Molecular Characterization of WCK 5222 (cefepime/zidebactam) Resistant Mutants Developed from a Carbapenem Resistant Pseudomonas aeruginosa Clinical Isolate

Xiaolei Pan, Xinrui Zhao, Yuqin Song, Huan Ren, Zhenyang Tian, Qi’an Liang, Yongxin Jin, Fang Bai, Zhihui Cheng, Jie Feng, and Weihui Wu

Corresponding Author(s): Weihui Wu, Nankai University

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(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

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January 23, 2022

Dr. Weihui Wu
Nankai University
State Key Laboratory of Medicinal Chemical Biology, Key Laboratory of Molecular Microbiology and Technology of the Ministry of Education, Department of Microbiology, College of Life Sciences
94 Weijin Road
Nankai District
Tianjin 300071
China

Re: Spectrum02678-21 (Molecular Characterization of WCK 5222 (cefepime/zidebactam) Resistant Mutants Developed from a Carbapenem Resistant Pseudomonas aeruginosa Clinical Isolate)

Dear Dr. Weihui Wu:

Thank you for submitting your manuscript to Microbiology Spectrum. When submitting the revised version of your paper, please provide (1) point-by-point responses to the issues raised by the reviewers as file type “Response to Reviewers,” not in your cover letter, and (2) a PDF file that indicates the changes from the original submission (by highlighting or underlining the changes) as file type “Marked Up Manuscript - For Review Only”. Please use this link to submit your revised manuscript - we strongly recommend that you submit your paper within the next 60 days or reach out to me. Detailed instructions on submitting your revised paper are below.

Link Not Available

Thank you for the privilege of reviewing your work. Below you will find instructions from the Microbiology Spectrum editorial office and comments generated during the review.

The ASM Journals program strives for constant improvement in our submission and publication process. Please tell us how we can improve your experience by taking this quick Author Survey.

Sincerely,

Beile Gao
Editor, Microbiology Spectrum

Journals Department
American Society for Microbiology
1752 N St., NW
Washington, DC 20036
E-mail: spectrum@asmusa.org

Reviewer comments:

Reviewer #1 (Comments for the Author):

In this manuscript, Pan et al., isolated a multidrug resistant P. aeruginosa clinical strain called NKPa-71, which is resistant to a variety of β-lactam antibiotics and ceftazidime-avibactam combination. A self-transmissible megaplasmid was identified in this strain, which harbored a metallo-β-lactamase gene blaDIM-2, conferred this strain resistance to β-lactam antibiotics, except WCK 5222. By in vitro passaging assay and whole genome sequencing the authors characterized substantial mutations in zidebactam target protein PBP2 encoding gene pbpa, and the authors verified the the roles of these mutations in reference strain PAO1, revealed that mutations in the pbpa gene might be a major cause of evolved resistance to WCK 5222 in clinical settings. The study is well designed and conducted, with a large number of solid experiments. The authors uncovered significant information about the underlying resistance mechanisms.

Minor Comments
1. I found that the authors did not present more discussion about the results that mutations of PBP2 caused different effect on bacterial growth as well as competition index (CI) in competition assay, what they thought might be the physiological significance.

2. A number of grammatical errors need corrected (a few examples below):
Line 20: bacteria-> bacterial 
Line 22 and 86: resistance-> resistant 
Line 78: mutation-> mutations 
Line 98 : and-> to 
Line 195 : of that of-> of

Reviewer #2 (Comments for the Author):

The authors have addressed previous comments.

Staff Comments:

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To submit your modified manuscript, log onto the eJP submission site at https://spectrum.msubmit.net/cgi-bin/main.plex. Go to Author Tasks and click the appropriate manuscript title to begin the revision process. The information that you entered when you first submitted the paper will be displayed. Please update the information as necessary. Here are a few examples of required updates that authors must address:

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• Manuscript: A .DOC version of the revised manuscript
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Thank you for submitting your paper to Microbiology Spectrum.
Review comments

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   Line 20: bacteria-> bacterial
   Line 22 and 86: resistance-> resistant
   Line 78: mutation-> mutations
   Line 98: and-> to
   Line 195: of that of-> of
Dear Editor:

Please accept my gratitude, to both you and your reviewers, for the attention given to our manuscript entitled “Molecular Characterization of WCK 5222 (cefepime/zidebactam) Resistant Mutants Developed from a Carbapenem Resistant Pseudomonas aeruginosa Clinical Isolate” [Spectrum02678-21]. The following is a point-by-point synopsis of the changes we have made to address the comments and concerns.

Reviewer #1 (Comments for the Author):

In this manuscript, Pan et.al., isolated a multidrug resistant P. aeruginosa clinical strain called NKPa-71, which is resistant to a variety of β-lactam antibiotics and ceftazidime-avibactam combination. A self-transmissible megaplasmid was identified in this strain, which harbored a metallo-β-lactamase gene blaDIM-2, conferred this strain resistance to β-lactam antibiotics, except WCK 5222. By in vitro passaging assay and whole genome sequencing the authors characterized substantial mutations in zidebactam target protein PBP2 encoding gene pbpA, and the authors verified the the roles of these mutations in reference strain PAO1, revealed that mutations in the pbpA gene might be a major cause of evolved resistance to WCK 5222 in clinical settings. The study is well designed and conducted, with a large number of solid experiments. The authors uncovered significant information about the underlying resistance mechanisms.

Minor Comments
1. I found that the authors did not present more discussion about the results that mutations of PBP2 caused different effect on bacterial growth as well as competition index (CI) in competition assay, what they thought might be the physiological significance.
   Response: We added the discussion about the effects of the PBP2 mutations on bacterial growth and competition index (lines 232-239).

2. A number of grammatical errors need corrected (a few examples below):
   Line 20: bacteria-> bacterial
   Line 22 and 86: resistance-> resistant
   Line 78: mutation-> mutations
   Line 98: and-> to
   Line 195: of that of-> of
   Response: The errors have been corrected. We also double checked the whole manuscript to correct grammatical errors.

Reviewer #2 (Comments for the Author):
The authors have addressed previous comments.

My coauthors and I agree that the changes made to the manuscript in addressing the reviewers’ concerns have greatly improved this manuscript. Please contact me if I can provide any additional information.

Sincerely,
Weihui Wu, Ph.D.
January 27, 2022

Dr. Weihui Wu
Nankai University
State Key Laboratory of Medicinal Chemical Biology, Key Laboratory of Molecular Microbiology and Technology of the Ministry of Education, Department of Microbiology, College of Life Sciences
94 Weijin Road
Nankai District
Tianjin 300071
China

Re: Spectrum02678-21R1 (Molecular Characterization of WCK 5222 (cefepime/zidebactam) Resistant Mutants Developed from a Carbapenem Resistant Pseudomonas aeruginosa Clinical Isolate)

Dear Dr. Weihui Wu:

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Thank you for submitting your paper to Spectrum.

Sincerely,

Beile Gao
Editor, Microbiology Spectrum

Journals Department
American Society for Microbiology
1752 N St., NW
Washington, DC 20036
E-mail: spectrum@asmusa.org

Supplemental table 2: Accept
Supplemental Materials: Accept