Author's response to reviews

Title: Emergence of sporadic non-clustered cases of hospital-associated listeriosis among immunocompromised adults in southern Taiwan from 1992 to 2013: effect of precipitating immunosuppressive agents

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Author's response to reviews: see over
Philippa Harris  
Editor-in-Chief  
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Dear Dr Harris

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Please find attached a revised version of our manuscript “Emergence of sporadic non-clustered cases of hospital-associated listeriosis among immunocompromised adults in southern Taiwan from 1992 to 2013: effect of precipitating immunosuppressive agents”, which we would like to resubmit for publication as a Research Article in *BMC Infectious Diseases*.

Your comments and those of the reviewers were highly insightful and enabled us to greatly improve the quality of our manuscript. In the following pages are our point-by-point responses to each of the comments of the reviewers as well as your own comments.

Revisions in the text are shown using yellow highlighting. In accordance with Reviewer 1 and the editor’s suggestions, we added a new Figure 1 and Table 4. We hope that the revisions in the manuscript and our accompanying responses will be sufficient to make our manuscript suitable for publication in *BMC Infectious Diseases*.

We look forward to hearing from you at your earliest convenience.

Yours sincerely

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Responses to the comments of Reviewer #1

1. No abstract was received for this manuscript
   
   *Response:*
   
   The abstract was added to the manuscript.

2. Initial presentation and their diagnosis of the 12 hospital-associated cases
   
   *Response:*
   
   Detailed information about the underlying diseases, cause for admission, clinical syndromes of listeriosis and indication for precipitating immunosuppressive agents for the 12 hospital-associated cases of listeriosis were added in the Results (Line 211) and in the new Table 4.

3. Categorize the immunosuppressive agents into groups
   
   *Response:*
   
   We added the information in the Results (Line 173) “Of those receiving a precipitating immunosuppressive agent, six (50%) patients received a corticosteroid and six (50%) received both a corticosteroid and chemotherapy.”

4. Rather than identifying these cases as hospital-associated, the alternative explanation may be that these cases tended to present with symptoms in a protracted manner and were diagnosed later in the hospital course.
   
   *Response:*
   
   We take your point and added a note in the Discussion (Line 241): “Although it may be argued that a hospital-associated case defined by symptoms developing ≥48 hours after admission could be a case of community-associated listeriosis with a protracted course and delayed diagnosis, detailed analysis of the cause of admission and prescription of a precipitating immunosuppressive agent in these 12 hospital-associated cases suggests these were truly hospital-associated cases rather than community-associated with a subacute course.”

5. The conclusion states that “It is likely that the incidence of this disease will increase in association with the development and widespread use of new and more potent immunosuppressants”. While this may be true, this conclusion may be
somewhat over reaching based on the data presented. This statement would depend on
the type of immunosuppression and the risk of listeriosis associated with different
immunosuppressive agents.

**Response:**

We rewrote the Conclusion (Line 302) as follows: “Sporadic non-clustered
hospital-associated listeriosis is an emerging infectious disease in
immunocompromised hosts. It should be included in the differential diagnosis of
sepsis, particularly in those receiving new or increased doses of immunosuppressive
agents, such as corticosteroids or chemotherapy within the previous 4 weeks. Whether
the incidence of this disease will increase in association with the development and
widespread use of new and more focused immunosuppressive agents needs further
study.”

6. Please clarify whether this APACHE score was at the time of admission or at the time
of diagnosis of listeriosis

**Response:**

We specified this in the Methods (Line 117): “The clinical manifestations and
laboratory findings, including APACHE II score, used in the analysis were recorded
at the onset of symptoms that were compatible with listeriosis.”

7. Clarify whether the others symptoms/labs listed in table 2 were at the time of
admission or at the time of diagnosis of listeriosis, as the healthcare-associated
patients were not diagnosed with listeriosis until days after
admission.

**Response:**

We added in the Methods (Line 117): “The clinical manifestations and laboratory
findings, including APACHE II score, used in the analysis were recorded at the onset
of symptoms that were compatible with listeriosis.”

8. In the fifth sentence of the discussion section the authors state that “In the present
study 32 of 35 healthcare associated cases were immunocompromised”. It seems like
the 35 cases encompass both healthcare and community associated cases. Please
clarify.

