RESEARCH NOTE

*In silico* analysis suggests repurposing of ibuprofen for prevention and treatment of EBOLA virus disease [version 1; peer review: 2 approved]

Veljko Veljkovic¹, Marco Goeijenbier², Sanja Glisic¹, Nevena Veljkovic¹, Vladimir R. Perovic¹, Milan Sencanski¹, Donald R. Branch³, Slobodan Paessler⁴

¹Center for Multidisciplinary Research, Institute of Nuclear Sciences Vinca, University of Belgrade, Mihajla Petrovica 12-14, 11001 Belgrade, Serbia
²Department of Viroscience, Erasmus MC, Rotterdam, The Netherlands
³Canadian Blood Services, Center for Innovation, 67 College Street, Toronto, M5G 2M1, Canada
⁴Department of Pathology, Galveston National Laboratory, University of Texas Medical Branch, 301 University Boulevard, Galveston, TX, USA

Abstract

The large 2014/2015 Ebola virus outbreak in West Africa points out the urgent need to develop new preventive and therapeutic approaches that are effective against Ebola viruses and can be rapidly utilized. Recently, a simple theoretical criterion for the virtual screening of molecular libraries for candidate inhibitors of Ebola virus infection was proposed. Using this method the ‘drug space’ was screened and 267 approved and 382 experimental drugs as candidates for treatment of the Ebola virus disease (EVD) have been selected. Detailed analysis of these drugs revealed the non-steroidal anti-inflammatory drug ibuprofen as an inexpensive, widely accessible and minimally toxic candidate for prevention and treatment of EVD. Furthermore, the molecular mechanism underlying this possible protective effect of ibuprofen against EVD is suggested in this article.

Keywords
drug space, NSAID, molecular libraries

This article is included in the Disease Outbreaks gateway.
Corresponding author: Veljko Veljkovic (vv@vinca.rs)

Competing interests: No competing interests were disclosed.

Grant information: This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant no. 173001).

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Copyright: © 2015 Veljkovic V et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. Data associated with the article are available under the terms of the Creative Commons Zero "No rights reserved" data waiver (CC0 1.0 Public domain dedication).

How to cite this article: Veljkovic V, Goeijenbier M, Glisic S et al. In silico analysis suggests repurposing of ibuprofen for prevention and treatment of EBOLA virus disease [version 1; peer review: 2 approved] F1000Research 2015, 4:104 (https://doi.org/10.12688/f1000research.6436.1)

First published: 01 May 2015, 4:104 (https://doi.org/10.12688/f1000research.6436.1)
Introduction
The recent Ebola virus outbreak in West Africa caused (as of April 8, 2015) a total of 25,556 confirmed cases, including 10,587 deaths (World Health Organization, Ebola data and statistics). Although reports of new, confirmed cases of Ebola seemed to decrease in April of 2015 to approximately 30 cases per week, medical and aid organizations are warning that the current crisis is not over. Furthermore, there remains a risk for future epidemics. Therefore, there is an urgent need to develop new preventive and therapeutic approaches that can be rapidly utilized. New drugs for the treatment of EVD should be safe, efficacious, easy to manufacture and inexpensive in order to be successfully deployed in African countries. In contrast to the significant progress recently achieved in development of an effective Ebola virus vaccine, therapeutic options are still limited. The main obstacle represents identification of an appropriate therapeutic target, which has been largely hampered by the time and money-consuming development of new drugs, especially for neglected diseases. Furthermore, the registration of newly identified drugs, like favipiravir, for potential usage in EVD patients takes a relatively long period of time, even though it has been shown to be effective in non-human primate models. In order to avoid these obstacles, several approaches for repurposing of approved drugs for the treatment of EVD patients have been proposed in literature\(^1\).

