Author's response to reviews

Title: Searching for the elusive typhoid diagnostic

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Author's response to reviews: see over
Dear Editor:

Enclosed is the modified manuscript entitled “Searching for the Elusive Typhoid Diagnostic” by Stephen Baker et al. We wish this article to be considered for publication as a debate article in your journal.

Typhoid “enteric fever” remains as a significant problem in many developing countries. Vaccination is effective, yet rarely introduced into national programs as the burden is often unknown. This is mainly due to the insensitive nature of current typhoid diagnostics which have shown no significant advances in recent years. In this manuscript we discuss why current diagnostics are limited due to the nature of the organism and the infection and suggest contemporary ideas on how they may be developed.

This manuscript has been subjected to peer review by journal and we believed we have improved the manuscript as directed by the four reviewers. Each of the reviewers comments is discussed below.

Reviewer 1.

General comments:
The article by Stephen Baker et al is a comprehensive, well informative and thought provoking review on typhoid diagnostics. The authors have critically analysed the potentials and limitations of existing diagnostic tests. Scopes for future studies were also extensively discussed with reasons.
In present situation, the diagnosis of typhoid fever is not very promising due to lack of suitable and ideal diagnostics, which is really a major global problem and needs to be addressed. Therefore the article warrants publication.

Specific minor revisions:
Line 51, Typhoid is caused by S. Typhi only, hence it is appropriate to replace "typhoid" with "enteric fever".

This is a constant headache, enteric fever is the generic name for all invasive Salmonellae in humans, yet typhoid is the well known name that everybody commonly uses. We have now explained the definition in the text and avoided using the two terms interchangeably.” Enteric fever is an all encompassing term for the disease caused by
several serovars of Salmonella enterica including (S.) Typhi and (S.) Paratyphi A.” and "This article focuses specifically on S. Typhi and the disease it causes; typhoid. With respect to other invasive Salmonella, S. Typhi causes a greater disease burden and there is a superior level of understanding of this organism. However, all of the arguments presented here are poignant for the diseases caused by other human invasive Salmonella pathovars."

It is important to discuss about currently accepted "gold standard" (culture? serology? or DNA based test?) for evaluation of any recently developed typhoid diagnostics.

This is discussed in the background section "Presently, direct blood culture, followed by microbiological identification is the gold standard, any potential new test needs to offer a higher diagnostic rate than this procedure".

Line 129, It is unlikely that many people who get infected by Typhi or paratyphi A donot develop the disease- please modify the sentence.

Actually this is not true, our data(and others) from Nepal suggests that people in endemic areas can have high S. Typhi antibody levels without every knowingly had the disease. In truth it is probably a wide spectrum ranging from asymptomatic to full blown infection. We have modified to clarify", it is highly feasible that many people who get exposed by S. Typhi do not progress to develop the recognized disease syndrome, or individuals have a small amount of constant boosting due to prolonged exposure”.

Line 135, Blood volume required for culture is usually 5-10 ml (20 ml is too much), as ratio of blood vol and broth is important for culture.

This has been modified

Reviewer 2.

Major Compulsory Revisions:
Background:
1. The background section requires more information such as the specific regions of the world where typhoid fever constitutes a major public health problem. Readers will benefit more from the article if information is provided on the current global epidemiological status and statistics of typhoid including morbidity and mortality figures per year.

This was not written as a article reviewing typhoid per se, more to discuss the limitation of diagnostics. We have added some statistics as required, yet the figures presented in this article are highly dubious!

2. Since the manuscript is a review article, it is important for authors to include the description of the strategies they used to search, identify and assemble the
publications included in the article. Issues such as date of the search, sources/databases used for the search, criteria for the selection and exclusion of publications etc should be adequately described to enable one to confirm or repeat the process.

Whilst the article is more in the form of review rather than novel data, it is written to form part of a discussion in this area and represents the thoughts of the authors and the available publications. We appreciate this is not exhaustive, yet we find this recommendation unnecessary.

Minor Essential Revisions:
Discussion section:
Typhoid infections: This section is well written, however readers will benefit greatly if authors would include information on the influence of other infections which usually occur concurrently with typhoid particularly in the tropics eg. malaria, enteric infections of viral origin and other bacteria species. This is important as they might affect the diagnosis of typhoid in such regions since their symptoms mimic those of typhoid.

This has been modified " . It is also worth noting that in a single tropical setting blood infections may be caused by a wide range of other gram negative and gram positive organisms (e.g. S. Typhi, Streptococci, Leptospira, etc.), parasites (e.g. Plasmodium) and viruses (e.g. dengue) [27, 28]. Blood culture may or may not be a suitable assay for a specific infection, depending on the pathogen and the location".

Discretionary Revisions:
Conclusions section: Authors have made recommendations on the possible direction the development of typhoid diagnostics could follow; readers from developing endemic countries would however benefit from suggestions that could improve the diagnosis of typhoid within the limits of available resources. Such suggestions could be included by authors in this section.

