OPEN PEER REVIEW

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Peer Review Report for "Sub-Inhibitory Concentrations of Sodium Houttuyfonate in Combination with Erythromycin Inhibit Biofilm Formation and Expression of IcaA in *Staphylococcus epidermidis*"

Author(s): Daqiang Wu, Qiangjun Duan, Ganfei Xu, Yan Guan

Review Timeline:

Submit Date: 11 Sep 2019
Revised Date: 29 Nov 2019
Accepted Date: 1 Dec 2019

Revision (0)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

Zohreh Mashak: Reviewer | **Revision** (0) — 27 Oct 2019

Dear AE,
- There are grammatical (, and the; synesthetic,....) and repeatable (broth (TSB) broth; SH in combination with erythromycin) words.
- Application of transition electron microscopy is very better for descriptive antibacterial effects.
My confident al decision: Minor revision.
Thanks.

Farhad Safarpoor Dehkordi: Reviewer | **Revision** (0) — 1 Nov 2019

Dear AE,
Well, this is an original research work about the effects of sodium houttuyfonate in combination with erythromycin on biofilm formation and expression of icaA gene in Staphylococcus epidermidis. It is interesting work and can help us to prevent the formation of biofilm in bacteria. However, there were some points that should revise before its full

acceptance.

Please, author, address all of the following comments:

1- Text is full of grammatical and typographical mistakes. Please use from a native English speaker person which is an expert in microbiology to revise your text.

2- How the quality and quantity of extracted cDNA were measured?

3- Table 1. Oligonucleotide primers used during qRT-PCR: Please add their references.

4- Please add the validity test for the real-time PCR.

5- Please add the figures for melting and amplification curve of the real-time PCR.

6- Please add the standard curve.

7- Please complete the discussion with some literature-based surveys and also explain and interpretation of your obtained findings.

8- Please also suggest the way that sodium houttuyfonate reduces the expression of the gene and also decreases the formation of biofilm.

9- Please complete the introduction part using the explanation about the characters of the studied gene and also sodium houttuyfonate.

10- Additionally, add some explanation about the fact that why other surveys cannot solve the issue of biofilm formation which caused that you and your colleague performed this research.

11- Some of your references were old. You should replace them with some novel references.

12- Is there any ethical consideration for your study? Please add it.

13- Please explain about the role of sodium houttuyfonate on biofilm formation of some rough strains or clinically isolated bacteria.

14- Are there any survey conducted on the role of sodium houttuyfonate on biofilm formation of other bacteria? Please add them.

15- Please complete the conclusion with some more relevant information about the exact finding of your survey and also their application in microbiology.

16- What are the main applications of your research in the field of microbiology based on the clinical view.

17- (Figure …) should change to (figure …). Make it throughout the text.

18- Some of the references attached in [] format and some other attached in () format. Please make them unique based on the instruction of the journal.

19- Please also unique the style of references throughout the reference lists.

20- The table should change to the table in the section of gene expression analysis and throughout the text.

21- Why authors used erythromycin? How about other antibiotic agents? Please add your reasons to the discussion.

Regards

FSD

Farhad Safarpoor Dehkordi: Associate Editor | **Revision (0)** 1 Nov 2019

Dear EIC,

The manuscript needs major revision before publication in JJM. The author should address the comments of 2 potential reviewers and send back the revised paper as soon as possible.

Regards
DR. F. Safarpoor Dehkordi
The Associated Editor

REFEREE: EIC | **Revision** (0) 1 Nov 2019

Dear Author,
Thank you for submitting your manuscript to JJM. I have completed the review of your manuscript and a summary is appended below. The reviewers recommend the reconsideration of your paper following major revision. I invite you to resubmit your manuscript after addressing all reviewer comments. Please answer point by point to the reviewer's comments on a separate sheet under WORD format and write your modifications in the revised version (WORD format) in colored or highlighted letters. Your answers to reviewers' queries are not only for the Editorial Board but for the readers and should be included, when appropriate, in the manuscript for improvement and a better understanding. When resubmitting your manuscript, please carefully consider all issues mentioned in the reviewers' comments, outline every change made point by point, and provide suitable rebuttals for any comments not addressed.
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Thanks.

OPEN PEER REVIEW

Revision (1)

Reply to Reviewers

Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

You can read the authors' responses to the reviewers' comments in the next page.