Recently, we proposed a relatively simple theoretical criterion for the fast virtual screening of molecular libraries for candidate inhibitors of Ebola virus infection\(^1\). Using this criterion, which is based on calculation of the average quasi-valence number (AQVN) and the electron-ion interaction potential (EIIP) - parameters determining long-range interaction between biological molecules\(^1\) - we selected 267 approved and 382 experimental drugs as candidates for treatment of EVD. Further detailed analysis of these drugs, including molecular docking, revealed ibuprofen as an inexpensive, widely accessible and minimally toxic candidate for potential prevention and treatment of EVD. The molecular mechanism underlying the possible protective effect of ibuprofen against EVD is suggested.

Material and methods
AQVN and EIIP molecular descriptors
Recently, we proposed a theoretical criterion for selection of candidate inhibitors of Ebola virus infection\(^1\). This criterion is based on the calculation of EIIP and AQVN which are determined by following equations:

\[
Z^* = \frac{1}{N} \sum_{i=1}^{m} n_i Z_i \tag{1}
\]

where:
- \(i\) - Type of the chemical element
- \(Z_i\) - Valence of the \(i\)-th chemical element
- \(n_i\) - Number of the \(i\)-th chemical element atoms in the compound
- \(m\) - Number of types of chemical elements in the compound
- \(N\) - Total number of atoms

The EIIP values calculated according to the equation (2) are in Rydbergs (\(\text{Ry} = 13.6 \text{ eV})\).

\[
EIIP = 0.25 \sin(1.04 \pi Z^*) \tag{2}
\]

The AQVN and EIIP molecular descriptors determine the long-distance (>5Å) intermolecular interactions in biological systems\(^1\). This approach showed that molecules which potentially block Ebola virus infection are placed within AQVN range (2.3–2.7) and EIIP range (0.829–0.954 Ry), respectively. Using this theoretical criterion the drug library encompassing 267 approved drugs selected as candidate inhibitors of the Ebola virus infection (Candidate Ebola Drugs Database, CEDD) was established\(^1\). This drug library was used for selection of an approved drug, which represents the optimal candidate for prevention and treatment of EVD.

GP1 receptor modelling
The modelling of glycoprotein GP1 from Ebola virus was previously described in\(^16\).

Ligand optimization
Ligands were built in VEGA ZZ\(^17\), protonated according to physiological conditions and optimized on semi empirical PM6 level of theory using MOPAC 2009.

Molecular docking
Ligand and receptor were prepared in VEGA ZZ\(^17\). The docking was carried out with Autodock Vina (version 1.1.2.)\(^18\). In both cases the whole receptor conformational space was searched, using grid boxes with dimensions 60×60×60 and 30×30×30Å\(^3\). The docking was carried out with weighting of hydrophilic interactions, and the corresponding parameter value in the docking configuration was set to -1.20 (compared to default weight: hydrogen = -0.587439). The exhaustiveness was set to 250. The conformations with lowest binding energy values were chosen. The calculations were carried out on the PARADOX Cluster computer.

Results and discussion
We screened the CEDD library\(^15\) for optimal candidates for prevention and treatment of EVD. To achieve this end, we used the following criteria: a) high efficacy of binding of drug to the Ebola virus glycoprotein GP1; b) low toxicity and accessibility (nonprescription drugs); and c) low cost. By mining CEDD using these criteria, we selected ibuprofen as the best candidate. Ibuprofen is a nonsteroidal anti-inflammatory drug (NSAID). Its mode of action, as for other NSAIDs, is not completely understood, but may be related to prostaglandin synthetase inhibition. It achieves this effect on prostaglandin synthesis by inhibiting cyclooxygenase (COX I and II isotypes), an enzyme that is present in at least one isoform most of the tissues of the body and which is responsible for production of not only prostaglandins but also prostacyclins and tromboxane.