This has been address somewhat in the discussion " The ultimate question is which direction do we follow in terms of developing typhoid diagnostics and how can these be applied to location with limited resources? In the short term, it appears that whilst current techniques are limited there is no real alternative without extensive research and culturing remains the inadequate gold standard. However, laboratories in developing countries with typhoid should be prepared to evaluate new diagnostics as they evolve. As a way forward for culture, it may be prudent to investigate specialized growth media that would favor the regeneration of S. Typhi from blood. Simple methods for enriching the small population of bacteria present in blood using simple direct enrichment procedures that do not rely on growth could be considered."
Reviewer 3.

Nice review, only needing minor changes to references on page 1
Ref 3 (line 49) has been superseded by a 2005 publication that includes more up
to date maps of antimicrobial resistance (Bhan et al Lancet 2005; 366: 749–62) and
this could be added, together with adding a ref on line 53 to the relative increase
in paratyphi A in travellers eg Connor & Schwartz Lancet Infect Dis 2005;
5: 623–28. Ref 9 (line 66 and ref list) could do with a full web reference as
difficult to find.

All these references have been added appropriately and the web link has been added in
the referencing section.

Reviewer 4

The manuscript is well-written, entertaining and provides insight into S. Typhi that
is useful and a pleasure to read.
While the focus is clearly on diagnostics, it would be nice to have a little focus
upfront on burden of disease and more on potential for vaccines in preventing
burden. Then, some discussion on the importance of diagnostic tests not only for
clinical management but for use in field studies of new vaccines.

We have added some information on this in the background, but wish to avoid a typhoid
epidemiology focus." Despite WHO recommendations, few countries have taken on
typhoid immunization [10], this is in part related to uncertainties about disease burden.
The best incidence assessment is based on available, sparse surveillance information,
estimated that in 2000 there were 21,650,974 illnesses and 216,510 deaths due to typhoid
and that paratyphoid caused 5,412,744 illnesses [2]. These data is extrapolated from
limited studies and such figures, therefore, may be imprecise, this is compounded by a
lack of accurate diagnosis. Therefore, new diagnostics will play a key role in decreasing
the incidence of typhoid fever, by permitting governments to accurately assess the
particular burden of disease and apply vaccination regimes accordingly. The
development of cheap and reliable enteric fever diagnostics would undoubtedly benefit
long term disease control and treatment."

My other comments are relatively minor.
Line 84/5, While I do think that the writing style is entertaining some of the
expressions used may get in the way of reader comprehension. “Typhi has
predominantly forsaken the luminal lifestyle of most enteric bacteria”. Maybe it is
the word “lifestyle” that is most distracting here. Do the authors mean “habitat” or
some version of that?

This has been edited for clarity " Whilst these mechanisms have never been directly
proven for typhoid, it is clear that S. Typhi has predominantly forsaken ongoing
transmission in the habitat of the mammalian gastrointestinal tract of most enteric bacteria, in the favor of systemic dissemination”.

Line 95, there is a word missing—probably “polysaccharide”

polysaccharide was missing and has been added

Line 117/8: Run on sentence. Not sure what it means, regardless. Please clarify.

“Another signature present in the Typhi genome is the lack of obvious evidence for immune selection, many systemic pathogens exhibit antigenic variation.

This has been modified “Analysis of multiple S. Typhi genomes shows a lack of obvious evidence for any amount of immune selection on the organism”.

Line 121: If authors want to use the term “stealth lifestyle”, will need to expand substantially on what they mean.

This has been modified “These data provide further evidence of the ability of S. Typhi to cause a systemic infection without stimulating a significant inflammatory response and transfer from the gastrointestinal lumen to the reticuloendothelial system in a relatively undetected fashion. Indeed, one may argue that the ability of S. Typhi to avoid immune detection constitutes the organism as a “stealth” pathogen and this has significant implications for diagnostics”.

Line 123 The sentence is not grammatically correct, but also needs explanation of what they mean by “immune privilege”

This has been modified “The lack of immune selection on the organism suggests that S. Typhi predominantly occupies an privileged niche within the host, a predominantly intracellular pathogen that can survive for long periods in this state”.

Line 139 What is meant by “bespoke” vessels

This has been edited for clarity and bespoke replaced ”Blood taken from patients is inoculated into vessels which are designed to fit in specific machines and contain specialized media, often there is minimal or no dilution of the sample into this media”.

Line 157 Could the authors expand for the uninformed on “metabolomic modeling”?

This has been modified “Understanding specific biochemical pathways that are up-regulated under defined conditions may permit some modeling of conditions in which S. Typhi can be grown more efficiently. In short, could we use a method that we define as “metabolomic modeling” to design recovery media to enrich for S. Typhi?”

Line 246 Regarding future for typhi diagnostics, the most cutting edge/interesting
part of the paper is the “Host factor other than antibody” section. Use of Mass spec, proteomics, NMR, and physiologic signatures would be nice to have discussed in greater detail, as they potentially relate to typhi diagnostics.

This section has been extended somewhat.

This manuscript has been read and approved by all co-authors in its current form. We think you will find that the concepts of this article are novel, entirely consistent scope of your Journal and will be of significant interest to your readership. We hope you will consider publishing this article.

Thank you for your attention to this matter.

Yours truly,

Dr Stephen Baker