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OPEN PEER REVIEW

Characterization of Clonal Groups of Antibiotic-Resistant Biofilm-Forming Staphylococcus *aureus* Strains Isolated from Patients with Urinary Tract Infections in Isfahan, Iran

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About this report

This open peer review (OPR) report shows the public comments made on this article during peer review, by the reviewers, the associate editor and the editor-in-chief, for every revision round, together with the authors' replies. Comments appear only with the consent of the people who wrote them. Reviewers who chose to sign their report are named; the others are shown as "Referee". Comments are edited for language only, and confidential remarks to the editors are not included.

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1 Original submission

2 comments · 1 Jun 2024 – 1 Jun 2024

Referee 1 Reviewer

MINOR REVISION 1 Jun 2024

Dear authors
Based on my review of the manuscript titled "Characterization of Clonal Groups of Antibiotic-Resistant Biofilm-Forming Staphylococcus aureus Strains Isolated from Patients with Urinary Tract Infections in Isfahan, Iran," I have the following comments:

-Abstract Line 4: "to establish the relationship" should be "to establish a relationship."
-Abstract Lines 7-8: "biofilm formation capacity was evaluated by a combination of the microtiter plate and Congo-red agar methods." This sentence would be clearer as "biofilm formation capacity was evaluated using a combination of microtiter plate and Congo-red agar methods."
-Results Section: Use of percentages can be confusing. Instead of "Results showed that 19, 57, and 24%," it is clearer to state "Results showed that 19%, 57%, and 24%."
-Methodology Section: Several sentences are excessively long and could be broken into shorter, clearer statements. For example, "All isolates were identified using specific primers for the nucA gene and their biofilm formation capacity was evaluated by a combination of the microtiter plate and Congo-red agar methods."
-Clarity of Objective: The objective should be more explicitly stated. Instead of "This study aimed to establish the relationship between clonal groups, biofilm formation, and antibiotic resistance," it should specify how these relationships are to be assessed and their significance.
-Sample Size Justification: There is no justification for the sample size of 119 isolates. Why was this number chosen? Was a power analysis conducted to ensure this number is statistically significant?
-Methodology Details: There are several details missing in the methodology that are crucial for reproducibility:
*Specific conditions for the PCR assays are not provided. Include details such as annealing temperatures, cycle numbers, and primer sequences.
*Clarify how the cut-off OD (ODc) was determined in the microtiter plate method.
-Discussion of Resistance Genes: The discussion should delve deeper into the implications of the presence of specific resistance genes. What does the high frequency of gyrA and grlA genes imply for treatment strategies?
-Comparison with Previous Studies: The study should compare its findings with those of previous studies more thoroughly. Are the resistance rates observed in this study consistent with those reported elsewhere? How do the clonal types found compare with those in other regions?
-Conclusion: The conclusion should be more specific. Currently, it is too general and does not provide concrete recommendations or implications for future research or clinical practice.

Referee 2 Reviewer

MAJOR REVISION 1 Jun 2024

Dear authors,

Thank you for your effort in preparing the manuscript on the characterization of antibiotic-resistant biofilm-forming S. aureus. While the topic is interesting, there are a few points that need to be addressed for potential publication. Please consider the following suggestions for revision:

Title and Abstract:
1. In lines 2-3, please expand all abbreviations upon first use (e.g., S. aureus, UTI, etc.). This expansion should be

consistent throughout the text.
2. In the Methods section, briefly outline the inclusion criteria for the study. Specifically, which isolates were considered for the study?
3. In the Conclusion section, discuss the clinical implications of your findings.
4. Utilize MeSH terms as keywords wherever possible.