Generally, ibuprofen is minimally toxic drug. A meta-analysis of eight placebo-controlled studies showed that application of non-prescription doses of ibuprofen (800–1200 mg/day) over 10 days caused no significant adverse events\(^19\). Similar results were obtained.
in a prospective study of ibuprofen use in healthy volunteers taking the maximum permitted, non-prescription dose of ibuprofen (1200 mg/day) for 10 days\(^6\). It was demonstrated that administration of ibuprofen in children is safe. A randomized, community-based study on the safety of ibuprofen in febrile children aged 2 years old (5 or 10 mg/kg) showed that the risk of hospitalization for gastrointestinal bleeding associated with this drug was 17 per 100,000\(^3\). It was also shown that fecal blood loss associated with application of prescription doses of ibuprofen (2400 mg/day) did not exceed the normal range\(^22\). Even in the case of post-surgical usage for pain control in children undergoing tonsillectomy, ibuprofen administration is considered to be safe and does not increase risk of hemorrhages\(^23\). Accordingly, we believe that it would be crucial to better understand the potential risk and benefits of ibuprofen usage in experimental models of EVD. Taken together, these data indicate that ibuprofen could be safely used in nonprescription doses in EVD patients, with potential antiviral effects as well as to alleviate the symptoms.

To assess specificity of ibuprofen as a potential inhibitor of the Ebola virus infection, distribution of all approved NSAIDs in AQVN/EIIP space was analyzed. Results presented in (Table 1 and Figure 1) show that only ibuprofen and its isomer dexibuprofen are located within the domain of the AQVN/EIIP space. Interestingly, most of the experimentally verified inhibitors of the Ebola virus infection are located in the same space (e.g. chloroquine, amodipine, brincidofovir, etc)\(^5\). This suggests that ibuprofen and dexibuprofen are the only drugs from the NSAID group that potentially have anti-Ebolavirus effects, which should be tested both in vitro and in vivo.

A previous in silico study suggested that Elastin Microfibril Interface Located Proteins (EMILINs) are involved in interaction between GP1 and endothelial extracellular matrix (ECM)\(^12\). Docking of ibuprofen to the GP1 model gave conformations which bound to EMILINs binding domain on GP1\(^14\). The binding site of ibuprofen on GP1 spans the edge of this region and consists of Thr 338, Ser 340, Gln 344 and Ala 415. The intermolecular interactions between ibuprofen and binding site amino-acids include hydrogen bonds of carboxyl group with Thr 338, Ser 340, Gln 344 and Ala 415. The binding site of ibuprofen to the GP1 model gave conformations which bound to the GP1/EMILIN-1 interaction allowing EMILIN-1 to keep control of TGF-β1 signaling pathway. These findings suggest the possibility that binding of GP1 to EMILIN-1 prevents its interaction with TGF-β1, which results in activation of TGF-β1 signaling pathway. Binding of ibuprofen to GP1 could prevent GP1/EMILIN-1 interaction allowing EMILIN-1 to keep control of TGF-β1 signaling pathway.

These findings suggest the possibility that binding of GP1 to EMILIN-1 prevents its interaction with TGF-β1, which results in activation of TGF-β1 signaling pathway. Binding of ibuprofen to GP1 could prevent GP1/EMILIN-1 interaction allowing EMILIN-1 to keep control of TGF-β1 signaling pathway.

