Center, Medical Informatics
REVIEW RETURNED	08-May-2021

GENERAL COMMENTS	**Methodology:** <u>Study design:</u> the authors call this a retrospective study but I'm not convinced. It feels more like a cross-sectional study where data is collected (at time of enrolment) with questionnaire data. It's not a prospective study as students are not followed over time (perhaps they aim to do so but then this paper is a result of the first cross-sectional part of a prospective study). It would fit criteria of a retrospective study if for instance electronic health care data (with data collected in the past) is used. Outcome: In the method section it states that Asthma, allergic rhinitis, and allergic conjunctivitis were self-reported according to doctors' diagnoses. However if I check on the questionnaire, it states the following: B02. Have you ever been diagnosed with any of the following allergic diseases? (*Multiple selections are allowed*) □ Asthma □ Allergic Rhinitis □ Allergic conjunctivitis □ Eczema □ Urticaria □ None of above ⇨ If this is indeed the question which was used to identify patients with asthma, allergic rhinitis and allergic

conjunctivitis, it means that they might have had these conditions in the past, but not longer at the time of the questionnaire and then making a statement on antibiotic use in the past and experiencing these conditions at present is not correct (you might have had asthma in the past, but this does not mean that you still have it at present)

- ⇨ I'm convinced that skin conditions were adequately captured but I'm not convinced anymore on asthma, allergic rhinitis and allergic conjunctivitis as this is not based on doctor diagnosis but based on questionnaire data and also they might not be existing anymore

Exposure: The authors state as if they asked for antibiotic use in general but in fact they only asked for antibiotic use for treatment of URTI! This implies that antibiotic use for treatment of LRTI, skin infections. Gastro-intestinal infections, URTI…. Is not collected which could also affect the microbiome. This is – in my opinion – a major limitation of the study and should be mentioned

Related to this, if you check the question from the questionnaire it asks for the following:

Which of the following methods can improve or cure your "Cold or fever"
in the early school age years? (*Single selection*)
□ It usually cured without □ By oral antibiotics occasionally
□ Often by antibiotics orally □ Often by antibiotics intravenously

- ⇨ This questions asks about the effect of treatment not whether student took it.

If you check how this phrase was mentioned in the method section, it states the following:

"How often did you receive antibiotics treatment when you had a URTI", with four responses: "rare", "occasional", "often, orally; and "often, intravenously".

This is different from the question from the questionnaire. Also "rare", "occasional", "often" is vague if no guiding is provided and might lead to information bias.

Analysis: I already asked before but it would be nice to understand why the authors choose to use the probit regression (and not a simple logistic regression) – because of the clustering effect of universities? (and why do they believe that there might be a clustering effect)

Result: With regard to health care seeking behaviour, around % reported that they would be treated with antibiotics when dealing with a cold or fever in the preschool age but this is not reflected by the actual numbers where proportion of antibiotic use is much lower

	Discussion: In the limitation section, nothing is mentioned about selection bias, which might hold for this study. Especially students with skin conditions might be interested to participate and this might bias the results Also the authors need to mention that they only asked about antibiotic use for treatment of URTI (and according to questionnaire only COLD or fever) so an important proportion of antibiotic use might have been missed. In addition, the authors state the following: "While recall bias on the frequency of antibiotics use and URTIs should not be ignored, this is a limitation in most retrospective studies" I don't agree as in my opinion this only holds for retrospective studies not using electronic data **General comment:** Although the text has improved – legibility is still an issue and sometimes I can not follow what they are trying to say As an example: Abstract - Objective: "To investigate the association of preschool use of antibiotics with atopic and allergic skin diseases in young adulthood for the association of antibiotics use with eczema." Discussion: "Furthermore, we have provided the data on the health seeking behavior dealing with a cold or fever in preschool age (shown in Table S3), and we found there were 66.2% in participants with the atopic march and 64.7% in participants allergic skin disease showed that they/their parents would like to receive antibiotics treatment when the participants had a cold/fever in their preschool age." "Keeping antibiotics at home for children was pervasive in China, as well as the parents sought medical care and use antibiotics in dealing with respiratory tract infections." Table and figures: Legend of figure 1: unclear – prevalence of conditions by URTI status and by use of Antibiotics

VERSION 2 – AUTHOR RESPONSE

Methodology:

Study design: the authors call this a retrospective study but I'm not convinced. It feels more like a cross-sectional study where data is collected (at time of enrolment) with questionnaire data. It's not a prospective study as students are not followed over time (perhaps they aim to do so but then this paper is a result of the first cross-sectional part of a prospective study). It would fit criteria of a retrospective study if for instance electronic health care data (with data collected in the past) is used.

