Review Report:

This study investigates the heteroresistance in Carbapenem-resistant Acinetobacter baumannii (CRAB) under cefiderocol treatment, an antibiotic showing decreased effectiveness in recent reports. Researchers identified unique chromosomal mutations in heteroresistant variants exposed to human pleural fluid. These mutations, alongside increased expression of certain genes, appear to contribute to the antibiotic resistance. The presence of human serum albumin was found to decrease cefiderocol's effectiveness, revealing the potential role of human biological factors in antibiotic resistance.

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Dear Authors,

Upon reviewing your manuscript, I find your study to be highly intriguing and potentially impactful in its respective field. The subject matter you've chosen to explore is compelling and addresses a significant area of research.

However, for your study to truly resonate with the readers of the International Journal of Molecular Sciences and leave a lasting impression, it necessitates some major revisions.

The first area where I would advise adjustments is the structure of your paper. Please ensure it follows the specific format: Title, Abstract, Key Words, Introduction, Materials and Methods, Results, Discussion, and Conclusion. This logical progression is a standard in scientific literature and will make your research more accessible to your readers.

In terms of visual presentation, it's essential to adopt a consistent font as per the journal's formatting guidelines. Maintaining a uniform appearance throughout will present your work professionally and in line with the journal's standards.

Additionally, the manuscript requires considerable attention to its grammar and usage of English. Although the research is promising, the present language issues may create barriers to comprehension. I highly recommend revising the text, perhaps with the help of a professional editor or a fluent English speaker.

Lastly, attention must be paid to the referencing style. Make sure your citations and bibliography are formatted as per the specifications laid out by the International Journal of Molecular Sciences. This will lend credibility to your research and ensure it's easy for readers to trace your sources.

While the need for revisions is substantial, I want to emphasize that the essence of your study is of significant interest. I'm confident that with these improvements, your research will be a valuable contribution to our journal. I look forward to reviewing your revised manuscript.

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Building upon my previous comments, there are several key parameters in your study that would benefit from further explanation:

Selection of Microorganisms and Strains: You should thoroughly describe the rationale behind the selection of the microorganisms and strains used in your experiments. What characteristics or behaviors of these organisms make them pertinent to your research questions? Additionally, the origin and unique characteristics of the strains should be described in detail. Where were

they isolated from? What are their known characteristics and how do these contribute to your study?

Choice of Antibiotics and Concentrations: It would be beneficial to provide a detailed explanation of why certain antibiotics were chosen for your study. Similarly, the concentrations used for these antibiotics should be justified. Were these concentrations chosen based on prior research, or is there another reasoning behind this decision?

Methodology Employed: The techniques and protocols utilized in your study need to be adequately explained. Why were these particular methods chosen over others available in the field? Additionally, any modifications made to standard protocols should be clearly stated and justified.

Experimental Conditions: The choice of specific conditions such as time, temperature, and other factors, need to be justified. How do these conditions reflect the natural environment of the microorganisms or the clinical setting?

Genes Studied: If you are investigating certain genes, it is important to describe why these particular genes were chosen. What is their significance in the context of your study?

In summary, every decision made during the experimental design phase should be thoroughly justified in your revised manuscript. Detailed explanations of these choices not only provide a more comprehensive understanding of your work, but also allow for better reproducibility and evaluation of your findings by peers in the field.

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TITLE

Considering the focus of the research and the results discussed in the paper, a potential rephrasing of the title could be:

"Induced Heteroresistance in Carbapenem-Resistant Acinetobacter baumannii (CRAB) via Exposure to Human Pleural Fluid (HPF) and its Impact on Cefiderocol Efficacy"

ABSTRACT

The abstract provides a concise overview of the study. However, there are a few issues that need revision:

Sentence structure: The abstract lacks proper sentence structure, making it difficult to follow. It would benefit from clearer and more organized sentences.

Objectives: The objectives are mentioned but not explicitly stated. It would be helpful to rephrase this section to clearly state the objectives of the study.

Lack of clarity: The abstract states that cefiderocol, an antibiotic, was approved by the FDA in 2019 for the treatment of CRAB infections. However, it does not clearly state the purpose of this study and what specific aspect of cefiderocol heteroresistance is being investigated.

Incomplete information: The abstract mentions recent studies reporting higher mortality rates in patients treated with cefiderocol, but it does not provide any specific details or references to support this claim. Adding more information or references would strengthen the abstract.