Point to point response

Reviewers' Comments:

Reviewer 1:
-There are grammatical(, and the; synesthetic,....) and repeatable (broth (TSB) broth;SH in combination with erythromycin)words.
-Application of transition electron microscopy is very better for descriptive antibacterial effects.
My confident al decision: Minor revision
Response:
We are truly grateful for the advices of reviewer. The manuscript has been revised by an English expert to correct the errors and repeatable words throughout the manuscript.

Reviewer 2:
Journal: Jundishapur Journal of Microbiology
Section: General Manuscript
Type: Research Article
Manuscript Full Title: Sub-inhibitory concentrations of sodium houttuyfonate in combination with erythromycin inhibit biofilm formation and expression of icaA in Staphylococcus epidermidis

Well, this is an original research work about the effects of sodium houttuyfonate in combination with erythromycin on biofilm formation and expression of icaA gene in Staphylococcus epidermidis. It is an interesting work and can help us to prevent from the formation of biofilm in bacteria. However, there were some points which should revise before its fully acceptance.
Please address all of the following comments:
1- Text is full from grammatical and topographical mistakes. Please use from a native English speaker person which is expert in microbiology to revise your text.
2- How the quality and quantity of extracted cDNA were measured?
3- Table 1. Oligonucleotide primers used during qRT-PCR: Please add their references.
4- Please add the validity test for the real-time PCR.
5- Please add the figures of melting and amplification curve of the real-time pcr.
6- Please add the standard curve.
7- Please complete the discussion with some literature-based surveys and also explanation and interpretation of your obtained findings.
8- Please also suggest the way that sodium houttuyfonate reduce the expression of gene and also decrease the formation of biofilm.
9- Please complete the introduction part using the explanation about the characters of the studied gene and also sodium houttuyfonate.
10- Additionally, add some explanation about the fact that why other surveys cannot solve the issue of biofilm formation which caused that you and your colleague performed this research.
11- Some of your references were old. You should replace them with some novel references.

12- Is there any ethical consideration for your study? Please add it.
13- Please explain about the role of sodium houttuyfonate on biofilm formation of some rough strains or clinically isolated bacteria.
14- Are there any survey conducted on the role of sodium houttuyfonate on biofilm formation of other bacteria? Please add them.
15- Please complete the conclusion with some more relevant information about the exact finding of your survey and also their application in microbiology.
16- What are the main applications of your research in the field of microbiology based on the clinical view.
17- (Figure …) should change to (figure …). Make it throughout the text.
18- Some of references attached in [] format and some other attached in () format. Please make them unique based on the instruction of the journal.
19- Please also unique the style of references throughout the references lists.
20- Table should change to table in the section of gene expression analysis and throughout the text.
21- Why authors used from erythromycin? How about other antibiotic agents? Please add your reasons into the discussion.

Response: We are grateful for the advices and suggestions of the reviewer. These suggestions would greatly prompt the scientific value of our manuscript. We have made the point to point response as follows:

Q1- Text is full from grammatical and topographical mistakes. Please use from a native English speaker person which is expert in microbiology to revise your text.
Response: Yes, we agree with the opinion of reviewer. The manuscript has been revised by an English expert to correct the errors throughout the manuscript.

Q2- How the quality and quantity of extracted cDNA were measured?
Response: Due to the low concentration of cDNA, we measured the quality and quantity extracted RNA by the concentration and OD 260/280 of samples using Nanodrop One to ensure the quality and quantity of cDNA. We have added the sentence in the methods section of revised manuscript as follows: "The quality and quantity of extracted RNA were measured by the concentration and OD 260/280 of samples using Nanodrop One (Thermo Fisher, Waltham, USA)."

Q3- Table 1. Oligonucleotide primers used during qRT-PCR: Please add their references.
Response: Yes, we have added the reference in the table 1 of *gyrB*. Because the oligonucleotide primers of icaA was designed in this work. We mentioned these in the method section as follows: "The primers of *icaA* was designed in this study, and the primers of gyrB was synthesized based on our previous study (27)."

Q4- Please add the validity test for the real-time PCR.
Response: Yes, due to the relative quantification PCR method used in this research, we validated the real-time PCR by sequencing the PCR products of *icaA* and *gyrB* to guarantee the correction of PCR reaction. We have added the sentence in the methods section of revised manuscript as

follows: "The real time PCR was validated by sequencing the PCR products of icaA and gyrB."