Methods and Results:
5. The data collection year is mentioned as 2017, which may be outdated. Please explain the reason for the delay in publishing the results, considering that the prevalence of antibiotic-resistant and biofilm-forming S. aureus may have changed over the past 6 years.
6. Provide a more detailed explanation of the statistical analysis. Specify the parameters used for descriptive statistics (e.g., frequency, %, mean, SD, etc.). Additionally, clarify the statistically significant p-value. If both Fisher's exact test and the Chi-square test were utilized, this should be explicitly stated. Confirm that ANOVA was not used for data analysis, considering the categorical or binomial nature of the variables.
7. Clarify whether data collection was based on a census method or sampling. If sampling was employed, include details on how the sample size was estimated and the sampling method. If the census method was used, explicitly state so. It appears that the census method was followed.

Discussion:
8. Expand on the clinical implications of your findings in the Discussion and Conclusion sections.
9. Include a section on study limitations at the end of the Discussion, mentioning the outdated data as a limitation.

Best regards,

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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.

To: Editor-in-Chief

Dear Prof. Masoud Mardani

Attached please find the revised version of our manuscript entitled “Characterization of Clonal Groups of Antibiotic-Resistant Biofilm-Forming Staphylococcus aureus Strains Isolated from Patients with Urinary Tract Infections in Isfahan, Iran” reference no. 143383.

In the revised version we have responded to the comments of the reviewer and if necessary, changed or added sections to clarify ambiguities mentioned by the reviewers. For each comment, apart from our response, we have also highlighted areas that are related to our response in the manuscript and have given the line numbers of the changes made.

We hope that the revised version of our manuscript will meet with the approval of the reviewers.

Sincerely yours,

Corresponding author

Reviewer 1

Title and Abstract:

1. In lines 2-3, please expand all abbreviations upon first use (e.g., S. aureus, UTI, etc.). This expansion should be consistent throughout the text.

Our response: Revised accordingly (lines 3 and 19).

2. In the Methods section, briefly outline the inclusion criteria for the study. Specifically, which isolates were considered for the study?

Our response: The inclusion criteria for the study were mentioned (lines 10-12).

3. In the Conclusion section, discuss the clinical implications of your findings.

Our response: The clinical implications of our findings were mentioned (lines 33-35).

4. Utilize MeSH terms as keywords wherever possible.

Our response: Keywords were rewritten based on MeSH (lines 36-37).

Methods and Results:

5. The data collection year is mentioned as 2017, which may be outdated. Please explain the reason for the delay in publishing the results, considering that the prevalence of antibiotic-resistant and biofilm-forming S. aureus may have changed over the past 6 years.

Our response: Clinical isolates were collected in 2017, but analyses were performed in subsequent years. Unfortunately, we were unable to work in our lab due to the COVID-19 pandemic. In addition, in our opinion, the results of this research can be useful and valuable because the main idea of studies like this is to predict the pattern of antibiotic resistance among bacterial pathogens over a period of several years. Therefore, by comparing the results of this study with other studies which have been published recently, it is possible to predict the antibiotic resistance pattern of *Staphylococcus aureus* strains.

6. Provide a more detailed explanation of the statistical analysis. Specify the parameters used for descriptive statistics (e.g., frequency, %, mean, SD, etc.). Additionally, clarify the statistically

significant p-value. If both Fisher's exact test and Chi-square test were utilized, this should be explicitly stated. Confirm that ANOVA was not used for data analysis, considering the categorical or binomial nature of the variables.

Our response: Thanks for your comment. The parameters used for descriptive statistics and the statistically significant level of *p*-value were mentioned in the revised version of the manuscript (lines 176-179). Usually, the Chi-square will not be performed if the value in any of the cells in the 2x2 contingency table is zero. However, a variant of the Chi-Square test, i.e. Fisher's exact test, will be performed. Also, in ANOVA, the dependent variable must be a continuous (interval or ratio) level of measurement. So, ANOVA may not be the most suitable test available in the present study.

7. Clarify whether data collection was based on a census method or sampling. If sampling was employed, include details on how the sample size was estimated and the sampling method. If the census method was used, explicitly state so. It appears that the census method was followed.
Our response: A census method is that process of the statistical list where all members of a population are analysed.