Plausible hypothesis: The abstract introduces the hypothesis of heteroresistance but does not provide any further explanation or context. It would be helpful to elaborate on the concept of heteroresistance and its potential implications.

Methods: The abstract briefly mentions the methods used for isolation, sequencing, and analysis but does not provide enough detail to understand the study's methodology comprehensively. Including a concise summary of the key methods employed would be beneficial.

Results: The abstract briefly mentions the chromosomal mutations, gene expression changes, and the impact on cefiderocol activity. However, it does not provide any specific findings or quantitative results. Adding a brief summary of the key results would enhance the abstract.

Conclusion: The conclusion lacks clarity and does not adequately summarize the study's findings. It would be beneficial to rephrase the conclusion to clearly state the main conclusions drawn from the study.

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Upon a critical analysis of the Abstract provided, there are a few potential areas for enhancement:

Line 35: It would be more precise to indicate the types of infections in which CRAB isolates pose a significant treatment challenge.

Suggested change: "Carbapenem-resistant Acinetobacter baumannii (CRAB) isolates are particularly challenging to treat in infections such as [provide examples]."

Line 38-39: The statement concerning higher all-cause mortality rate in patients treated with cefiderocol might benefit from further clarity. In particular, it could be useful to add a comparison with a different treatment or the average mortality rate for these types of infections.

Suggested change: "Recent studies reported a higher-than-average all-cause mortality rate in patients treated with cefiderocol compared to [alternative treatment or the average mortality rate]."

Line 47: It could be beneficial to give a brief explanation or some relevant characteristic of the AMA40 IHC1 and IHC2 variants to better inform the reader about these specific strains.

Suggested change: "Two AMA40 variants, AMA40 IHC1 and IHC2, which are [brief description/characteristic], were subjected to..."

Line 49: The sentence is quite long and complicated. You could simplify it by splitting it into two sentences.

Suggested change: "To identify mutations and expression changes associated with cefiderocol heteroresistance, we subjected these variants to whole genome sequencing and transcriptional analysis. We then assessed the impact of these mutations on the pharmacodynamic activity of cefiderocol via susceptibility testing, EDTA and boronic acid inhibition analysis, biofilm formation, and static time-kill assays."

Line 54-55: Here it would be beneficial to clarify what you mean by 'these functions'. Do you refer to iron-uptake and β-lactam resistance, or other functions?

Suggested change: "...transcriptional analyses showed significant modifications in levels of expression of genes associated with [specifically mention the functions]."

Line 62-63: The sentence could be more precise by stating what "fluids containing high HSA" refers to, assuming not all readers may know the abbreviation.

Suggested change: "We conclude that exposing CRAB to fluids with high concentrations of Human Serum Albumin (HSA) facilitates..."

General: The abstract could benefit from including a statement about the implications or importance of this study for the scientific community or for clinical practice. This would typically be included at the end of the conclusions section.

Suggested addition: "These findings have significant implications for [the development of new treatments/the understanding of CRAB resistance mechanisms/etc.]."

Overall, the abstract should be revised to provide a clearer and more structured summary of the study, including the objectives, methods, key results, and conclusions.

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KEYWORDS:

The keywords are relevant to the study but could be more comprehensive. Adding additional keywords related to the specific methods used and the outcomes observed would enhance the keyword section.

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INTRODUCTION:

The introduction section of the paper provides a comprehensive overview of the importance of Acinetobacter baumannii infections and the need for effective treatment options. However, there are a few issues and areas that could benefit from revision and correction:

Sentence structure and organization: The introduction contains several lengthy sentences, making it challenging to follow the information being presented. It would be helpful to break down the sentences into shorter, more concise statements to improve readability.

Lack of clarity: While the introduction discusses the approval of cefiderocol by the FDA for the treatment of CRAB infections, it does not clearly state the purpose of the study or what specific aspect of cefiderocol heteroresistance is being investigated. It would be beneficial to provide a clearer research objective or hypothesis.

Incomplete referencing: The introduction mentions previous studies and clinical trials, but it lacks specific references to support the claims made. Adding proper citations to support the statements would strengthen the introduction and provide readers with the opportunity to explore the referenced studies.

Heteroresistance concept: The introduction briefly introduces the concept of heteroresistance but does not provide a detailed explanation or context for readers who may not be familiar with the term. It would be beneficial to provide a more thorough explanation of heteroresistance, its significance, and its potential implications in the context of the study.