**Response:**

This was an ambiguous and we deleted “healthcare-associated”.
Responses to the comments of Reviewer #2

1. Paragraph 1 (page 3 of manuscript). While ok to start by providing a general description of the clinical manifestations of listeriosis (perinatal and non-perinatal cases), would be good to provide a separate description of reported clinical manifestations in the non-perinatal immunosuppressed host. See cited reference 2. Would be good to categorize infections in the immunosuppressed host as focal and non-focal and describe the reported manifestations in that way.

Response:
We added more relevant background as suggested (Line 66): “Unusual localized infections such as pneumonia, hepatitis, arthritis and endophthalmitis have also been reported. The clinical presentation of non-perinatal listeriosis depends on predisposing factors. Patients with a severe immunocompromised status tend to have bacteremia without a focus, localized infection, and central nervous system (CNS) infection with coma or encephalitis.”

2. Paragraph 2 (page 3). Reference 8. Would add more detail of the immune response to listeria infections (innate and adaptive). Current description is too brief. Since the article is about listeriosis in the immunosuppressed host and the impact of immunosuppressive agents, essential to provide that detail.

Response:
We added more information as suggested (Line 79): “In innate immunity, macrophages play an important role in initial control of infection because replication of \textit{L. monocytogenes} occurs primarily within the macrophages that mediate clearance of bacteria. After specific recognition of pathogen-derived products by Toll-like receptors on macrophages, dendritic cells are activated and initiate CD4 and CD8 T cell responses that result in a stable population of \textit{L. monocytogenes}-specific memory T cells.”

3. Paragraph 1 (page 5). Study design section. Line 5. What are the other body fluids where listeria monocytogenes was isolated? Would specify.

Response:
These other body fluid include cerebrospinal fluid, ascites, pleural effusion and joint fluid. We revised this sentence (Line 111) to “\textit{L. monocytogenes} was isolated from blood and other sterile sites, including CSF, ascites, pleural effusion and joint fluid.”
4. Paragraph 1 (page 6). Line 7. In patients without CSF cultures positive for listeria monocytogenes, how was the diagnosis of CNS listeriosis made microbiologically? i.e. In these cases, was another body fluid culture like blood etc positive for listeria monocytogenes?
Response:
We added in definition (Line 136): “For cases without a positive CSF culture, the diagnosis of CNS infection was based on a positive blood culture with compatible CSF or imaging findings.”

5. Paragraph 1 (page 8). Line 2. You mention that all cases were non-clustered. How did you define non-clustered cases for your study? In other words, how was that determination made? Would specify.
Response:
The definition of non-clustered cases is in Definitions (Line 128): “Non-clustered cases were patients unrelated to any other in time or place.” The evidence is shown in the new Figure 1.

6. Paragraph 1 (page 8). You mention that all cases were adults. Did you look at pediatric cases at all? I.e. non-perinatal infections in children?
Response:
We reviewed all cases, and no non-perinatal infections in children were identified. This is mentioned in the first paragraph of the Results (Line 157).

7. Paragraph 1 (page 9). Line 2. You state that L monocytogenes was isolated from blood cultures in 32 out of 35 cases. Where was it isolated from in the remaining 3 cases?
Response:
This was a mistake. It was corrected to “33 of the 35……”. We also added the information (Line 182): “The other two cases with negative blood cultures had L monocytogenes isolated from joint fluid or from CSF.”

8. Paragraph 2 (page 9). Refer to figure-1. Would add detail here to provide a better description of the sites of infections. See line 2. 12 (34.3%) had bacteremia in association with other sites of infection. What were these other sites of infection? Would specify.
Response:

A detailed description is added in the figure legend of the figure (now Figure 2): “The majority (n = 20, 57%) presented as primary bacteremia without obvious site of involvement. Central nervous system involvement (n = 10, 28.6%) was the next most common. Other clinical syndromes included infective endocarditis (n = 2, 6%), infectious diarrhea (n = 2, 6%), and septic arthritis (n = 1, 3%).”

