Reviewer’s report

Title: Antimicrobial activity of amlodipine against extensively drug-resistant Acinetobacter baumannii isolates in vitro

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Reviewer: Shan-Chwen Chang

Reviewer’s report:

Multidrug resistant Acinetobacter baumannii infection has become an important issue in many parts of the world. Selection the appropriate antimicrobial agents to treat MDR of XDR A. baumannii infection is difficult because limited active agents can be used. The authors studied the antimicrobial activity of amlopidine (AML) against 42 isolates of MDR and XDR A. baumannii, and the combination effect of AML and imipenem against these 42 A. baumannii isolates. Their results showed AML could decrease the MIC of imipenem and increase the imipenem susceptible percentage of these 42 isolates when 40 ug/ml of AML was added. Using the checkerboard methods, 14.3% and 35.7% of the isolates demonstrated synergistic or partial synergistic effects when MAL and imipenem were combined. There are several major comments and minor comments on this manuscript.

Major comments:

(1) The title of this manuscript is not good. The bacterial isolates studied were not extensively drug-resistant (XDR) A. baumannii only. “Antimicrobial activity of amlopidine against extensively drug-resistant Acinetobacter baumannii isolates in vitro” also did not express the major parts of the study, that is the effects of combination of amlopidine with imipenem. The authors should modify the title of this manuscript.

(2) According to the study results, AML need to be used up to 40 ug/ml to improve the activity of imipenem against these resistant A. baumannii isolates. However, AML serum level can be achieved to the level of ng/ml if used in current recommended dosage in human. How to achieve the level of 40 ug/ml? How large dosage of AML need to be used in order to achieve 40 ug/ml? What kind of effects will be appeared in human body (eg. blood pressure effect) with such kind of large dosage? The authors should discuss the possibility to achieve this kind of AML concentration in human and its possibility of clinical application.

(3) In “Results” section, Paragraph 1, the authors said among the 42 A. baumannii isolates, 11 were MDR strains (MDRAB) and 31 were XDR strains (XDRAB). However, according to the definition described in “Background” section and the susceptibility results of individual isolates in Table 1, the numbers of MDR and XDR strains were not correct. The authors need to calculate again or check the S, I, R results of individual isolates again. Besides, the definition of MDR and XDR used in this study should be put in the “Methods” section, not in the “Background” section.
(4) To study the combination effects of two different agents, it’s better to do the
time-killing study in addition to the checkerboard method. The authors should
choose several isolates with different FICindex results to demonstrate the
time-killing curve, which may prove or disprove the killing synergistic effect
against the specific isolates.

(5) Although the authors collected 42 consecutive isolates of A. baumannii from
different 42 patients, many isolates had totally the same antibiogram. It’s better to
do the molecular typing of these 42 isolates, such as PFGE, to know whether
there was clonal spreading in this hospital or not, how many isolates were
belonged to the same clone, and whether different clones have different effects
for AML and imipenem combination.

(6) In “Background” section, including the “Abstract Background” section, the
authors mentioned that “In this work …….its potential mechanism of action was
explored.” However, they only checked whether these 42 isolates had
metallo-beta-lactamase (MBL) production by E-test MBL method. They did not
check other carbapenem resistance mechanism or the antimicrobial mechanism
of AML. How could they say they would explore the potential mechanism of
action for either AML alone or AML in combination with imipenem. In addition, the
MBL production was checked by E-test MBL method only. It is not enough. They
should check what kind of MBL was produced in this isolate by other methods,
such as PCR with appropriate primers.

(7) Fig. 2 can be deleted. The results of Table 3 for the change of imipenem MIC
with adding 10 ug/ml, 20 ug/ml, 40 ug/ml of AML, compared to the MIC without
adding AML, can be demonstrated in figure showing the distribution of imipenem
MICs. This will show more clearly the effect on imipenem MIC change when
different concentrations of AML were added.

Minor comments:

(1) In “Methods” section Paragraph1, “The First People’s Hospital” in what city or
what province should be clarified. Is it Guangzhou First People’s Hospital?

(2) In “Discussion” section Paragraph 1, the authors should not extend the
description to Acinetobacter infection. They should only say A. baumannii
infection because this study only tested the isolates of A. baumannii.

(3) In “Discussion” section Paragraph 1, the sentence “According to our data, A.
baumannii is widely involved in hospital infections, resulting in respiratory, blood,
wound, urine and cerebrospinal infections, ……” is not correct because the
authors mentioned that they collected consecutive isolates from clinical
specimens of different patients, but did not mention whether these patients had
true A. baumannii infection or not, especially when the isolates were from
respiratory specimens.

(4) In “Discussion” section Paragraph 2 : “staphylococcus aureus NCTC6571”
should be “Staphylococcus aureus NCTC6571”. “Escherichia coli” and “Klebsiella
pneumoniae” should be “E. coli” and “K. pneumoniae” when they appeared
second time in the text.
(5) In “Discussion” section Paragraph 2: After the sentence “A further study demonstrated that AML in combination with streptomycin has a synergistic effect.” references should be added.

(6) Typo error in the sentence “Our research demonstrates the antimicrobial activities of AML against clinical A. baumannii strains, and the MICs ranged from 40 to 320 ug/ml in combination with imipenem. Half of the isolates (N=21, 50.0%) demonstrated synergy or partial synergy.” in “Discussion” section, Paragraph 2.

(7) In “Conclusion” section, the sentence “Our research indicates that AML alone or in combined with imipenem showed antibacterial activities against clinically resistant Acinetobacter baumannii isolates in vitro.” is not correctly described, because AML alone did not have good antibacterial activity. “Acinetobacter baumannii” in this sentence should use abbreviation as “A. baumannii”.

(8) The detailed antibiogram of individual isolates in Table 1 can be put in the “Appendix”. Table 1 can be modified to show only the statistical results of “S, I, R” percentage of total 42 isolates for individual antibiotics tested. In this way, data in Fig. 1 can be showed in this new Table. Therefore Fig. 1 can be deleted.

**Level of interest:** An article of limited interest

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

None.