Author’s response to reviews

Title: Sequence analysis of genes mediating extended-spectrum beta-lactamase (ESBL) production in isolates of Enterobacteriaceae in a Lagos Teaching Hospital, Nigeria.

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Author’s response to reviews: see over
Editor, BMC Infectious Diseases

Dear Editor

Thank you very much for sending us the reviewers’ comments for our perusal and necessary action. We have, as much as possible, reviewed our manuscript according to the suggested changes recommended. These changes are indicated in yellow highlighted sentences/words. Where we did not agree, we have given our reasons for the difference of opinion. We wish to thank the referees for their valuable criticisms of the manuscript and the effort and energy spent on the paper. Below please find point-by-point response to the referees’ concerns:

Reviewer 1

1. “In Materials and Methodology, The authors described that MBL was screened for by EDTA-synergy test, modified Hodge test, and E-test by MBL strips for detecting MBL-producing Enterobacteriaceae. However, this manuscript is not described for data of this test in Results. I suggest that is described that addition for data of this test in Results of this manuscript”.

   **Our response:**
   We must apologize profusely for this insertion error on our part. Our study was all about prevalence of ESBL and not MBLs. The sentences relating to MBL screening have all been deleted throughout the manuscript. See page 6, lines 117-122; page 7, lines 124-125 and page 9, lines 167-168.

2. “In Discussion, page 9, lane 177, the authors described “over 96% of these isolates 178 also produced a narrow-spectrum TEM #lactamase”. How determined as “narrow-spectrum TEM #lactamase”? If need, describe methods in manuscript”.

   **Our response**
   “Narrow-spectrum” has been deleted. See page 9, line 180

3. “In Table 1 and Table 2, remove vertical lines”.

   **Our response**
   Done. Vertical lines removed.

4. “In Table 1, present No. of E. coli and K. pneumoniae”
Our response
Done. See Table 1

5. “In Table 2, What ESBL-type is from M. morganii”.

Our response
We apologize for this lapse in Table 2. It has been re-constructed.

6. “In Table 2, if “CTX-M15”, “TEM-1”, and “SHV” are name of genes, “CTX-M15”, “TEM-1”, and “SHV” exchanged to italic style that will be required”.

Our response.
Done. The error has been corrected.

Reviewer 2

Major compulsory revision:

Our response
We have now included the reference in the introduction, page 5, line 85-89, page 10, line 193 and under reference as no.21, page 15, line 315.

2. “References are given for some of the BLA primers but for others the sequence is given. Could the authors explain how these primers were designed?”

Our response
Once again we apologize for this mix-up. The entire sentences relating to the primers for MBL have been deleted.

Results section.
3. “there is inconsistency between the results given in the text and table 2. E cloacae is described in table 2 but not in the text”

Our response
Table 2 has been reformatted and E. cloacae deleted.

4. “table 2 cannot explain all the BLSE described in the study. Indeed, at least 2 E. coli and 1 Proteus have no CTX-M and no SHV, and TEM-1 cannot be responsible for ESBL. Therefore this should be explained in the text in a
sentence explaining that the ESBL gene could not be found for...

Our response
A sentence to this effect has been inserted on page 9, lines 169-170.

5. "As demonstrated in Table 1, inspection of the MICs for piperacillin-tazobactam together with ceftazidime and cefoxitin could predict CTX-M-15 positive isolates as their MIC90s were >256 µg/ml each and 12 µg/ml, respectively." I don't understand the meaning of this sentence. Do you mean that MICs > 256 for ceftazidime and Pipe-tazo and MIC <12 for cefoxitin is typical of CTX-M15? If so, table 1 should also show the results for other ESBL? If not, this sentence should be omitted"

Our response
To include the MICs of antibiotics against other isolates positive for different ESBL-types would make the Table to be crowded and so the sentence has been deleted as recommended. See page 8, lines 163-164.

A new sentence regarding the susceptibility of isolates to imipenem and meropenem was added on page 9, lines 167-168.

Discussion.
6. "I am surprised that when the authors describe the prevalence of CTX-M 15 they only cite studies from Europe, Middle-East or America. Studies from Africa would have been more relevant. They say "The ESBL-producing E. coli that had been reported from Nigeria [34, 35] were not characterized making it difficult to compare our findings with others in the country." However, when I search CTX-M + Nigeria in PubMed, 11 references are found. I don't know why the authors chose to ignore these data but it is important that they change the discussion".

Our response
Unfortunately, our manuscript has spent close to one year with the journal and some articles have come out from studies conducted in Nigeria since then. It was not our intention to exclude any contemporary study from our region. However, we have cited about 5 relevant articles from Nigeria in the introduction, page 5, lines 79-83 and lines 85-89 as well as on page 10, line 193. The whole paragraph on page 10, lines 196-200 has been rewritten for clarity.
Reviewer 3

Major comments
1. The introduction is not well documented, poorly written. It should emphasize the predominance of the CTX-M-type ESBLs in the world and give some data about the situation in Nigeria in terms of ESBLs and carbapenemases dissemination.

Our response
We politely disagree with comments of this referee concerning the documentation of and poorly written introduction. We have documented the relevant information from Nigeria and omitted elaborating on carbapenemase dissemination as this was not part of our study.

Materials and methods
2. Which clinical sample (urine, blood, rectal swabs)? What hospitalization unit? 38 among how many isolates were ESBLs?

Our response
The clinical samples are clearly mentioned on page 8, lines 147-148. We have included the total number of Enterobacteriaceae isolates processed during the study period and gave the overall prevalence of ESBL-positive strains on page 8, lines 144-147. The samples were from in-patients from different units in the hospital.

3. One month is too short; the date of the study is too old (2011). A more recent study would be more relevant.

Our response
We disagree that one month is too short. Point-prevalence study is a recognized epidemiological study and the literature is full of such studies. It is not our fault that the process of reviewing this article has taken almost a year. As agreed to by the other two referees the information contained in this manuscript is worthy of publication after revision.

“PCR amplification”
4. This part should begin with the blaTEM, blaSHV, blaCTX-M specific primers, all others primers are not adequate for this study. Moreover, the isolates have been selected for the presence of ESBLs, all PCR experiments with primers specific for the detection of carbapenemases genes are then useless, unless carbapenemases would have been detected, but apparently this is not the case. Noticeably, the authors give sequence of primers specific for the detection of carbapenemases that are only produced in Pseudomonas spp. (AIM, SPM...), inappropriate in this study.
Our response
We agree with the referee that we included some irrelevant primers in this study in error for which we apologize. They have been deleted from the entire text. The specific primers used for the \textit{bla}_{TEM}, \textit{bla}_{SHV} and \textit{bla}_{CTX-M} were adequately referenced. See page 7, lines 131-132.

The recommended procedure for insertion sequence was followed.

Results
5. Many precisions are needed in this part

Our response
There were 3 ESBL-positive isolates which were negative for the ESBL \textit{bla} genes. It is possible these isolates harbored genes mediating other forms of resistance which we could not pursue for logical reasons. The remaining 5 non-\textit{bla}_{CTX-M} harboring isolates were positive for \textit{bla}_{TEM}. Unfortunately, we could not sequence these genes and so unable to determine the type of TEM enzyme produced.

We have deleted the MBL investigation which was included in error.

We hope the revised manuscript will now be acceptable for publication in your esteemed journal.

Best regards

Sincerely

Dr. MA Raji,
Corresponding author