The section in the Results was re-written (Line 189) as: “The clinical presentation of the 35 cases of listeriosis is shown in Figure 2. The most common clinical syndrome was primary bacteremia in 20 (57%) patients. Ten (28%) patients had CNS involvement, seven with meningitis and three with a brain abscess. Localized infection presenting as septic arthritis of the knee was noted in one case (2.9%).”

9. Paragraph 1 (page 10). Line 3. The fact that only 11.4% were treated with effective/appropriate antibiotic therapy is concerning. Would highlight this as a contributing factor along side the risk of immunosuppression and would try to ascertain and describe in the paper what the underlying cause is leading to a very low rate of effective antimicrobial therapy in these cases and also mention future directives that can be taken in this regard to improve the rates of effective empiric antimicrobial therapy. For example: Clinical education initiatives.

Response:

We agree that this is an important point and added a paragraph in the Discussion (Line 261): “In the current study, only four of the 35 (11.4%) cases were treated with an appropriate antibiotic within 24 hours after the onset of listeriosis. The rate was low for both community-associated and hospital-associated cases (4.3% and 25%, \( p = 0.16 \)). This may arise because of the non-specific manifestations and rare occurrence of listeriosis, combined with the primary physician being unfamiliar with the disease. Education of physicians about the presentation of listeriosis, especially sepsis and meningitis in the elderly and immunocompromised patients, and in the setting of new or increased treatment with immunosuppressive agents in hospital may improve the rate of appropriate prescribing of antimicrobial agents.”

10. Paragraph 1 (page 12). Line 6. Correction in reported statistics is needed. It currently says "32 out of 35 hospital associated cases - 91.4% were immunocompromised." The numbers reported earlier in the manuscript and table-1 on HA cases are different from
these. Please clarify/correct as needed.

Response:
These numbers represent different meanings. The 91.4% (32/35) refers to patients in total who were immunocompromised, with neoplastic, autoimmune or steroid-dependent obstructive pulmonary diseases, alone or in combination with other diseases such as diabetes, chronic renal failure, and hepatic cirrhosis (mentioned in Results - Immunocompromised status of the patients with listeriosis; Line 165). The 91.7% (11/12) in Table 1 refers to patients receiving precipitating immunosuppressive agents (within the 4 weeks prior to symptoms of hospital-associated listeriosis).


Response:
This is an observation of the current study (Table 4). We added a paragraph to clarify that the data related to the current study (Line 211). “Detailed information about the underlying diseases, cause of admission, clinical syndromes of listeriosis, and reason for precipitating immunosuppressive therapy for the 12 hospital-associated cases of listeriosis are shown in Table 4.”

12. Please review pages 12,13 and 14. The references should be cited in numerical sequence.

Response:
The references have been checked and are in numerical order throughout the paper.

13. Table 2. Page 25. Please provide site HA and community associated cases.

Response:
The site of the isolated pathogen was added in Table 2.

14. Please be consistent in the use of the term immunosuppressed or immunocompromised. These are used interchangeably in the current manuscript.

Response:
Both words (immunocompromised and immunosuppressive) were used in the manuscript, but stand for different meanings. The specific term immunosuppressed was not used in the manuscript.
--**Immunosuppressive** was used as in immunosuppressive agent(s).

--**Immunocompromised** was used as immunocompromised (host or patient or status), which may include patients receiving immunosuppressive agent or have underlying diseases such as autoimmune disease/solid organ tumor/hematologic tumor.
Responses to comments of editor:

1. The authors define mortality as "death directly related to Listeria." How did they define related vs. unrelated death? Were other deaths not included in this analysis? All cause mortality at 60 days should be presented if this is not what is being presented.

Response:
The definition of mortality was revised (Line 142): “Mortality was all-cause death within 60 days after the onset of symptoms compatible with listeriosis.” The 12 deaths in this study were all-cause deaths, and were all directly related to listeriosis.

2. The authors state that 32 of the 35 cases involved bacteremia. Would be interesting to know the sites of infection not associated with bacteremia in the other three patients.

Response:
This was a mistake. It was corrected to “33 of the 35……”. We also added the information (Line 182): “The other two cases with negative blood cultures had L. monocytogenes isolated from joint fluid or from CSF.”