<table>
<thead>
<tr>
<th>NSAID</th>
<th>Formula</th>
<th>AQVN</th>
<th>EIIP [Ry]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin</td>
<td>C9H8O4</td>
<td>3.238</td>
<td>0.1179</td>
</tr>
<tr>
<td>Diflunisal</td>
<td>C13H8F2O3</td>
<td>3.077</td>
<td>0.0720</td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>C7H6O3</td>
<td>3.250</td>
<td>0.1202</td>
</tr>
<tr>
<td>Salsalate</td>
<td>C14H10O5</td>
<td>3.310</td>
<td>0.1296</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>C13H18O2</td>
<td>2.485</td>
<td>0.0954</td>
</tr>
<tr>
<td>Dexibuprofen</td>
<td>C13H18O2</td>
<td>2.485</td>
<td>0.0954</td>
</tr>
<tr>
<td>Naproxen</td>
<td>C14H14O3</td>
<td>2.839</td>
<td>0.0169</td>
</tr>
<tr>
<td>Fenoprofen</td>
<td>C15H14O3</td>
<td>2.875</td>
<td>0.0036</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>C16H14O3</td>
<td>2.909</td>
<td>0.0092</td>
</tr>
<tr>
<td>Dexketoprofen</td>
<td>C16H14O3</td>
<td>2.999</td>
<td>0.0925</td>
</tr>
<tr>
<td>Flurbiprofen</td>
<td>C15H13O2</td>
<td>2.774</td>
<td>0.0390</td>
</tr>
<tr>
<td>Oxaprozin</td>
<td>C18H15N3O3</td>
<td>2.973</td>
<td>0.0337</td>
</tr>
<tr>
<td>Loxoprofen</td>
<td>C15H18O3</td>
<td>2.667</td>
<td>0.0693</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>C19H16CIN4O4</td>
<td>2.976</td>
<td>0.0347</td>
</tr>
<tr>
<td>Tolmetin</td>
<td>C15H15N3O3</td>
<td>2.882</td>
<td>0.0008</td>
</tr>
<tr>
<td>Sulindac</td>
<td>C20H17FO3S</td>
<td>2.905</td>
<td>0.0076</td>
</tr>
<tr>
<td>Etodolac</td>
<td>C17H21N3O3</td>
<td>2.667</td>
<td>0.0693</td>
</tr>
<tr>
<td>Ketorolac</td>
<td>C15H13N3O3</td>
<td>3.000</td>
<td>0.0439</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>C14H11C2N02</td>
<td>2.867</td>
<td>0.0067</td>
</tr>
<tr>
<td>Aceclofenac</td>
<td>C16H13C2N04</td>
<td>3.000</td>
<td>0.0439</td>
</tr>
<tr>
<td>Nabumetone</td>
<td>C15H16O2</td>
<td>2.667</td>
<td>0.0693</td>
</tr>
<tr>
<td>Piroxicam</td>
<td>C15H13N3O4S</td>
<td>3.277</td>
<td>0.1251</td>
</tr>
<tr>
<td>Meloxicam</td>
<td>C14H13N3O4S2</td>
<td>3.333</td>
<td>0.1319</td>
</tr>
<tr>
<td>Tenoxicam</td>
<td>C13H11N3O4S2</td>
<td>3.454</td>
<td>0.1317</td>
</tr>
<tr>
<td>Droxican</td>
<td>C16H11N3O5S</td>
<td>3.500</td>
<td>0.1260</td>
</tr>
<tr>
<td>Lornoxicam</td>
<td>C13H10CIN3O4S2</td>
<td>3.454</td>
<td>0.1317</td>
</tr>
<tr>
<td>Isoxicam</td>
<td>C14H13N3O5S</td>
<td>3.333</td>
<td>0.1319</td>
</tr>
<tr>
<td>Mefenamic acid</td>
<td>C15H15N2O2</td>
<td>2.788</td>
<td>0.0345</td>
</tr>
<tr>
<td>Meclomenamic acid</td>
<td>C14H11C2N02</td>
<td>2.867</td>
<td>0.0067</td>
</tr>
<tr>
<td>Fluemenamic acid</td>
<td>C14H10F3N02</td>
<td>2.867</td>
<td>0.0067</td>
</tr>
<tr>
<td>Tolmenamic acid</td>
<td>C14H12CIN02</td>
<td>2.867</td>
<td>0.0067</td>
</tr>
<tr>
<td>Celecoxib</td>
<td>C17H14F3N02S</td>
<td>2.950</td>
<td>0.0249</td>
</tr>
<tr>
<td>Rofecoxib</td>
<td>C17H14O4S</td>
<td>3.111</td>
<td>0.0835</td>
</tr>
<tr>
<td>Valdecoxi</td>
<td>C16H14N2O3S</td>
<td>3.111</td>
<td>0.0835</td>
</tr>
<tr>
<td>Parecoxi</td>
<td>C19H18N2O4S</td>
<td>3.046</td>
<td>0.0608</td>
</tr>
<tr>
<td>Lumaracoxib</td>
<td>C15H13CIFN02</td>
<td>2.788</td>
<td>0.0345</td>
</tr>
<tr>
<td>Etoricoxi</td>
<td>C18H15CIN2O2S</td>
<td>2.974</td>
<td>0.0342</td>
</tr>
<tr>
<td>Firocoxib</td>
<td>C17H20OSS</td>
<td>2.884</td>
<td>0.0003</td>
</tr>
<tr>
<td>Nimesulide</td>
<td>C13H12N2O5S</td>
<td>3.333</td>
<td>0.1319</td>
</tr>
<tr>
<td>Chlorain</td>
<td>C13H11CIN2N02</td>
<td>2.966</td>
<td>0.0308</td>
</tr>
<tr>
<td>Licofelone</td>
<td>C23H22CINO2</td>
<td>2.694</td>
<td>0.0626</td>
</tr>
</tbody>
</table>
In conclusion, presented results should encourage further investigation of ibuprofen and ibuprofen-inspired drugs as inexpensive, low-toxic and wide-accessible candidates for prevention and its usage in the treatment of EVD.