Q5- Please add the figures of melting and amplification curve of the real-time pcr.
Response: Yes, we have added the melting and amplification curve in the revised manuscript as figure 1B-D.
Q6- Please add the standard curve.
Response: Due to the relative quantification PCR method used in this research, the calculated cycle threshold (CT) of icaA was normalized to the CT for gyrB amplified from the corresponding sample, and the fold changes of icaA were calculated by relative quantification method according to the 2-ΔΔCT method (28). Thus, there is no standard curve in our relative quantification PCR assay.

Q7- Please complete the discussion with some literature-based surveys and also explanation and interpretation of your obtained findings.
Response: Yes, we are grateful for the suggestion of reviewer. We have revised the discussion to complete our findings as follows: "*S. epidermidis* is an important conditional pathogen involved in device related infection due to biofilm formation activity (1). The formation of epidermal staphylococcus biofilm is a dynamic process (30). Firstly, the bacteria surface hydrophobic protein or polysaccharide adhesion to the host organism. The initial attachment of the material forms the bacterial community; then the bacterial cells gather together to build biofilm structure (30). Among them, PIA is a bacterial biofilm material which is necessary for the aggregation stage (30). *IcaA* is the first gene of the operon and plays a decisive role in the biosynthesis of PIA, and could be a possible target for antibiofilm formation of *S. epidermidis*. Blocking the expression of the biofilm formation related gene requires by the inhibition of QS mechanism and biosynthesis of polysaccharides and extracellular proteins of biofilm compositions (31). Currently, Macrolide antibiotics including erythromycin and vancomycin are commonly anti-staphylococci agents in clinic by repression protein biosynthesis of bacterial cell, but also easily to induce the antibiotic resistance of staphylococci (32), such as medical device infection related *S. epidermidis* strains. Therefore, it is important to seek effective anti-biofilm drugs or multi-drug combination application in traditional alternative medicines in order to achieve high drug efficiency against *S. epidermidis* biofilm and reduce toxicity of drug treatments. Erythromycin has lower adverse reaction and price than the third line antibiotic, vancomycin (32). Thus, we used erythromycin as positive control of antimicrobial agents and selected combination agents in this research.

Recently, SH are found to inhibit the biofilm formation of *P. aeruginosa*, *S. aureus* and *C. albicans* in vitro (20-25), but the detail mechanisms remains unclear. Previously, we found that SH in combination with erythromycin groups at sub-inhibitory concentrations can effectively inhibit the biofilm formation of *S. epidermidis* at adhesion and maturation stages (26, 27). Furthermore, we have explored the effect of SH in combination with erythromycin on QS system which regulates biofilm system of *S. epidermidis*. The results (27) indicated that SH in combination with erythromycin can quickly up-regulate expression of *luxS* at adhesion stage, and significantly reduce the expression of *agr* and *RNAIII* at adhesion and maturation stages. These three genes code the protein and RNA which are key members of quorum sensing system of *S. epidermidis*. Notably, LuxS can inhibit biofilm formation of *S. epidermidis* via inactivation of

gene expression of *icaADBC* operon by an *icaR*-activation pathway (33, 34). Therefore, our previous results suggested that SH may affect QS system to repress the biofilm formation of *S. epidermidis*.

Here, our presented results indicated that the morphology of the biofilm cells of *S. epidermidis* were destructed significantly by SH alone and in combination with erythromycin at sub-inhibitory concentrations. Furthermore, the qRT-PCR data showed that the gene expression *icaA* of *S. epidermidis* was significantly repressed by SH alone and in combination with erythromycin at sub-inhibitory concentrations. Because SH are proved to affect quorum sensing system in bacteria (25). Thus, the inhibitory effects of SH against *S. epidermidis* may be cell density and anti-QS dependent of promotion in the early stage and repression in the maturation stage to cause the intriguing effects of SH against transcript levels of *icaA* at 6 and 24 hours of exposure. And the interesting effects of SH counteracts the inhibitory action of erythromycin at the time point of 6 hour but amplifies inhibition at the 24 hour time may also due to the QS dependent reason. Consistent with the gene expression results, production of IcaA is also significantly inhibited by SH alone and in combination with erythromycin at sub-inhibitory concentrations, especially at maturation stage of biofilm formation of *S. epidermidis*. Based on our previous results of repressing of quorum sensing system of *S. epidermidis* by SH in combination with erythromycin (27), the down-regulation of *icaA* may be due to the repressing of quorum sensing system of *S. epidermidis* by combing SH and erythromycin. Furthermore, reducing production of IcaA may lead to the decreasing of biofilm matrix PIA, and result in decreasing of formation of biofilm. Additional in vivo experiments will be required to assess the future applicability of SH in combination with erythromycin, and effective activity of combing SH and erythromycin in against biofilm formation of bacteria holds great promise."

