

September 29, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 12239-review.doc).

Title: «Patents on antivirulence therapies»

Author: María López, Beathriz Barbosa, Eva Gato, Germán Bou, and María Tomás

Name of Journal: World Journal of Pharmacology

ESPS Manuscript NO: 12239

Answering Reviewers

1) REFEREE 1

Ok

2) REFEREE 2

All changes have been added.

3) REFEREE 3

1. Please provide a definition of 'virulence' at the beginning of the article.

Ok, it has been added (Line 61)

2. Please state which patent databases were searched, which search terms were used and which years were included in the search will **stop**.

References number: 6- European Patent Office and 7- United States Patent and Trademark Office.

3. Is antibiotic resistance actually a virulence factor? I think you are mixing up "multidrug resistance" and "antimicrobial resistance". If you want to discuss patents on antimicrobial resistance then you will have to include patents that involve the development of antibiotics.

The relationship between antibiotic resistance and virulence is ever closer (Beceiro et al., Clin Microbiol Review 2013).

In this review, we include as virulence factor the efflux pump systems, which are responsible of the resistance to several antibiotics and as well in the microbial virulence. For this, we include the patents about inhibitors of the efflux pumps systems (Amaral et al., Frontiers in Pharmacology 2014).

4. Line 83 should read "multidrug resistance" and not "antimicrobial resistance" (see also above).

The efflux pumps are implicated in the resistance to several antibiotics and disinfectants (drugs) for this that it is better to use the word "drugs".

5. In the quorum sensing chapter - from my understanding AI-1 are acyl homoserine lactones, AI-2 are heterocyclic furanosyl-borates, AI-3 are catecholamines and AI-4 cyclic peptides

Ok, it has been modified (Lines 152-157).

6. Please add 1 to 2 sentences on the five main families of efflux pumps i.e. the MATE, MFS, SMR, RND and ABC (super) families. I presume that you have searched the patents related to all of these different types of efflux pumps?

Of course. All inhibitors of efflux pumps classes has been studied by patents a A small introduction about the efflux pumps have been included (Lines 201-207).

7. Line 187 to 189 - "Furanones, which are naturally occurring compounds, appear to be the most widely studied quorum quenching compounds. However, they are toxic to artemias and rotifers, which will limit their use" - I do not understand the significance of this sentence. If anti-quorum sensing compounds are to be used in preventing infections in animals and plants, then the use of such compounds in a wide variety of settings should be stressed in the introduction to the quorum sensing chapter.

Ok, the sentence has been modified. (Lines 183-187)

8. Line 191 - 193 - "Other compounds involved in QQ include honaucin A, 2-methylthiopyrrolidines, lovastatin and hydroxytyrosol. Finally, one enzyme (OLB-26) is known to be involved in QQ." - So what is the conclusion? Are there any patents associated with these compounds?

Yes, the sentence has been improved for greater understanding.(Lines 187-190)

9. The chapter "Future prospects: resistance to antivirulence compounds" only deals with quorum sensing compounds. Therefore this chapter should be incorporated within the quorum sensing chapter (without a title). It would also be interesting to know if resistance to any of the other mechanisms mentioned in the paragraphs of this manuscript had been described. This information can then be added to the end of the relative chapter.

Vibrio cholerae virstatin resistant strains have been described. It has been explained in text (Lines 231-233) Reference: Shakhmovich EA et al., Mol Microbiol 2007

10. Line 141 - Change "Search of the patent database (up to April 2014)..." to " A search of the patent database (up to April 2014)..."

Ok, changed it

11. Line 169 - Change "Numerous quorum sensing inhibitors are now begin to be reported in the literature" to "Numerous quorum sensing inhibitors have been reported in the literature"

Ok, changed it

12. In the concluding remarks, you mention nothing of the virulence mechanisms that are explained in this manuscript! For example the authors state "It is therefore imperative to develop new molecules, therapies and / or new combinations of these for the eradication of these pathogens." This sentence should be followed by 2 to 4 sentences giving examples based on the work presented manuscript

Ok, it has been added (Lines 268-272)

13. Figure 1 - I do not understand why are you are showing antibiotics resistance mechanisms here. Further, there are no chapters specifically relating to antibiotic resistance. As previously mentioned, if you include a chapter on antibiotics resistance and you have to include patents on antibiotics, which would involve a lot of extra work! In any case, I am not convinced that antimicrobial resistance is a virulence mechanism. Figure 1 should show the effect of quorum sensing molecules on global cellular virulence factor production and microbial growth

I agree with you, new Figure has been worked.

14. You do not say anything about proteases, one of the most important virulence factors that bacteria possess. For example, https://www.google.com/patents/US20070231334?dq=protease+inhibitors+virulence&hl=nl&sa=X&ei=eQGxU66WHcPSPO_6gMAP&ved=0CFQQ6AEwBg

In this review paper, we provide details about patents concerning inhibition of virulence mechanisms except specific toxins (proteases) produced by pathogen determined. It has been described in Anthrax infection and Clostridium spp. (Rasko et al., Nature Reviews 2010) Lines 73-77

15. You should add a chapter describing patents relating to therapy u....

¿?

It is difficult to include in the text patents about inhibition of the toxins(proteases), too much information