Structure and organization: The introduction could benefit from a clearer structure and organization. It should include a clear statement of the research objective, a brief background on the topic, and a logical flow of information to set the stage for the study.

Overall, the introduction should be revised to provide a clearer and more organized presentation of the research objective, the significance of the study, and the existing knowledge gap regarding cefiderocol heteroresistance in CRAB infections. Proper referencing and a more comprehensive explanation of heteroresistance would further enhance the introduction section.

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Reviewing the introduction section, the authors have provided a good amount of background information and context about the study. However, I noticed a few areas that can be improved or clarified:

Line 71-72: The terms “pneumonia” and “bacteremia” are introduced without any brief explanation or context. It is important to provide a brief explanation of these terms to make it clear to the reader, especially those who might not be familiar with clinical terminology.

Suggestion: “The two principal clinical manifestations are pneumonia, an infection that inflames the air sacs in the lungs, and bacteremia, a condition where bacteria enter the bloodstream...”

Line 74: The abbreviation “CRAB” is introduced without providing a clear definition. It is mentioned as “Carbapenem-resistant A. baumannii” but should ideally be clearly defined for readers. Suggestion: Instead of “Carbapenem-resistant A. baumannii (CRAB)”, it should be “Carbapenem-resistant Acinetobacter baumannii (CRAB)”.

Line 78: The use of the term "highly resistant A. baumannii" is ambiguous and could be better defined. What constitutes "high resistance" should be explained. Suggested correction: "Additionally, the global emergence and spread of A. baumannii strains resistant to multiple classes of antibiotics highlights the need for new antimicrobial therapies."

Lines 80-81: The authors mention that cefiderocol is the only novel drug approved by the FDA against A. baumannii. It could be helpful to add more context about the time frame they're referring to. This can be addressed by specifying the years or time period. E.g., "In the past decade, cefiderocol is the only novel drug approved by the FDA active against A. baumannii."

Line 81-82: The reference to the FDA's website seems to break the flow of the sentence and could potentially be considered unprofessional in an academic context. The authors could simply state that cefiderocol has been approved by the FDA for use against A. baumannii and provide the reference at the end of the sentence in parentheses.

Suggestion: "the only new novel drug approved by the U.S. Food and Drug Administration (FDA) active against A. baumannii is cefiderocol [reference]."

Line 86-88: The transition between these lines is a bit abrupt. It would be better to introduce the concept of heteroresistance before mentioning its possible impact on cefiderocol efficacy.

Suggested correction: "Although positive outcomes abound, there are recent reports indicating decreased cefiderocol efficacy against multi-drug resistant (MDR) CRAB, which may be suggestive of a phenomenon known as heteroresistance. Heteroresistance is a phenotype

wherein a small fraction of bacteria within a bacterial community develop resistance under antibiotic pressure."

Line 89-90: The explanation of heteroresistance is given too late in the introduction. The abstract already uses this term, so it is better to explain it earlier in the introduction.

Suggestion: Provide a brief explanation of heteroresistance early in the introduction, shortly after introducing CRAB and its resistance to carbapenem.

Lines 92-94: The authors should explain what the acronym "CREDIBLE-CR" stands for in its first mention. This will make the text more accessible for readers unfamiliar with this specific study.

Line 94-101: The description of the CREDIBLE-CR study is a bit hard to follow. It may be helpful to rephrase for clarity and provide more details about the study (e.g., control group, treatment duration).

Line 96-101: The sentences seem long and complex which might complicate comprehension. Simplifying these sentences could enhance readability and understanding.

Suggestion: "Among the 118 patients in the intent-to-treat population, A. baumannii infected 54 patients, making it the most frequent carbapenem-resistant pathogen [reference]. Most previous trials that included A. baumannii were focused on colistin-based regimens [8]. The cefiderocol group had a higher all-cause mortality rate (19/39), especially in patients with nosocomial pneumonia, bloodstream infection, or sepsis with Acinetobacter spp at baseline [19]."

Line 107-108: The phrase “Enterobacterales and A. baumannii ESBLs genes” is used without introducing or explaining the term “ESBLs”.

Suggestion: Define ESBLs when it is first mentioned. For example, “Enterobacterales and A. baumannii extended-spectrum β-lactamases (ESBLs) genes”.

Line 108-110: The terms "NDM-producing Enterobacterales" and "blaNDM-5 expression" could be confusing for some readers. Consider explaining what NDM (New Delhi metallo-beta-lactamase) is and how it contributes to antibiotic resistance.