Q8- Please also suggest the way that sodium houttuyfonate reduce the expression of gene and also decrease the formation of biofilm.
Response: Yes, we agree with the suggestions of reviewer. We explained the way in the discussion section of revised manuscript as follows: "Based on our previous results of repressing of quorum sensing system of S. epidermidis by SH in combination with erythromycin (27), the down-regulation of icaA may be due to the repressing of quorum sensing system of S. epidermidis by combing SH and erythromycin. Furthermore, reducing production of IcaA may lead to the decreasing of biofilm matrix PIA, and result in decreasing of formation of biofilm."

Q9- Please complete the introduction part using the explanation about the characters of the studied gene and also sodium houttuyfonate.
Response: Yes, we are grateful for the advices of reviewer. We revised the introduction part using the explanation about the characters of the *icaA* gene and also sodium houttuyfonate as follows:
"Staphylococcus epidermidis is an abiding gram positive bacterium of the normal human microbiota, usually found on mucous membranes, and skin (1). S. epidermidis can establish a lifelong commensal relationship with its host that begins from born by adhering to tissue surface moieties of the mucous membranes and skin via specific adhesions. Although commensal S. epidermidis strains display high rates of antibiotics resistance, their features as commensal

bacteria renders this phenomenon largely unconcerned with the normal healthy host (2). However, S. epidermidis has become an important opportunistic pathogen, causing infections on medical implants such as contact lenses, urinary catheters, prosthetic heart valves, central venous catheters and orthopedic devices in specific person which is hard to cure (3-5).

First step in biofilm formation is the capability to adhere to the surface of implant, commonly believed to be the most dominant virulence factor of S. epidermidis. Biofilm formation of S. epidermidis facilitates resistance against host immune system (6-8) and confers resistance of antibiotic (6, 9). Medical and surgical treatment protocols of implant medical device are also complicated by biofilm formation due to remove the biofilm. Biofilms of microorganism are defined as intricate communities of adherent microorganism cells enclosed in a matrix of self-biosynthesized extracellular polymeric substances (10). The adhesion and maturation of the S. epidermidis biofilm occurs by several mechanisms. Polysaccharide intercellular adhesin (PIA), synthesized by icaADBC coding enzymes (11) is responsible for biofilm formation in the most S. epidermidis strains and was proved to be the most common macromolecule associated with the adhesion and maturation of S. epidermidis biofilm (12). This was demonstrated by the finding that the ica operon was absent in most non-pathogenic S. epidermidis isolates (13, 14). IcaA, which is an N-acetylglucosaminyltransferase that synthetizes PIA oligomers from UDP-N-acetylglucosamine, exert a primary role in the PIA synthesis to form biofilm (12). Thus, IcaA is a potential candidate of target of antibiofilm agents against S. epidermidis.

In China, Houttuynia cordata Thunb (Saururaceae family) is an edible medicinal vegetable which has been widely applied in traditional Chinese medicine for centuries to cure plenty of inflammatory and infectious diseases (15, 16). Sodium houttuyfonate [SH, $CH_3(CH_2)_8COCH_2CHOHSO_3Na$], is a product of addition reaction of sodium bisulfite and houttuynin [decanoyl acetaldehyde, $CH_3(CH_2)_8COCH_2CHO$] which is the most important active constituent of the volatile oil derived from H. cordata (17). Thus, SH is commonly used for treating chronic bronchitis, respiratory tract and purulent skin infections (17). Recently, SH are found to inhibit LPS-induced mastitis in mouse via the NF-κB signaling pathway (18) and alleviate post-infarct remodeling in rat depending on AMP-activated protein kinase pathway (19). However, despite its effective and widespread medical applications especially antimicrobial application, the detail mechanism of antimicrobial effects of SH remains unknown.