**Data availability**

F1000Research: Dataset 2. Approved and experimental drugs selected as candidate for treatment of EVD, 10.5256/f1000research.6110.d4287

**Author contributions**

VV, SP and MG conceived and designed the study. VP developed the analysis tools. VV, SG, NV, MS and DB analyzed the data. VV, SP and MG wrote the paper. All authors agreed to the final content of the manuscript.

**Competing interests**

No competing interests were disclosed.

**Grant information**

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant no. 173001).

I confirm that the funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**References**


Open Peer Review

Current Peer Review Status: ✔ ✔

Version 1

Reviewer Report 23 June 2015

https://doi.org/10.5256/f1000research.6904.r8796

© 2015 Darji A. This is an open access peer review report distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Ayub Darji
Centre for Research into Animal Health (CReSA), Autonomous University of Barcelona, Barcelona, Spain

Current manuscript by Veljkovic et al., deals with the identification of inhibitors against Ebola virus infection. Authors took advantage of existing approved and experimental drugs and, based on in silico analysis, proposed ibuprofen as a potential candidate for prevention and treatment of Ebola virus infection. EIIP and AQVN calculation, previously described by authors, was employed to define ibuprofen as the best candidate against Ebola virus infection.

The manuscript is adequately presented and can be considered as acceptable for indexation.

Minor points:

Ebola virus infection is initiated by interaction between the virus glycoprotein GP1 and its cognate receptor(s). Putative receptor binding domain of GP1, key residues involved in GP1 protein folding or structure and a putative receptor binding pocket has been proposed and mapped to the N-terminal 150 amino acids of GP1 (33-185 residues).

Authors should include in the discussion as how interaction of ibuprofen with GP1 (residues, Thr 338, Ser 340, Gln 344 and Ala 415) will influence the receptor binding domain.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 24 Aug 2015

Veljko Veljkovic, University of Belgrade, Mihajla Petrovica 12-14, 11001 Belgrade, Serbia

It was previously suggested that Elastin Microfibril Interface Located Proteins (EMILINs) are involved in interaction between GP and endothelial extracellular matrix (ECM) (Veljkovic et al. 2015)
1. The binding site of ibuprofen on GP overlaps with the proposed domain of GP involved in GP/EMILINs interaction. This suggests that ibuprofen could modulate the virus/ECM interaction.

**Competing Interests:** No competing interests were disclosed.

---

Reviewer Report 05 June 2015

https://doi.org/10.5256/f1000research.6904.r8913

© 2015 Mori M. This is an open access peer review report distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Mattia Mori

Center for Life Nano Science@Sapienza, Istituto Italiano di Tecnologia, Rome, Italy

In the work of Veljkovic et al., *in silico* methods were used with the aim of identifying inhibitors of Ebola virus infection. Particularly, authors focused their investigation on approved and experimental drugs and proposed ibuprofen as a candidate for prevention and treatment of Ebola virus. It is well known that Ebola virus represents an international health emergency and that there is an urgent need for therapeutics and prophylactic drugs.

In the present manuscript, authors applied a previously described and well-established criterion, based on EIIP and AQVN calculation, to select ibuprofen as the best candidate for Ebola virus disease treatment. Moreover, a mechanism of action consisting on GP1 inhibition has been proposed.