Line 112: The abbreviation "CFDC" appears for the first time without previous explanation. It might be helpful to explain or define it before using it.

Suggested correction: Define the term "CFDC" in its first occurrence.

Line 116: The authors should clarify what they mean by "selected" in the context of "selected heteroresistant variants". Does this refer to naturally occurring variants that were isolated for the study, or were they somehow induced or engineered?

Line 116-122: Again, this paragraph appears complex and might be hard for readers to comprehend. Break down into simpler sentences.

Suggestion: "We carried out molecular and phenotypic analyses of two selected CRAB heteroresistant bacterial subpopulations obtained after exposure to HPF. The selected variants showed acquired chromosomal mutations impacting genes coding for numerous functions, including those potentially related to iron metabolism. These heteroresistant variants showed

increased expression of genes related to β-lactamases, high-affinity iron uptake systems, and biofilm formation when compared to the parental strain. In contrast, transcripts of genes coding for outer membrane proteins were reduced in these mutant variants."

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MATERIALS AND METHODS:

Here are some potential issues and corrections in the Materials and Methods section:

Part I:

Line 404-409: The authors should clarify what they mean by 'recovered within the inhibition ellipse zones after exposure.' If this refers to a technique used in the study, it would be beneficial to briefly describe this process for the reader's understanding. Additionally, the authors should specify how the stability of the increased cefiderocol resistance of these isolates was determined.

Line 416: The authors used the FASTQ software for sequence quality check. However, FASTQ is not software, it's a file format. They might be referring to FastQC software instead.

Line 417-419: The authors mentioned using SPAdes and QUAST for de novo sequence assembly and quality assessment. It would be better to provide the versions of these tools used in the study to replicate the methods in the future. This applies to all software tools mentioned in this section.

Line 424: The authors have deposited the gene expression profiles for the AMA40 wild type strain and the IHC1 and IHC2 derivatives in the ArrayExpress database. It would be beneficial to provide direct links to these datasets to improve access for the readers.

Line 430-432: The authors stated that RNA extractions were performed using three independent biological replicates for each condition. It would be helpful if the authors clarify what they mean by 'each condition.' Does this refer to each strain (AMA40, IHC1, and IHC2) or does it refer to different experimental conditions?

Line 434: The authors have mentioned the adjustment of cDNA concentrations to 50 ng/µL. However, they haven't specified how they have measured these concentrations.

Line 444-454: In the antibiotic susceptibility assays, the authors should specify the concentrations of antibiotics used and the number of replicates for these tests. Additionally, the time points at which measurements were taken should be indicated.

Line 461-462, 468: In the EDTA and boronic acid inhibition assays, the authors defined an increase of >3 mm as indicative of resistance contribution. However, it's unclear how this value was chosen, as the standard protocols usually specify >5 mm as significant.

Line 471-482: In the time-kill studies, the authors need to specify the number of replicates used and the statistical tests used for analysis. It would be beneficial to detail if any controls were used in these experiments.

Line 484-485: In the biofilm assays, the authors have to clarify the number of replicates, the

statistical analysis used, and any control conditions included in the assay.

In general, the authors should also mention any potential issues or challenges encountered during their methodology implementation and how they overcame them. This would be of great help for other researchers trying to reproduce these results.

Part II:

Line 404-409: Preservation and Recovery of Strains:

Issue: The text explains that the strains were kept at -80°C, but does not provide enough detail on the preservation method or the recovery process.

Suggestion: The authors should include details on how the strains were preserved (e.g., in a cryopreservative) and the procedures used to recover them for the experiments.

Line 415-416: Quality Control of Sequencing Data:

Issue: It is mentioned that the sequence quality was checked by FASTQ software analysis, but it is not clear what specific quality control measures were used.

Suggestion: Specify the quality thresholds that were applied and any trimming or filtering steps that were taken to clean the sequencing data.

Line 417-418: De Novo Assembly:

Issue: There is a mention of using SPAdes for de novo assembly, but no parameters or configurations are mentioned.

Suggestion: The authors should provide specific information about the parameters used for assembly, such as k-mer sizes, to allow for reproducibility.

Line 419: Variant Calling:

Issue: The paper mentions using breseq and gdtools for variant calling but does not specify parameters or filters used.

Suggestion: Include detailed information on the variant calling process, including any filtering steps (e.g., minimum read depth) and specific parameters used.

