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OPEN PEER REVIEW

Phenotypic and Genotypic Antibiotic Resistance Patterns of Clinical *Shigella* Isolates from Tehran, Iran

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1 Original submission

2 comments · 13 Feb 2024 – 3 Mar 2024

Seyed Hossein Shahcheraghi Reviewer

MAJOR REVISION 13 Feb 2024

Your manuscript is good with major revisions.
1- PCR products are very long. Please check.
2- Shigella should be italicized throughout the text.
3- The genes (ial, virF, invE, sigA, and pic) and others should be italicized.
4- At its first mention, Shigella should be written in full and thereafter as SH.
5- Extraction using the Pishgaman kit is very good.
6- The legends are not clear to readers. Please clarify them further.

Referee 1 Reviewer

REJECT 3 Mar 2024

Comments:
The present study investigated phenotypic and genotypic antibiotic resistance patterns and virulence gene distribution in clinical Shigella isolates from patients in Tehran, Iran. I think there are some drawbacks and several points throughout the manuscript that need clarification. In general, the structure of the text and style are fair; however, the English writing needs attention.

Abstract:
-The exact place & time of the study and important statistical analyses should be added to the abstract.

Introduction:
- Specific mention of similar studies in Tehran, Iran, and other cities in Iran is needed for a better understanding of the necessity of the study.
Material and methods:
-The exact place of sampling, along with a description of the hospitals, is needed.
-The justification for such a sample size is not clear and should be clarified. Is the sample size sufficient to represent the claimed area of the study?
- Why didn't you detect the Shigella species or groups? This would have been useful for better clarification and understanding of the clinical and epidemiologic situation and the antibiotic resistance of your Shigella isolates.
-The standards and controls for antibiotic susceptibility testing need to be described precisely (according to the latest edition of CLSI).
- How did you check the quality and quantity of the extracted DNA?
-Could you calculate confidence intervals as well?
-Do you have any accession numbers for those sequenced genes?

Results:
-The p-value is a value between 0 and 1, but in Table five, some p-values are more than 1.
- As you detected intermediate resistance in your isolates, how did you deal with these isolates, and how did you account for them in the calculations?
- Illustrations in Figure 4 are not of sufficient quality, and some descriptions and numbers cannot be read and are not clear.
-Tables and figures should be self-standing. Abbreviations should be described under the tables.
- What did you mean by writing those cumulative percentages of 100% in Table 2?

Discussion:
-"Limitations" and "suggestions" should be added to the discussion.
-Please clearly discuss the clinical relevance of the virulence genes and detected antibiotic resistance genes in relation to the corresponding antibiotics you tested.
- Conclusions should be directly relevant to what you have studied.

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Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Dear Editor,

We sincerely thank the editor and reviewers for their valuable feedback on our paper. We have revised the manuscript accordingly by following the reviewers' suggestion. Below is our point-by-point response to the reviewers' comments:

Respond to respected reviewer 1:

1- PCR products are very long. Please check.

Respond: Thank you for your feedback. We have carefully checked the PCR products in our study and compared them with following similar articles. The length of the PCR products obtained using the same primers we utilized in our study was consistent with what was expected and appropriate. Therefore based on this comparison, we believe the PCR products are accurately sized. If there are any special concern or criteria you would like us to address regarding the PCR products, please let us know, and we will be happy to provide further clarification or evidence.

- Komijani M, Bouzari M, Rahimi F. Detection of TEM, SHV and CTX-M antibiotic resistance genes in Escherichia coli isolates from infected wounds. Medical Laboratory Journal. 2017 Mar 10;11(2):30-5.
- Ibrahim AS, Youssef N. Prevalence of CTX-M, TEM and SHV beta-lactamases in clinical isolates of Escherichia Coli and Klebsiella Pneumoniae isolated from Aleppo University Hospitals, Aleppo, Syria. Archives of Clinical Infectious Diseases. 2015 Apr 1;10(2).

2- *Shigella* in all text should be italic.

