

January 21, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 1527-review.doc).

Title: Virulence factors in *Enterococcus* strains isolated from patients with Inflammatory Bowel Disease.

Author: Golińska E, Tomusiak A, Gosiewski T, Więcek G, Machul A, Mikołajczyk D, Bulanda M, Heczko PB, Strus M.

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 1527

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

Introduction:

- (1). Right. This was rejected.
- (2). More information about involvement of virulence factors in pathomechanisms of enterococcal infection + appropriate literature was added.
- (3). Done.

Materials and Methods:

- (1). Explained.
- (2). Information added.
- (3). Reference to the standard procedure used in our university hospital is given. We do not think that it is worthy to discuss such a standard preparation of children before colonoscopy commonly used in children gastroenterology.
- (4). It is stated in 4 sentence of Bacterial strains para in M&M section.
- (5). Added.
- (6). Yes, we do counting of bacteria per field and then categorize their numbers. Appropriate reference was added.
- (7). This issue is discussed in Discussion (hope it was also before).

Results:

- (1). This was described in detail and two more sentences added.
- (2). The sentence was removed, since the same information is given in Results section.
- (3). Yes, an information about PFGE typing and cluster analysis was added, as per item 1.
- (4). Appropriate mean values + comment were added to this para.
- (5). Our group developed a standard method to measure hydrogen peroxide decomposition by lactobacilli producing catalase and published several paper on that subject. The same method was used here. A borderline of 1 mM H₂O₂/4 hours was therefore arbitrarily chosen on the basis of the previous studies (see:

Strus M, Brzychczy-Włoch M, Gosiewski T, Kochan P, Heczko PB. The in vitro effect of hydrogen peroxide on vaginal microbial communities. FEMS Immunol Med Microbiol. 2006 Oct;48(1):56-63 and related papers).

- (6). Distribution of the tested genes in *E.faecalis* and *E.faecium* was equal; *E.avium* had less genes but there were no significant differences found.
- (7). No, there were no significant statistical differences.
- (8). A new para on that was added to Discussion.
- (9). We believed that it is so but no statistical link was found. The matter is far more complicated since enterococci are able to produce at least three enzymes deactivating ROS while their ability to produce extracellular hydrogen peroxide is highly dependent on oxygen tension.

Discussion:

- (1) Faeces contains predominantly planktonic microflora which has no direct contact with gut mucosa and therefore in human studies only samples obtained by biopsy are commonly taken and regarded as containing microbes adherent to mucosa (or underlying tissue in IBD ulcerations) and colonizing it. Therefore biopsies were taken both from children with IBD and children from control group. It is stated in M&M.
- (2) More information about origins of the strains was added. Caveats related to in vitro vs. in vivo situations are in a new para as described above [Results, 8].
- (3) The matter is highly speculative. If large aggregates form more biofilm and are embedded in its matrix they should not be removed as easily as single cells.
- (4) Corrected, although enterococci are in a common sense also streptococci.
- (5) Activated macrophages are typical cells present in inflammatory infiltrate in IBD lesions as well as in other inflammatory conditions [like as endocarditis].
- (6) The sentence and both citations were rejected.
- (7) Word „random“ was rejected although it is obvious that colonization is a random process: in a given time only a few bacteria of the same clone find free receptors on a host surface and adhere while the rest is lost since the receptors are already blocked. The sentence was also changed.
- (8) Yes, bleeding from gut ulcerations is common in IBD children.
- (9) Both paragraphs were abbreviated and merged.
- (10) Explained and changed.
- (11) As above.
- (12) This sentence was rejected
- (13) It is discussed above on the basis of our observations [Strus et al. (14)] and paper of Rezaie et al. [35].
- (14) Reference 35 but not 31.
- (15) The whole paragraph on oxidative stress and IBD and the role of hydrogen peroxide producing bacteria in this mechanism was rewritten. Please also notice a new para on in vitro – in vivo relations as above.
- (16) One reference added and one corrected [Denning et al., Kruidenier et al.].

- (17) Corrected. Re in vitro – in vivo, again, please look at new paragraph as above and observe that this paper is based on in vitro but not vivo studies but this should not limit our discussion on implications of in vitro studies on disease pathomechanisms.
- (18) Corrected.
- (19) Disagree: „Efficient“ according to Webster’s dictionary means „causing effects“. This expression should not be defined. Objections of the reviewer that not enough isolates were tested are subjective: who is able to judge if it should be 10 or 100 strains. Our data were supported with statistical analysis showing significant differences.
- (20) The expression „assume“ was changed but we think that a spiteful remark of the reviewer on scientist mission was not objective. We also added a citation supporting our observations to be published in JCM.
- (21) Right: „May“ is used now instead of „seem to“. To answer the question of the reviewer how do we know that bacterial biofilm is 3D I suggest reading following paper:

Beyenal H, Donovan C, Lewandowski Z, Harkin G. Three-dimensional biofilm structure quantification. J Microbiol Methods. 2004 Dec;59(3):395-413.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,



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February 26, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 1527-review.doc).

Title: Virulence factors in *Enterococcus* strains isolated from patients with Inflammatory Bowel Disease.

Author: Edyta Golińska, Anna Tomusiak, Tomasz Gosiewski, Grażyna Więcek, Agnieszka Machul, diana Mikołajczyk, Małgorzata Bulanda, Piotr B Heczko, Magdalena Strus.

Name of Journal: *World Journal of Gastroenterology*

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Answers to the reviewer:

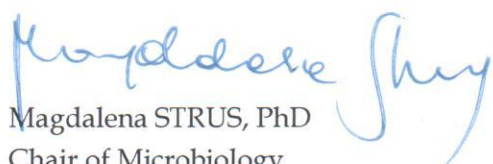
Many thanks for your efforts spent to increase scientific level and understanding of our paper. Below are our answers

- 1) Statistics section was re-written in order to clarify how our calculations were made.
- 2) The word "subdue" was incorrect. Therefore, this sentence was changed and appropriate citation was added.
- 3) We followed your suggestions.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,



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