3. The authors offer a clear definition of community vs. hospital-onset Listeria. However, it would be important to indicate the reason for admission for the hospital-onset cases to properly assess for the possibility that some of these might have been subacute community-onset cases with delays in diagnosis.

Response:
The detailed information of the 12 hospital-associated listeriosis was added in Table 4. We also added a note in the Discussion (Line 241): “Although it may be argued that a hospital-associated case defined by symptoms developing ≥48 hours after admission could be a case of community-associated listeriosis with a protracted course and delayed diagnosis, detailed analysis of the cause of admission and prescription of a precipitating immunosuppressive agent in these 12 hospital-associated cases suggests these were truly hospital-associated cases rather than community-associated with a subacute course.”

4. The authors state that these are non-clustered but do not show the evidence. Perhaps a Figure might be shown to confirm that this is the case. I suggest a graph with the years on the X-axis and the number of cases on the Y axis. Would specify community and
hospital onset cases on this graph.

Response:
A new Figure 1 was added to illustrate this point.

5. The authors refer to "listeriosis-related symptoms" but do not define what these are in the methods.

Response:
It was defined (Line 138): “Listeriosis-related symptoms included fever, reduced consciousness, headache, seizure or nausea, which had no alternative cause.”

6. In the discussion the authors state that all 35 cases were "healthcare-associated" but do not define this term.

Response:
“Healthcare associated” was initially used in the study for patient who meets any one of below criteria:
(1) hospitalized in an acute care hospital for two or more days within 90 days of the infection;
(2) resided in a nursing home or long-term care facility;
(3) received recent intravenous antibiotic therapy, chemotherapy, or wound care within the past 30 days of the current infection;
(4) attended a hospital or hemodialysis clinic.
However, it is easy to confuse “Hospital-associated” and “healthcare-associated”. We therefore deleted the term “healthcare-associated”.

7. Were any of the patients treated with corticosteroids receiving trimethoprim-sulfamethoxazole prophylaxis? If not, should they have been? Could this have prevented the infections? Worth discussing.

Response:
No patients treated with corticosteroids received trimethoprim-sulfamethoxazole prophylaxis for listeriosis or other pathogen in this study. Whether trimethoprim-sulfamethoxazole prophylaxis in patients treated with corticosteroids protects against listeriosis needs further study. However, it is an important point to mention the TMP-SMX prophylaxis in listeriosis, and we added a paragraph in the Discussion (Line 278) “Prophylaxis with trimethoprim-sulfamethoxazole (TMP-SMZ) for Pneumocystis jiroveci and Toxoplasma gondii is given in patients with advanced
human immunodeficiency virus (HIV) infection and organ transplant recipients. In the current study, no patients had HIV infection or organ transplantation and they didn’t receive TMP-SMZ prophylaxis at the onset of listeriosis. Mark et al. reveal that prescription of TMP-SMZ for *Pneumocystis jiroveci* prophylaxis in persons with HIV infection also significantly decrease risk for other infections, including *Haemophilus*, *Salmonella*, and *S. aureus* disease, but not listeriosis. However, estimation of TMP-SMZ prophylaxis for listeriosis in HIV-infected patient and other hosts with a severe immunocompromised status needs further studies.” We also added the information in the Results (Line 169) “No patients had HIV infection or organ transplantation.”

8. Were the conditions listed in Table 1 selected based on literature showing association with Listeria? If so, references should be provided. Why are hypertension and peptic ulcer disease included in Table 1? Have these diseases been associated with Listeria in prior studies?

*Response:*
The condition selected is mainly based on reference 1 (Line 466). It has been reported that use of drugs that decrease gastric acidity may lead to predisposition to listeriosis, and we included peptic ulcer disease in the analysis. Reference 43 was added (Line 451). We deleted the row with hypertension.

9. Did any of the patients receive both long-term and precipitating immunosuppressants? This should be clarified.

*Response:*
It was revised in the Results (Line 171): “Eight (44.4%) of these 18 patients received long-term immunosuppressive agents, 12 (66.7%) received a precipitating immunosuppressive agent, and two (11.1%) received both long-term and precipitating immunosuppressive agents.”