Line 427-428: Growth Conditions:

Issue: It is mentioned that cultures were incubated with agitation for 18 hours, but the rpm of agitation is not provided.

Suggestion: Specify the rpm at which the cultures were agitated to allow for reproducibility.

Line 433-436: RT-PCR:

Issue: The concentration of cDNA used is mentioned, but there is no mention of the specific volume of cDNA used in the RT-PCR reactions.

Suggestion: Provide the volume of cDNA that was used in each RT-PCR reaction.

Line 444-451: Antibiotic Susceptibility Assays:

Issue: The methods mention using CLSI guidelines, but it is not clear which version of the guidelines was used. Guidelines can change over time.

Suggestion: Specify the version/year of the CLSI guidelines that were followed.

Line 456-470: Inhibition Assays:

Issue: The incubation conditions (35°C ± 2°C) for the inhibition assays with EDTA and boronic acid are different from the standard 37°C used in microbiology. The reason for this difference is not explained.

Suggestion: The authors should provide an explanation for the choice of incubation temperature, or clarify if it was a typographical error.

Line 477-478: Quantification of Bacteria:

Issue: The authors mention using a spiral plater for plating but do not explain why this specific method was used.

Suggestion: A brief rationale for using the spiral plating method should be provided, especially if it has particular advantages relevant to the research.

Line 484-485: Biofilm Formation:

Issue: The section is cut off and does not provide complete information on how biofilm formation was assessed.

Suggestion: The authors should include complete information on how biofilm formation was measured, including details

RESULTS:

Overall, the Results section is well written, but there are several areas where clarity could be improved and some potential issues that need to be addressed.

General comment: Use of passive voice throughout the section makes the text more difficult to read and understand. Consider using active voice for clarity.

Suggestion: Re-phrase the sentences using active voice.

Lines 127-133: It is not clear what is meant by "HPF" and "5 doubling dilutions". These terms should be defined or explained. Also, there needs to be a clear explanation of how the A. baumannii CRAB model strain was exposed to HPF and if there were any control strains used in the study.

Suggestion: Clearly define "HPF" and "5 doubling dilutions". Additionally, provide a brief description of the experimental method used to expose the A. baumannii CRAB model strain to HPF and mention if control strains were used.

Line 136-147: The detailed breakdown of mutations is helpful, but it could be made clearer by

providing some context. How does this mutation rate compare to what is typically seen in similar studies or with other organisms? Are these mutations likely to be responsible for the observed phenotypes?

Suggestion: Provide context for the reported mutation rates and discuss the potential significance of these mutations.

Lines 150-154: The study states that there were no mutations in genes related to iron uptake, yet a mutation was found in the ppiA gene which may be linked to iron regulation. This seems contradictory.

Suggestion: Clarify this point by explaining whether or not ppiA is typically considered an iron uptake gene and what its potential role in iron regulation might be.

Line 177-180: The authors should explain why the balance between the expression of certain genes contributes to the increased resistance to cefiderocol.

Suggestion: Elaborate on the mechanism by which the balance of gene expressions could contribute to increased cefiderocol resistance.

Line 191: The authors should explain the statistical methods more comprehensively to provide a clear understanding of how the data was analyzed.

Suggestion: Add more detail about the statistical methods used, including any assumptions or preconditions for their use.

Lines 193-198: It is not clear what methodology is being referred to when the authors mention "E-strips, and microdilution assays". These terms should be properly defined or explained.

Suggestion: Clearly define or explain the "E-strips, and microdilution assays" methodology.

Line 235-248: The authors should specify what HSA stands for before using the acronym.

Suggestion: Define "HSA" before using the acronym in the text.

Lines 237-240: The comparison of bacterial growth in the presence and absence of HSA lacks clarity and needs to be expressed more precisely.

Suggestion: Explicitly state the comparative growth rate difference in the presence and absence of HSA.

DISCUSSION:

General Issue: The Discussion section is quite dense and could benefit from better structuring to help the reader follow the argument.

Suggestion: Use subheadings to separate different aspects of the discussion, such as comparison with previous literature, implications of new findings, and future research directions. This will make the text more readable and help the reader understand the contribution of the paper.

Here are some points that could be improved or clarified in the Discussion section of the paper:

Line 303-304: “The genomic, transcriptional, and phenotypic analysis of the two randomly selected isogenic variants indicated that multiple factors may be responsible for the cefiderocol resistance phenotype of IHC1 and IHC2 derivatives, including genomic mutations, increased expression of β-lactamases, reduced expression of porins, and increased biofilm formation.”