Respond: Italic font of *shigella* was checked in all text. The changes were highlighted in yellow color.

3- (*ial*, *vir*F, *inv*E, *sig*A, and *pic*) genes and others should be italic.

Respond: Italic font of them was checked in all text. The changes were highlighted in yellow color.

4- First time *Shigella* be complete and other times as SH.

Respond: All *shigella* except first one (line 4) was changed to SH. The changes were highlighted in yellow color.

5- Very good for extract by Pishgaman kit.

6- Legends are not clear for readers. Please clarify them more.

Respond: Some explanations were added to the legends. They were highlighted in yellow color (lines 180, 241, 256 and 305).

Respond to respected reviewer 2:

1-The present study investigated phenotypic and genotypic antibiotic resistance pattern and virulence gene distribution in clinical *Shigella* isolates from patients in Tehran, Iran. I think there are some drawbacks and several points throughout the manuscript which need clarification. In general, the structure of the text and style are fair however, English writing needs attention.

Respond: The whole text was checked for grammar and the changes were highlighted in yellow color.

Abstract:

2 -Exact place & time of study and important statistical analyses are better be added to the abstract.

Respond: Exact places and time period and statistical analyses were added and they were highlighted in yellow color (line 10).

Introduction:

3 - Specific mention to similar studies in Tehran, Iran and other cities in Iran, for better understanding of the necessity of study is needed.

Respond: Two studies were mentioned and they were highlighted in yellow color in the text and list of references (lines 75, 451).

Material and methods:

4 -The exact place of the sampling along with description of hospitals are needed.

Respond: they were added in the method section of manuscript (line 96).

5 -Justification of such a sample size is not clear and should be clarified. Is the sample size sufficient to represent the claimed area of the study?

Respond: Based on the study conducted by Seyyed Amiri Mir Hassan and colleagues, it was observed that the prevalence of *Shigella* in 1850 samples with acute diarrhea was 14%. Therefore, using an 8% margin of error and a 5% Type I error rate, the sample size was calculated to be 94 samples using the Cochran formula.

The sample size calculation formula is as follows:

$$n = \frac{Z^2 \cdot p \cdot (1-p)}{E^2}$$

Where:

- (n) represents the required sample size.
- (Z) is the Z-score corresponding to the desired confidence level (e.g., 1.96 for a 95% confidence level).
- (p) denotes the estimated prevalence (proportion) of the condition (in this case, 14% or 0.14).
- (E) represents the margin of error (8% or 0.08).

Therefore, a sample of 94 individuals was calculated to ensure adequate statistical power for the study.

In this study, **101** samples suspected of *Shigella* were investigated, of which **60** samples were found to have at least one of the *Shigella* strains.

6 - Why didn't you detect the *Shigella* species or groups? It was useful for better clarification and understanding clinical, epidemiologic situation and also antibiotic resistance of your Shigella isolates.

Respond: In our study**,** the focus was primarily on investigating the phenotypic and genotypic antibiotic resistance patterns of clinical *Shigella* isolates from Tehran, Iran. While detecting *Shigella* species or groups could provide additional insights, our main objective was to assess the antibiotic resistance profiles of the isolated strains. We believe that by focusing on the resistance patterns, we can contribute valuable information to the understanding and management of *Shigella* infections in our region. However, we appreciate your suggestion and acknowledge the importance of identifying bacterial (*Shigella*) species or group in future studies to provide more comprehensive analysis. Furthermore, it is worth mentioning that this research project was conducted as part of an MSc thesis, where budget constraints limited our ability to conduct comprehensive bacterial identification.

7- Standards and controls of the Antibiotic Susceptibility testing needed to be exactly described (according to the latest edition of CLSI).

Respond: Reference strains were added and highlighted in yellow color (line 111).