Response: Thanks for your suggestion. We divided the study as a retrospective cohort as this cohort study divided a specific population into different subgroups according to whether or not they were exposed and the degree of exposure to antibiotics. The incidence of outcomes in each group was tracked, to observe whether there was a causal association between variables and outcomes and the degree of association.

Outcome: In the method section it states that Asthma, allergic rhinitis, and allergic conjunctivitis were self-reported according to doctors' diagnoses. However if I check on the questionnaire, it states the following:

B02. Have you ever been diagnosed with any of the following allergic diseases?
(*Multiple selections are allowed*)

□ Asthma □ Allergic Rhinitis □ Allergic

conjunctivitis

□ Eczema □ Urticaria □ None of above

- □ If this is indeed the question which was used to identify patients with asthma, allergic rhinitis and allergic conjunctivitis, it means that they might have had these conditions in the past, but not longer at the time of the questionnaire and then making a statement on antibiotic use in the past and experiencing these conditions at present is not correct (you might have had asthma in the past, but this does not mean that you still have it at present)

- □ I'm convinced that skin conditions were adequately captured but I'm not convinced anymore on asthma, allergic rhinitis and allergic conjunctivitis as this is not based on doctor diagnosis but based on questionnaire data and also they might not be existing anymore

Response: Thanks for your precious suggestion. All the disease conditions were diagnosed by our certificated doctors, but there is still possibility that asthma, allergic rhinitis and allergic conjunctivitis patients might have had these conditions in the past, but not longer at the time of the questionnaire. We design the items in the questionnaire, because 'atopic march' was usually interpreted as the sequential development of symptoms from eczema in infancy, to asthma, and then allergic rhinitis while we could not ignore the co-occurring conditions of eczema, wheeze, and rhinitis were more often than would be expected by chance. Therefore, we collected the information both by the questionnaire and clinical diagnose.[Allergy. 2000 Jul;55(7):591-9. ;Ann Allergy Asthma Immunol. 2010 Aug;105(2):99-106; Expert Review of Clinical Immunology, 16:9, 873-881]. We add your suggestion into the limitation part.

Exposure: The authors state as if they asked for antibiotic use in general but in fact they only asked for antibiotic use for treatment of URTI! This implies that antibiotic use for treatment of LRTI, skin infections. Gastro-intestinal infections, URTI…. Is not collected which could also affect the microbiome. This is – in my opinion – a major limitation of the study and should be mentioned

Related to this, if you check the question from the questionnaire it asks for the following:

Which of the following methods can improve or cure your "Cold or fever" in the early school age years? (*Single selection*)

□ It usually cured without □ By oral antibiotics occasionally

□ Often by antibiotics orally □ Often by antibiotics intravenously

- This questions asks about the effect of treatment not whether student took it.

If you check how this phrase was mentioned in the method section, it states the following:

"How often did you receive antibiotics treatment when you had a URTI", with four responses: "rare", "occasional", "often, orally; and "often, intravenously".

This is different from the question from the questionnaire. Also "rare", "occasional", "often" is vague if no guiding is provided and might lead to information bias.

Response: Thanks for your suggestion. Firstly, in the questionnaire, we inquiry about antibiotic use in the condition of having a URTI, but we could not ignore there is the possibility that antibiotic use for the treatment of LRTI, skin infections, gastrointestinal infections….was not collected could also affect the microbiome. We added it in our limitation. Secondly, there is some translation mistake in items of the questionnaire in our last version, we modified them into the original and right contents in accordance with manuscript. Indeed, we asked about the "rare", "occasional", "often" in URTI frequency in our questionnaire, which was defined as "≤1 time/year", 2-3 times/year", and "4 or more times/year, respectively.

Analysis: I already asked before but it would be nice to understand why the authors choose to use the probit regression (and not a simple logistic regression) – because of the clustering effect of universities? (and why do they believe that there might be a clustering effect)

Resoponse：Thanks for your suggestion. One of our corresponding authors was a professor of public health and statistics, and we inquiried about the statistic advice and recognized the two-level Probit regression models (also called bivariate probit regression) as suitable models in our analysis. Bivariate probit regression was also used in some similar studies with binary variables as dependent variables. (Perry BI et al. Schizophr Res. 2021 Mar 5;230:69-76. doi: 10.1016/j.schres.2021.02.008.)