Our response: Data collection was based on a convenience sampling method. Further details on how the sample size was estimated were included in the revised version of the manuscript (lines 88-93).

Discussion:

8. Expand on the clinical implications of your findings in the Discussion and Conclusion sections.

Our response: Revised accordingly (lines 288-303, 316-321, and, 358-364).

9. Include a section on study limitations at the end of the Discussion, mentioning the outdated data as a limitation.

Our response: We agree with the reviewer and we have added this as a limitation at the end of the Discussion section (lines 343-349).

Reviewer 2:

1. Abstract Line 4: "to establish the relationship" should be "to establish a relationship."

Our response: Revised accordingly (line 8).

2. Abstract Lines 7-8: "biofilm formation capacity was evaluated by a combination of the microtiter plate and Congo-red agar methods." This sentence would be clearer as "biofilm formation capacity was evaluated using a combination of microtiter plate and Congo-red agar methods."

Our response: Revised accordingly (line 14).

3. Results Section: Use of percentages can be confusing. Instead of "Results showed that 19, 57, and 24%," it is clearer to state "Results showed that 19%, 57%, and 24%."

Our response: The modification was done (line 21).

4. Methodology Section: Several sentences are excessively long and could be broken into shorter, clearer statements. For example, "All isolates were identified using specific primers for the nucA gene and their biofilm formation capacity was evaluated by a combination of the microtiter plate and Congo-red agar methods."

Our response: As suggested by the reviewer, the sentences excessively long were broken into shorter, clearer statements (lines 13-18).

5. Clarity of Objective: The objective should be more explicitly stated. Instead of "This study aimed to establish the relationship between clonal groups, biofilm formation, and antibiotic resistance," it should specify how these relationships are to be assessed and their significance.

Our response: Revised accordingly (lines 5-9).

6. Sample Size Justification: There is no justification for the sample size of 119 isolates. Why was this number chosen? Was a power analysis conducted to ensure this number is statistically significant?

Our response: Cochran's formula was used to adjust sample size based on the prevalence of biofilm formation (95.45%) which was reported in previous studies. Further details on how the sample size was estimated were included in the revised version of manuscript (lines 88-93).

7. Methodology Details: There are several details missing in the methodology that are crucial for reproducibility:

*Specific conditions for the PCR assays are not provided. Include details such as annealing temperatures, cycle numbers, and primer sequences.

Our response: PCR conditions and primer sequences were added in the Supplementary Table.

*Clarify how the cut-off OD (ODc) was determined in the microtiter plate method.

Our response: In this method, the cut-off value is established based on the OD value of the negative control (ODc), and the biofilm ability of each tested strain is expressed as the OD value of the strain reduced by ODc value (OD = OD of the tested strain - ODc) (further details were mentioned in the Material and Methods section (lines 120-128).

8. Discussion of Resistance Genes: The discussion should delve deeper into the implications of the presence of specific resistance genes. What does the high frequency of gyrA and grlA genes imply for treatment strategies?

Our response: The clinical significance of the determination of the prevalence of resistance genes was mentioned (lines 316-321).

9. Comparison with Previous Studies: The study should compare its findings with those of previous studies more thoroughly. Are the resistance rates observed in this study consistent with those reported elsewhere? How do the clonal types found compare with those in other regions?

Our response: The resistance rates observed in this study were compared with those reported elsewhere (lines 288-299). The prevalence of each clone in different parts of the world has been reported as quantitative data in previous studies. So, by comparing the results of different studies with values obtained in our study, we can report which clone is dominant in each geographical region.

10. Conclusion: The conclusion should be more specific. Currently, it is too general and does not provide concrete recommendations or implications for future research or clinical practice.

Our response: We added these contents to the conclusion section (lines 358-364).

2 Revision 1

0 comments

No public comments in this round.