8 - How did you check the quality and quantity of the extracted DNA?
Respond: To evaluate the quantity and quality of the extracted DNA, we employed the nanodrope and gel electrophoresis, respectively. However, due to word and figure constraints, the detailed were not included in the manuscript. The brief explanation for qualitative and quantity assessment of the extracted DNA was added in the method section of manuscript and highlighted in yellow color (line 120).
9 -Could you calculate Confidence intervals as well?
Respond: The only statistical test that had been done in this article is the Chi-square test to assess the relationship between two categorical variables and I guess we couldn't compute a 95% CI.
10 -Have you got any accession number for those sequenced genes?
Respond: They were submitted to the GenBank database. Their accession numbers were added in the manuscript and highlighted in yellow color (line 272).

Results:
11 -The p-value is a value between 0 and 1 but in Table five some p values are more than 1.
Respond: This had been a typo and will be corrected. The corrections were highlighted in yellow color (line 309).
12 - As you detected intermediate resistance for your isolates, how did you deal with them and how did you considered them in calculations?
Respond: They were treated as non-resistant and the antibiotics were generally classified into resistant and non-resistant.
13 - Illustrations in Figure 4 have not sufficient quality and some description and numbers cannot be read and are not clear.
Respond: The quality of Figure 4 (descriptions and numbers) was improved (line 294).
14 -Tables and Figures should be self-standing. Abbreviations should be described under the tables.
Respond: All tables and figures were checked and if an abbreviation was not described, it was mentioned below the corresponding figure or table and highlighted in yellow color (lines 183, 244, 258 and 309).
15 - What did you mean by writing those cumulative percentages of 100% in Table 2?
Respond: Those cumulative percentages do not have a specific meaning and they were eliminated.

Discussion:
16 -"Limitations" and "suggestions" are better to be added in the discussion.
Respond: a paragraph was added to discussion and highlighted in yellow color (line 392).
17 -Please clearly discuss the clinical relevance of the virulent genes and detected antibiotic résistance genes to those related antibiotic you have checked.
Respond: This paragraph was added to the discussion and highlighted in yellow color. Additional discoveries resulting from the analysis of this study's outcomes were a presence of bla CTX-M for many (19/22) of the MDR isolates. Similarly, several virulence genes (invE, virF, sigA, ial, and pic) were also present in the MDR isolates. However, it is important to note that not all MDR isolates carried both studied genes. In some MDR patterns, beta-lactamase genes were not detected (marked as "-ND"), and a few patterns had MDR isolates without any beta-lactamase genes. Likewise, some patterns with MDR isolates did not have all virulence genes

listed. The examination of more genes within this group might likely lead to a modification in the results (line 384).

18 - Conclusions should be exactly relevant to what you have studied.

Respond: The conclusion section of the manuscript has been revised. Irrelevant parts have been removed and only the parts relevant to the conducted study have been retained. This part was highlighted in yellow color (line 400).

Respond to respected reviewer 3:

The subject of the article and the obtained results are interesting. However, an improvement in the several aspects of the paper could be necessary. In this situation, some points could be considered:

Honestly, the proposed results were rather limited, and these limitations were poorly presented throughout the manuscript, lacking an appropriate style, especially regarding the antibiotic resistance pattern of each isolate. It is highly recommended to utilize scientific terminology instead of simple phrases. Numerous high-quality papers exist in the field, and it would be greatly appreciated if the authors referred to them and adjusted their writing style accordingly (https://doi.org/10.1016/j.jgar.2019.04.020) (https://doi.org/10.1016/j.ijfoodmicro.2019.108252).

Respond:

The text was edited and the references were added. All the editions were highlighted in yellow color (lines 350, 355).

1 - Please replace "*Shigella*" with "*Shigella* spp." throughout the text.

Respond: *Shigella* spp. was replaced and highlighted in yellow color.

2 - Why didn't you identify the species through serological or molecular tests?

Respond: In our study**,** the focus was primarily on investigating the phenotypic and genotypic antibiotic resistance patterns of clinical *Shigella* isolates from Tehran, Iran. While detecting *Shigella* species or groups could provide additional insights, our main objective was to assess the antibiotic resistance profiles of the isolated strains. We believe that by focusing on the resistance patterns, we can contribute valuable information to the understanding and management of *Shigella* infections in our region. However, we appreciate your suggestion and acknowledge the importance of identifying bacterial (*Shigella*) species or group in future studies to provide more comprehensive analysis. Furthermore, it is worth mentioning that this research project was conducted as part of a MSc thesis, where budget constraints limited our ability to conduct comprehensive bacterial identification.

