Reviewer's report

Title: Characterization of Salmonella enterica in Guangdong China 2007-2012: serovars distribution, antimicrobial resistance and PFGE typing

Version: 2 Date: 14 April 2014

Reviewer: Markus Woegerbauer

Reviewer's report:

1. General comments:
The study “Characterization of Salmonella enterica in Guangdong China 2007-2012: serovars distribution, antimicrobial resistance and PFGE typing” by Ke et al. is dealing with the taxonomic characterization and the establishment of antimicrobial resistance profiles of 1764 Salmonella enterica strains isolated during a period of 6 years from outpatients in a province of China. Facing a global crisis in antibiotic resistance the monitoring of the antibiotic resistance status of pathogens causing a substantial burden of disease and for public health like Salmonella is necessary to develop and implement strategies for reducing the spread of antibiotic resistant strains or resistance genes.

Efforts as described in the present study are warranted, highly recommended and should be supported in any case.

However, the manuscript as presented by Ke et al. shows distinct deficits in linguistic competence impeding the understanding of some core statements made by the authors and some scientific flaws which may be overcome by thoroughly reworking the text. The manuscript requires major revisions before it is eligible for publication in BMC Infectious Diseases. For details please see below.

2. Is the question posed original, important and well defined?
The topic of the manuscript is easily identifiable and clearly defined, but not original. The work is performed with well-known methodology and comprises a not-innovative standard approach to solve the given problem. However, in the area of antibiotic resistance dissemination and pathogen diagnostics the present manuscript provides some essential monitoring data which are of interest for the involved scientific community and eventually for the broader public. The authors have collected a reputable number of strains which have been characterized with standard technology, a circumstance which is of some advantage if the data should be compared to the available amount of information in the field.

3. Are the data sound and well controlled?
A random check of the presented data in the text and some tables did not reveal any obvious inconsistencies. If the authors adhered to the protocols described in the manuscript then there are no obvious reasons why the data should be
contested. For MHC-determination an appropriate control strain was applied, however for RFLP analysis with pulse field gel electrophoresis the authors do not report the use of type strains but refer to clinical routine diagnostic procedures, where, hopefully, some quality control measure have had been implemented.

4. Is the interpretation well balanced and supported by the data?
It is very hard to follow the line of argumentation due to linguistic deficiencies of the manuscript. However, these flaws may be overcome by a thorough revision of the text with the help of a native speaker and should not be the argument for a rejection of the paper. The authors disseminate commonplace statements (e.g. “Several other serovars that are common in many countries…”). Multilocus sequence typing results would have been beneficial to better track clonal distributions of strains. However, obtaining these data for all tested strains would have certainly busted the budget. For the discussion I would prefer a strict focusing on the obtained data which are then compared point for point with the already available information for the local Chinese situation and then with the relevant data in Western countries.

5. Are the methods appropriate?
The methods are standard and appropriately described. The experiments may be reproduced by other scientists relying on the provided information in the manuscript.
Statistical procedures were not evaluated.

6. What are the strength and the weaknesses of the methods?
Serotyping by slide agglutination, MHC determination by agar diffusion and PFGE are state of the art technologies in the field. MLST would have substantially added value to the work, but is not necessarily required to fulfill the tasks mentioned in the title.

7. Can the writing, organization, tables and figures be improved?
The usage of English is certainly below the standard expected for scientific publications. However, the data are of some significance and should be made available to the public. To achieve this goal some hints may be found below (see section 10 onwards).
Figure 5 may not be eligible for publication.

8. When revisions are requested.
BMC Infectious Diseases is strictly disregarded to publish the manuscript in the present form. The authors are recommended to have a native speaker re-check their paper.
Additionally, the authors have to provide some essential information concerning the sampling plan. Otherwise the relevance of the results cannot be assessed.

9. Are there any ethical or competing interests’ issues?
The authors have acquired an ethic approval from the Ethics Committee of the
local Center for Disease Control and Prevention. If patients’ data are kept confidential and serotyping results cannot be traced back to the original donor there are no ethical issues obvious.

10. Discretionary revisions

Title page
Line 1: Restructuring the title to pinpoint and achieve a focus on the desired message in the following way is recommended:

“Serovar distribution, antimicrobial resistance profiles, and PFGE typing of Salmonella enterica strains isolated from 2007-2012 in Guandong, China”

Line 1: Please establish a continuous line numbering system covering the whole text – not only a single page, each. Otherwise it is extremely difficult to locate the exact positions for corrections.