Result: With regard to health care seeking behaviour, around % reported that they would be treated with antibiotics when dealing with a cold or fever in the preschool age but this is not reflected by the actual numbers where proportion of antibiotic use is much lower

Resoponse：Thanks for your suggestion. We have revised the results and discussion parts of seeking behavior.

Discussion:

In the limitation section, nothing is mentioned about selection bias, which might hold for this study. Especially students with skin conditions might be interested to participate and this might bias the results

Also the authors need to mention that they only asked about antibiotic use for treatment of URTI (and according to questionnaire only COLD or fever) so an important proportion of antibiotic use might have been missed.

In addition, the authors state the following: "While recall bias on the frequency of antibiotics use

and URTIs should not be ignored, this is a limitation in most retrospective studies"

I don't agree as in my opinion this only holds for retrospective studies not using electronic data

Resoponse：Thanks for your suggestion. We have revised the limitations in the manuscript.

General comment:

Although the text has improved – legibility is still an issue and sometimes I can not follow what they are trying to say

As an example:

Abstract - Objective: "To investigate the association of preschool use of antibiotics with atopic and allergic skin diseases in young adulthood for the association of antibiotics use with eczema."

Discussion: "Furthermore, we have provided the data on the health seeking behavior dealing with a cold or fever in preschool age (shown in Table S3), and we found there were 66.2% in participants with the atopic march and 64.7% in participants allergic skin disease showed that they/their parents would like to receive antibiotics treatment when the participants had a cold/fever in their preschool age."

"Keeping antibiotics at home for children was pervasive in China, as well as the parents sought medical care and use antibiotics in dealing with respiratory tract infections."

Table and figures:

Legend of figure 1: unclear – prevalence of conditions by URTI status and by use of Antibiotics

Resoponse：Thanks for your precious suggestions. We have modified the description to improve the manuscript legibility.

Responses to reviewers：

Reviewer: 1
Prof. Morten Lindbaek, University of Oslo
Comments to the Author:
SEE MY COMMENTS UNDER EACH POINT, stated as ML: .
One point needs revision, the health seeking behaviour, where there seems to be a moderate, but statistical significant difference.

Also look at the statistical comments from referee 2.
Reponse：Thanks for your suggestion. There is indeed a moderate but statistically significant difference in the health-seeking behavior in different disease conditions. We edited and added those contents in our manuscript (see the part of results)

Responses to the Editor's Comments :

3. Furthermore, we know from other studies that doctor seeking behavior varies a lot in populations. In this setting, both children with AD will seek doctor frequently and more likely get a URTI-diagnosis if they also have a cold. Equally, children with asthma and wheezing will more frequently get a URTI diagnosis and are more likely to get an antibiotic. So the topic of doctor seeking behavior should be discussed more in the paper.
Response: Thanks for your advice. We have provided the data on Health seeking behavior on a cold or fever in Table S3, and we found there were 66.2% in participants with the atopic march and 64.7% in participants allergic skin disease showed that they/their parents would like to receive antibiotics treatment when the participants had a cold/fever in their preschool age. In those without atopic/allergic diseases, this proportion ratio was 61.5%. We did not observe a significant difference in health seeking behavior in our study, as most of the Chinese parents could pay close attention to the preschool health of children, and keep a non-exclusion attitude to antibiotics use. Keeping antibiotics at home for children was pervasive in China, as well as the parents sought medical care and use antibiotics in dealing with respiratory tract infections.

ML: This is a new result that should be described also in the results section, and then discussed as has been done. With such a large population, the difference between atopic march of 66.2% and 61.5% in those without seems to be significant. By this there is a difference in doctor seeking behavior in the study, statistically significant, but still not so large.

Response：Thanks for your suggestion, we have described the part of doctor seeking in the part of results and discussed the results.

Responses to reviewer 2's Comments :

-With regard to the analysis, I do not understand why adding an interaction term would solve the issue on what came first - the skin disease or the use of antibiotics/URTI
Response: Thanks for your advice. We cannot solve the issue of what came first - the skin disease or the use of antibiotics/URTI. Indeed, there is a possibility of reversed association, as we stated. The two variables are highly correlated with the use of antibiotics in the context of China where antibiotics have been overused for the treatment of URTI.

ML: SHOULD BE DISCUSSED WITH STATISTICIAN
Response: Thanks for your suggestion. We have discussed this problem with the statistician. Indeed, there is a possibility of a reversed association, as we stated, and we added the interaction to identify it, which also a limitation shown in our study.