It is the opinion of the present referee that some minor issues should be fixed before the manuscript may be considered as acceptable for indexation:

1. Since molecular docking of ibuprofen was performed against the putative target GP1, it would be important to report the predicted binding energy (in kcal/mol) or score, and to compare this value with estimated or experimental affinity of reference GP1 ligands. Moreover, in a repurposing perspective, it would be interesting if authors could comment on the approximated dosage of ibuprofen they expect for providing anti-Ebola activity.

2. Ibuprofen has a serum half-life of 1.8 to 2.0 hours. Did authors consider this aspect when claiming that ibuprofen could be also administered to patients to prevent Ebola virus disease.

3. Liver and gastrointestinal tissues are among the most injured by Ebola virus infection. Moreover, it is well known that ibuprofen can cause serious gastrointestinal toxicity and should be used with caution in person with coagulation defects. Did authors considered ibuprofen side effects in the context of Ebola infection treatment? Following the question 1., a comment on the expected dosage would help to clarify most of these issues.

4. It is not clear how ibuprofen can alleviate the symptoms of Ebola virus disease, also considering the proposed mechanism of action.

**Competing Interests:** No competing interests were disclosed.
I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reader Comment 23 Jun 2015

**Thomas Hoennen**, National Institute of Allergy and Infectious Diseases, National Institutes of Health, USA

With respect to reviewer comment #3 it is worth pointing out that Medicines Sans Frontieres, the WHO, the CDC, and other health organizations specifically advise AGAINST the use of Ibuprofen and other NSAIDs due to the risk of adverse effects. This is an important point that should, in my opinion, be addressed by the authors.

See also:
http://www.ammi.ca/media/69846/Ebola%20Clinical%20Care%20Guidelines%202%20Sep%202014.pdf


The short version:
http://www.who.int/csr/disease/ebola/photos/ebolablock-poster15.jpg

**Competing Interests:** No competing interests were disclosed.

Author Response 24 Aug 2015

**Veljko Veljkovic**, University of Belgrade, Mihajla Petrovica 12-14, 11001 Belgrade, Serbia

**Response to Mattia Mori**

1. It was demonstrated that several host proteins interact with GP1. This indicates (i) that more than one receptor/co-receptor is involved in the Ebola virus infection and (ii) that entry inhibitors have different mode of actions because they could bind different targets. All experimentally proven entry inhibitors of Ebola virus are selected *in vitro* and their exact binding site on GP or receptor/co-receptor is not known. For these reasons, there are no “reference GP ligands” whose binding properties could be compared with ibuprofen.

Without initial experimental data any suggestion of dosage of ibuprofen in treatment of EVD would be highly speculative because the low toxicity of this drug allows its application in a very broad dosing range (100 – 2400 mg per day).

2. Although the plasma half-life of ibuprofen is short (1.9 to 2.2 hours), without experimental data it is not possible to predict antiviral effect of the residual concentration of this drug. Fourteen to twenty-four hours after administration of single dose of 800 mg of ibuprofen, the plasma level of this drug and its metabolites is < 250 ng/mL. For comparison, after administration of the therapeutic dose of 300 mg of Maraviroc, the plasma concentration of this HIV entry inhibitor after 10 hours is <100 ng/mL (Abel *et al.*, 2009).
3. Application of ibuprofen in the early stage of disease should be safe because hemorrhagic effect of EVD appears in the late stage of the illness. This is confirmed by the safe use of ibuprofen in the treatment of Ebola patients in Sierra Leone during the 2014/2015 outbreak without side effects (Ansumana et al., 2015).

4. We proposed that ibuprofen acts as an entry inhibitor preventing spread of virus in the body of Ebola patients (like HIV entry inhibitor Maraviroc which prevents spread of virus in the body of HIV patients).

Response to reader’s comment (Thomas Hoenen)

Despite the recommendation of WHO, CDC and some other organizations against ibuprofen in therapy of EVD, this drug was safely used in treatment of EVD patients in Sierra Leone during 2014/2015 Ebola outbreak (Ansumana et al. 2015).

Competing Interests: No competing interests were disclosed.