Previously, our group found that SH is promising effective at repressing pathogenic related biofilm formation and motility of P. aeruginosa (20-22), and can inhibit S. epidermidis and Candida albicans (20, 23), and act synergistically with Na2-EDTA and levofloxacin against biofilm formation of P. aeruginosa, S. aureus and C. albicans (23, 24). In addition, we found that SH is a possible natural analog of N-acyl-homoserine lactone, and can effectively inhibit the Las quorum sensing (QS) system and regulated virulence factors of P. aeruginosa (25). However, due to the high resistance of antimicrobial agents of biofilm, we found that SH alone cannot effectively inhibit the biofilm formation of S. epidermidis. Thus, we have chosen the commonly used macrolide antibiotic, erythromycin to combine with SH to inhibit the biofilm formation of S. epidermidis. Interestingly, our previous results indicate that SH alone can only mildly inhibit the biofilm formation of S. epidermidis, and SH in combination with erythromycin can significantly inhibit the biofilm formation of S. epidermidis, and up-regulate the transcription of luxS, and down-regulate the expression of agr/RNA III which are members of regulatory QS system involved in biofilm formation of S. epidermidis (26, 27). However, the mechanism of SH in

combination with erythromycin against biofilm development cycle and PIA biosynthesis especially the adhesion and maturation stages of S. epidermidis remains unclear."

Q10- Additionally, add some explanation about the fact that why other surveys cannot solve the issue of biofilm formation which caused that you and your colleague performed this research.
Response: Yes, we have made the explanation in the revised manuscript as follows: "… However, due to the high resistance of antimicrobial agents of biofilm, we found that SH alone cannot effectively inhibit the biofilm formation of S. epidermidis. Thus, we have chosen the commonly used macrolide antibiotic, erythromycin to combine with SH to inhibit the biofilm formation of S. epidermidis… However, the mechanism of SH in combination with erythromycin against biofilm development cycle and PIA biosynthesis especially the adhesion and maturation stages of S. epidermidis remains unclear."

Q11- Some of your references were old. You should replace them with some novel references.
Response: Yes, we have replaced the No. 7, 10, 11, 12 of old references into newly related published references in the revised manuscript as follows:
"7. Paharik AE, Horswill AR. The Staphylococcal biofilm: adhesins, regulation, and host response. Microbiol Spectr. 2016;4(2). doi:10.1128/microbiolspec.VMBF-0022-2015. [PubMed:27227309]. [PubMed Central: PMC4887152].
10. Buttner H, Mack D, Rohde H. Structural basis of Staphylococcus epidermidis biofilm formation: mechanisms and molecular interactions. Front Cell Infect Microbiol. 2015;5:14. doi:10.3389/fcimb.2015.00014. [PubMed:25741476]. [PubMed Central: PMC4330918].
11. Arciola CR, Campoccia D, Ravaioli S, Montanaro L. Polysaccharide intercellular adhesin in biofilm: structural and regulatory aspects. Front Cell Infect Microbiol. 2015;5:7. doi:10.3389/fcimb.2015.00007. [PubMed:25713785]. [PubMed Central: PMC4322838].
12. Rohde H, Frankenberger S, Zahringer U, Mack D. Structure, function and contribution of polysaccharide intercellular adhesin (PIA) to Staphylococcus epidermidis biofilm formation and pathogenesis of biomaterial-associated infections. Eur J Cell Biol. 2010;89(1):103-11. doi:10.1016/j.ejcb.2009.10.005. [PubMed:19913940]. "

Q12- Is there any ethical consideration for your study? Please add it.
Response: No, there no ethical consideration in our in vitro study. We have added in the revised manuscript as follows: "Ethical Approval: This is an in vitro study and ethical approval was not required."

Q13- Please explain about the role of sodium houttuyfonate on biofilm formation of some rough strains or clinically isolated bacteria.
Response: Yes, we have made the explain the role of sodium houttuyfonate on biofilm formation of some rough strains or clinically isolated bacteria in the revised introduction section as follows: "Previously, our group found that SH is promising effective at repressing pathogenic related biofilm formation and motility of P. aeruginosa (20-22), and can inhibit S. epidermidis and Candida albicans (20, 23), and act synergistically with Na2-EDTA and levofloxacin against biofilm formation of P. aeruginosa, S. aureus and C. albicans (23, 24). In addition, we found that SH is a possible natural analog of N-acyl-homoserine lactone, and can effectively inhibit the Las

quorum sensing (QS) system and regulated virulence factors of P. aeruginosa (25)."