11. Minor essential revisions

Abstract (p. 2):
Line 6: Please remove “further”.
Line 7: Please add “tests” instead of test
Line 12: Please remove “the”
Line 13: Please replace “resistant” by “resistance”
Line 14: “20 – 30% increasing percentage”. This is not comprehensible. Please clarify and modify the text accordingly.
Line 17: What is “severe” MDR? How is it quantified? I would recommend to omit this word in the text.
Line 19: Please use plural for “cephalosporin” and “quinolone”
Line 20: Please start the sentence with “Dominant PFGE patterns were…”.
Please be aware that you are actually talking about RFLP patterns visualized with pulsed field gel electrophoresis.
Line 22: ACGNaSSuTTm, ASSuTNa: Please explain these abbreviations in the main text of the manuscript. There is no single Pubmed citation available mentioning this string in the title and/or abstract.

Background (p. 4):
Line 2: As main topic the manuscript is dealing with the taxonomic characterization (serovars, PFGE analysis) of Salmonella strains. Please provide some background information about the taxonomy of Salmonella enterica and name the major serovars (e.g. Thyphi; Paratyphi A, B, C; Enteritidis,
Typhimurium) causing most of the clinically relevant Salmonella infections at the beginning. Please refer to nomenclature and eventually to problems with the taxonomic designation of Salmonella strains. This is a paper about the characterization of bacterial strains (in the laboratory) not a report about special disease characteristics of salmonelloses. This fact should be reflected in the introduction/background section.

Line 7: Non-typhoidal Salmonella serovars: Please add some important representatives as examples (e.g. S. Enteritidis, Typhimurium)

Line 8: Please replace “disease burden” by “burden of disease”.

Please be aware that Majowicz et al. (2010) report on “gastroenteritis” not on “diarrhea”. Both terms are not congruent, thus, please replace “cases” by “cases of gastroenteritis”. Majowicz et al. refer to 2.8 billion of “diarrheal illness” each year worldwide.

Line 11: Infections caused by NTS via a contaminated food chain is also the main route of infection in developed countries. Please correct it in the text accordingly.

Line 14: The fatality rate of 216.000 is the number of global cases in 2000 – not restricted to Asia. Please correct it accordingly.

Line 16: The citation is referring to the situation in Africa. Please be careful and do not mix up data from Asia, Africa and global data. Please correct it accordingly.

Line 17: Please avoid the term “first generation antibiotics”. This kind of designation is uncommon and usually restricted to the class determination of cephalosporins. “First generation antibiotics” is also not covered by the cited references (7, 8).

Line 18: “…is usually high”. Where? In China, Africa, Asia or worldwide? Please indicate in the text.

Line 21: Please replace “NTS infection” by “the rate of infections by NTS”.

Page 5:

Line 3: Please be aware to use the correct tense: please replace “still is” by “was”

Line 4: Please replace “while the non-susceptibilities” by “strains non-susceptible”

Line 5: increased

Line 6: increased

Line 7: Please replace “2006-2007 isolates” by “isolates collected during 2006 and 2007”

“Are” by “were”

Line 11: “are found” by “were”
Line 12: Please replace “are MDR isolates” by “were multidrug resistant”
Replace the next sentence by “However, these studies did not provide sufficient information concerning prevalence, serovar distribution ....”
Line 14: Please remove “Decreased”
Remove lines 16-18 and start with: “The present study describes serovar profiles and potential profile shifts, characterizes MDR isolates with a special focus on susceptibility to quinolones and third generation cephalosporins and reveals associations between MDR profiles and PFGE fingerprint pattern.”

Materials and methods (p. 6):

Line 5: Please provide the relevant references after “....World Health Organization and international guidelines on global surveillance.”
Please remove the sentence “The procedure of sampling...” This sentence is commonplace. It does not add any useful information.
Line 9: Please explain why you have selected exactly those 15 cities? Why have some districts (like Yunfu, Quingyuan etc...) not submitted samples for analysis?
Line 11: “positive results” for what? Please explain. The legend to Figure 1 is not clear. Please indicate Guandong province in the Figure. Exactly this designation is missing in the picture. Replace the first sentence in the legend by “the 15 isolation sites are marked in red and green”.
Line 11: Please provide information about the medium which was used for the strains during transport. It is not interesting that the colonies were transferred into vials. The crucial information is which environment was applied to guarantee viability of the isolates.
Line 12: Where is the Guangdong Provincial Center for Disease Control and Prevention located exactly? You never mention the location.
Line 15: Please provide supplier name for Kligler Agar.
Line 22: Reference 19 is incorrectly reproduced in the reference section: a more correct wording is:

Antimicrobial susceptibility
Line 5: Please put the antimicrobial compounds in brackets after “agents” and
use the plural e.g. “…agents (aminoglycosides, cephalosporins, penicillins….)