Issue: The sentence seems to be loaded with information and could be confusing.

Suggestion: Break down the information into simpler sentences or bullet points to improve readability and comprehension.

Line 307-315: The authors mention a possible connection between the ppiA mutation and increased resistance to cefiderocol, and they bring up examples from Mycobacterium tuberculosis.

Issue: This section does not clearly differentiate between the findings of the current study and what has been found in other organisms.

Suggestion: Explicitly state that the role of the ppiA mutation in cefiderocol resistance in A. baumannii is based on speculation and that direct evidence is still lacking.

Line 317-331: The paper discusses increased expression of β-lactamases contributing to cefiderocol resistance.

Issue: There is no discussion of how the results of the current study compare or contrast with previous studies.

Suggestion: The authors should discuss whether their findings are in agreement or contrast with previous literature and propose possible reasons for any discrepancies.

Line 332-346: This portion discusses other factors such as down-regulation of porin coding genes and increased expression of biofilm-associated genes in contributing to increased resistance.

Issue: It is unclear how much weight each of these factors has in contributing to resistance based on the current study.

Suggestion: The authors should be explicit in discussing the relative importance of these factors in resistance based on their data and discuss any limitations in the ability to determine this.

Line 349-361: The discussion about unstable A. baumannii heteroresistance subpopulations being found in other samples versus the stable resistance traits found in this study.

Issue: The significance of this difference is not clear.

Suggestion: The authors should discuss the implications of finding stable resistance traits in this study compared to unstable heteroresistance in other studies.

Line 362-374: Discusses the combination of cefiderocol and diazabicyclooctane derivatives to restore antibacterial activity.

Issue: The mechanism of the synergistic activity is stated as not understood, but there is no

discussion or speculation on possible mechanisms or the need for future research in this area.

Suggestion: The authors should briefly discuss why understanding the mechanism is important and suggest directions for future research.

Line 377-384: The section addresses the reduction in the killing activity of cefiderocol due to the presence of HSA.

Issue: This section discusses a significant finding but does not provide any further analysis or potential implications.

Suggestion: The authors should discuss the clinical implications of this finding and how it might affect treatment strategies.

CONCLUSION:

In the Conclusion section of the paper, I found a few areas that could benefit from some revision:

Lines 390-392: "In the present work, we found that two independent cefiderocol heteroresistant derivatives showed no mutations in genes coding for active iron acquisition or β-lactam resistance functions. However, both derivatives showed the same point mutation in pipA, a gene associated with iron homeostasis in other species."

Issue: The term "pipA" is used here, while "ppiA" was used in the previous section. Is it the same gene or are they different? It's important to maintain consistency throughout the paper.

Suggestion: If the genes are the same, use the same name consistently to avoid confusion. If they are different, make it clear in the text.

Line 395-396: "Notably, static time kill assays showed that the cefiderocol killing activity was considerably reduced in the presence of HSA."

Issue: There's no mention of what this finding means in the broader context of treating A. baumannii infections.

Suggestion: Consider adding a sentence that explains the clinical implications of this finding. For example, you could discuss how this impacts the use of cefiderocol for treatment in a clinical setting.

Line 397-398: "In sum, HSA-containing fluids result in reduced susceptibility to cefiderocol through mechanism(s) that might include genomic point mutations or phenotypic modifications like increased biofilm formation or changes in gene expression."

Issue: This sentence is very complex and may be hard for readers to follow.

Suggestion: Break this into two sentences for improved readability. For example, "In sum, our study shows that the presence of HSA-containing fluids can reduce the susceptibility to cefiderocol. This might be due to mechanisms including genomic point mutations, phenotypic modifications such as increased biofilm formation, and changes in gene expression."

Line 399-401: "Further studies focused on understanding the mechanisms through which HSA-

rich human fluids elicit antibiotic resistance may provide the basis for designing more effective strategies for treating A. baumannii infections."

Issue: The last sentence is a bit vague in terms of the direction of future research.

Suggestion: Elaborate on the types of studies or methodologies that could be used to further investigate these mechanisms. This would give the reader a clearer picture of the proposed research trajectory.

In conclusion, the Conclusion section generally provides a concise summary of the findings and their implications, but it could benefit from more detailed comments on the implications of the findings for future research and treatment strategies. Also, it would be helpful to maintain consistency in the names of genes and other technical terms throughout the paper.