-With regard to the analysis, did the authors conduct a logistic regression analysis (as for a case-control analysis) or was this a retrospective cohort study where a relative risk was calculated?

Response: Thanks for your advice. In our manuscript, we only used the two-level Probit regression models to obtain the estimates of relative risks.

ML: NEED FOR STATISTICAL ADVICE?

Response: Thanks for your suggestion. One of our corresponding authors was a professor of public health and statistics, and we inquired about the statistic advice and recognized the two-level Probit regression models (also called bivariate probit regression) as suitable models in our analysis. Bivariate probit regression was also used in some similar studies with the binary variable as the

dependent variable. (Perry BI et al. Schizophr Res. 2021 Mar 5;230:69-76. doi: 10.1016/j.schres.2021.02.008.)

-As an additional limitation, the authors should refer to fact that they did not have info on all covariates to adjust for such as prematurity, smoking status (parents).
Response: Thanks for your advice. We have added in the limitation that we did not have information on all covariates to adjust for such as prematurity. Actually, we have assessed the passive smoking conditions in the previous version (as shown in item C04 of Material S1) and adjusted it in the final results. While we have omitted information on passive smoking in the previous version and now provided it in Table 1.

ML: I COULD NOT FIND THIS UNDER LIMITATIONS?

Response: Thanks for your suggestion. We have added this point as one of the limitations.

Reviewer: 2
Dr. Katia Verhamme, Erasmus Medical Center
Comments to the Author:
** Please find additional comments from this reviewer attached to this email**
The paper has improved but as additional information has been provided, I have some additional questions.
It is very important to be clear on the design, and how you assessed exposure but also outcome and exposure data

Reviewer: 1
Competing interests of Reviewer: No competing interest

Reviewer: 2
Competing interests of Reviewer: KV works for a research group who receives/received unconditional research grants from Yamanouchi, Pfizer-Boehringer Ingelheim, Novartis, GSK, U

CB, Chiesi, Amgen, Astra-Zeneca, none of which relate to the content of this work

VERSION 3 – REVIEW

REVIEWER	Lindbaek, Morten University of Oslo
REVIEW RETURNED	02-Jul-2021

GENERAL COMMENTS	The manuscript has improved in most parts. The authors have done a relevant analysis on health seeking behavior in the results with moderate, but significant differences. However, this is in contrast with the following statement in the discussion lines 177-178: We did not observe significant difference in health seeking behavior in our study, as most of the Chinese parents could pay a close attention to preschool health of children, and keep a non-exclusion attitude to antibiotics use. This part should be taken out or changed

REVIEWER	Verhamme, Katia Erasmus Medical Center, Medical Informatics
REVIEW RETURNED	01-Jul-2021

GENERAL COMMENTS	Paper has been improved and I'm happy that part on limitations has been extended. In my opinion, this is a cross-sectional study and not a retrospective cohort study but this will not influence the interpretation of the manuscript. English has improved but the manuscript will need thorough review (to improve readability) but this will be dealt with by the editors I suppose

VERSION 3 – AUTHOR RESPONSE

Responses to reviewers：

Reviewer: 2

Dr. Katia Verhamme, Erasmus Medical Center

Comments to the Author:

Paper has been improved and I'm happy that part on limitations has been extended.

In my opinion, this is a cross-sectional study and not a retrospective cohort study but this will not influence the interpretation of the manuscript.

English has improved but the manuscript will need thorough review (to improve readability) but this will be dealt with by the editors I suppose.

Reponse：*Thanks for your precious suggestions which greatly improved the quality of our manuscript. We have edited text and made some spelling correction after a careful thorough review to improve the readability. Thanks again for your time and consideration.*

Reviewer: 1

Prof. Morten Lindbaek, University of Oslo

Comments to the Author:

The manuscript has improved in most parts. The authors have done a relevant analysis on health seeking behavior in the results with moderate, but significant differences.

However, this is in contrast with the following statement in the discussion lines 177-178:

We did not observe significant difference in health seeking behavior in our study, as most of the Chinese parents could pay a close attention to preschool health of children, and keep a non-exclusion attitude to antibiotics use.

This part should be taken out or changed.

Reponse：Thanks for your precious suggestions which greatly improved the quality of our manuscript. We have deleted the part of the improper statement of 'We did not observe significant difference in health seeking behavior in our study', which we ignored in the last revision. Thanks again for your time and consideration.