3 - While it’s common to follow CLSI as a standard guideline but the authors also need to pay attention to routine antibiotic for Shigellosis.

Respond: Thank you for your valuable feedback. We acknowledge the importance of considering routine antibiotics for Shigellosis treatment in addition to following CLSI guidelines for antimicrobial susceptibility testing. In our study, we performed antimicrobial susceptibility testing according to the CLSI guidelines to assess the phenotypic antibiotic resistance patterns of the clinical *Shigella* isolates. Additionally, the selection of antibiotics for our study was based on recent and reputable literatures, which provided insight into the commonly used antibiotics for Shigellosis treatment. Furthermore, as we aimed to identify multidrug resistant (MDR) *Shigella* isolates in our study, we intentionally selected antibiotics from various antibiotic classes to ensure a comprehensive assessment of antibiotic resistance patterns. This approach allowed us to evaluate the resistance profiles of the *Shigella* isolates against a range of commonly used

antibiotics, including those from different antibiotic families. By including antibiotics from diverse classes, we aimed to capture the full spectrum of antibiotic resistance among the clinical *Shigella* isolates from Tehran, Iran

4 - Please double check the company name and country in Materials and Methods section.

Respond: The company name and country in Materials and Methods section were double checked and corrections were highlighted in yellow color (line 143).

5 - In “3.3. Antimicrobial Susceptibility Testing” section; pleas added CLSI version.

Respond: the version of CLSI (2021) was added to the text and highlighted in yellow color (line 105).

6 - It is better to use “ND” instead of “a” in table 3.

Respond: ND was replaced and highlighted in yellow color (line 245).

7 - All *Shigella* spp are resistance to ampicillin these days. Choosing a noneffective antibiotic and then using its resistance phenotype for determining MDR isolates would not be reasonable.

Respond: Thank you for your insight. We acknowledge that *Shigella* spp. have shown widespread resistance to ampicillin in recent years. In our study, while we included ampicillin in the panel of antibiotics tested, we did so with the awareness of its limited effectiveness against *Shigella* isolates. Our intention was not to rely solely on ampicillin resistance to determine multidrug-resistant (MDR) isolates. Instead, we considered resistance to multiple antibiotics (more than 3 different antibiotics) across different classes to identify MDR strains. By assessing resistance patterns against a range of antibiotics, including those with known limited efficacy like ampicillin, we aimed to comprehensively characterize the antibiotic resistance profiles of the clinical *Shigella* isolates from Tehran, Iran. Additionally, it's important to note that in our study, we classified *Shigella* isolates as multidrug-resistant (MDR) if they demonstrated resistance to more than three antibiotics from different antibiotic classes, which equates to four or more antibiotics in total. By adopting this definition, we aimed to identify isolates with broad-spectrum resistance, thereby ensuring that reliance on ampicillin resistance alone did not significantly impact our findings. This approach allowed us to account for the limited effectiveness of ampicillin against *Shigella* isolates and to focus on identifying MDR strains based on resistance to multiple antibiotics across diverse classes.

8 - The results were not carefully discussed, and the discussion section is a repetition of results. There are sentences without reference. Moreover, with a simple search I found studies on MDR isolates of Shigella in the region Iran which would be helpful to promote MS quality if authors would consider them (https://doi.org/10.1186/s40001-023-01570-0) (https://doi.org/10.1016/j.jgar.2019.04.020).

Respond:

The text was edited and the references were added (line 370).

9- Finally, I believe this paper needs reviewing by a professional editor as the English used, although good, still is not satisfactory for publication.

Respond: The whole text was double checked for grammar by a professional editor and the changes were highlighted in yellow color.

2 Revision 1

0 comments

No public comments in this round.