Q14- Are there any survey conducted on the role of sodium houttuyfonate on biofilm formation of other bacteria? Please add them.
Yes, we have made the description in the revised introduction section as follows: "Previously, our group found that SH is promising effective at repressing pathogenic related biofilm formation and motility of P. aeruginosa (20-22), and can inhibit S. epidermidis and Candida albicans (20, 23), and act synergistically with Na2-EDTA and levofloxacin against biofilm formation of P. aeruginosa, S. aureus and C. albicans (23, 24).". We also have added the description in the discussion section as follows: "Recently, SH are found to inhibit the biofilm formation of P. aeruginosa, S. aureus and C. albicans in vitro (20-25), but the detail mechanisms remains unclear."

Q15- Please complete the conclusion with some more relevant information about the exact finding of your survey and also their application in microbiology.
Response: Yes, according to the suggestions of reviewer, we have revised the conclusion section as follows: "Our results indicate that the potent antimicrobial activity of natural plant products, SH, in combination with erythromycin may be partially due to its inhibitory effect on biofilm formation in S. epidermidis via inactivation of icaA. Therefore, our findings may provide a new possible option of treatment of medical device related infection by S. epidermidis biofilm, which is combing SH and erythromycin. Furthermore, the above results also imply that IcaA could be a potent drug target in the exploring new treatment application against biofilm formation of S. epidermidis."

Q16- What are the main applications of your research in the field of microbiology based on the clinical view.
Response: Yes, we have added the main applications of your research in the field of microbiology based on the clinical view in the revised conclusion section as follows: ". Therefore, our findings may provide a new possible option of treatment of medical device related infection by S. epidermidis biofilm, which is combing SH and erythromycin. Furthermore, the above results also imply that IcaA could be a potent drug target in the exploring new treatment application against biofilm formation of S. epidermidis."

Q17- (Figure …) should change to (figure …). Make it throughout the text.
Response: Yes, we have changed (Figure …) into (figure …) in the revised manuscript.

Q18- Some of references attached in [] format and some other attached in () format. Please make them unique based on the instruction of the journal.
Response: Yes, we have changed [] format of attached references into () format in the revised manuscript.

Q19- Please also unique the style of references throughout the references lists.
Response: Yes, we have corrected the style of references throughout the references lists according to the instruction and publication articles of JJM.

Q20- Table should change to table in the section of gene expression analysis and throughout the text.

Response: Yes, we have moved the table 1 into the section of gene expression analysis and throughout the text of revised manuscript.

Q21- Why authors used from erythromycin? How about other antibiotic agents? Please add your reasons into the discussion.

Response: Yes, we are grateful for the advices of reviewer. Due to the high efficiency, adverse reaction and price of erythromycin, we have selected the erythromycin as combination drug. We have made the explanation in the discussion section of the revised manuscript as follows: "Currently, Macrolide antibiotics including erythromycin and vancomycin are commonly anti-staphylococci agents in clinic by repression protein biosynthesis of bacterial cell, but also easily to induce the antibiotic resistance of staphylococci (32), such as medical device infection related S. epidermidis strains. Therefore, it is important to seek effective anti-biofilm drugs or multi-drug combination application in traditional alternative medicines in order to achieve high drug efficiency against S. epidermidis biofilm and reduce toxicity of drug treatments. Because, commonly used antibiotic, erythromycin, has lower adverse reaction and price than the third line antibiotic, vancomycin (32). Thus, we used erythromycin as positive control of antimicrobial agents and selected combination agents in this research."

OPEN PEER REVIEW

Revision (1)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

REFEREE: EIC | **Revision (1)** 1 Dec 2019

Dear authors
According to the associated editor report, your manuscript is accepted for publishing in JJM and send to the publisher for the next process.
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Please add ORCID number into you and your collages account, then automatically appear in your published papers in JJM both in HTML and pdf. Pleases follow the below link for the process. http://single.demo.pub/en/knowledgebase/category/tree.html#orcid.html
Thanks.