Line 10: Please use indicative and replace “would be” by “were”. Please add the minimum inhibitory concentrations (MIC) cut offs which have been used as breakpoints to classify a strain as resistant to the respective antimicrobial.

Line 20: Please replace “with comparison settings as the Dice similarity coefficient and UPMGA dendrogram type (optimization 0.50%, position tolerance 1.50%)” by “using the Dice similarity coefficient and UPMGA dendrogram type (…) as settings”.

Please explain UPMGA (unweighted pair group method using average linkages) in the text.

Results (p. 8):

Line 11: Please replace first 3 sentences by “In total 1764 isolates, which were isolated from stool samples of diarrhea outpatients (Table-S1), could be classified into 128 serovars. The most prevalent strains isolated during the test period were NTS…”

Line 20: Please replace sentence by “Most NTS (Top 11 serovars, n=1401, 79.42%) showed increasing incidence from 2007 to 2012 except for Salmonella Enteritidis.


Page 9:

Lane 2: “as a replacement”: What does this mean? Please remove. “Since 2008 Salmonella Typhimurium and Salmonella 4,5,12:i:- are the predominate endemic serovars in diarrhea outpatients in Guangdong (Fig 2).”

Lane 5: Please replace “Patients’ male/female ratio” by “The male/female ratio of the patients”

Please correct the next sentence to “The age distribution of the patients with the predominant Salmonella serovars …”

Lane 15: Please replace “were” by “are”. Start the next sentence with “Of all isolates 63.49%, 59.97%...”

Page 10:

Line 5: Please remove “For the third…in clinic” to the discussion.

Line 6: Please continue with “From all isolates (?) 8.05% showed a reduced susceptibility to ciprofloxacin”. Please explain for which diseases cephalosporins and fluoroquinolones are first line antibiotics in the text.

Line 14: Please add “all” preceding “isolates”. Please replace “decreased” by
“reduced”. This replacement is also recommended for other similar positions in the manuscript.

Line 15: Please explain the “few exceptions”.

Line 21: Did you mean “between the sampling locations”? If so, please replace “The ratio of MDR within each geography location keeps in comparable level (46.5-71.4%) (Table 2)” by “The ratio of MDR between the sampling locations was comparable (46.5-71.4%) (Table 2)”

Page 11:

Line 1: Please replace “geography location” by “sampling area”

Line 5: Please define “decreased susceptibility” or replace the first 3 words of the sentence by “Reduced …”

Line 19: Without additional comments “ACGNaSSuTTm, ASSuTNa and ASSuNa” are not comprehensible for the common reader in this context. Please explain the abbreviations.

Discussion (p. 12):

Line 9: Please be aware that it is common to use the plural for cephalosporins.

Line 11: In 2008 the dominant serovar changed to Salmonella Typhimurium. Please explain if changes in the laboratory protocol concerning handling of the samples, installation of new detection devices, new collaborators etc… have occurred between 2007 and 2009.

Page 13:

Lines 2 - 4: Therefore, it seems that the much wider reservoir pattern of Salmonella Typhimurium and Salmonella 4,5,12:i:- offers more opportunities to get into contact with new hosts, which results in a fast expansion of these two serovars.

Line 6 - 7: Please remove “instead of Salmonella Typhimurium and Salmonella 4,5,12:i:-.”

Line 7: Different eating habits of infants and adults or special host adaptations may be the reason for the observed distribution pattern.

Line 15: “Europe”

Line 16: Please remove “could be able”

Line 17: “in a large surface”: What does this mean? Please explain.
A similar increase of resistance to antibiotics was observed in fish and chicken in Guangdong.

“differing from higher ciprofloxacin…”. Please explain, what do you want to tell the reader exactly?

WHO reference is missing. Please provide.

Reference is missing. Please provide.

The dramatic increase of resistance to ciprofloxacin and third generation cephalosporins in infants is a warning signal for a prudent application of these antibiotics in clinical settings.

Reduced susceptibility to ciprofloxacin

implicating

“unrelated epidemiologic background”: Please provide evidence for this statement.

Do you mean “few dominant PFGE patterns in past and recent isolates of Salmonella Typhimurium …”?

Page 15

“PFGE patterns perhaps …”. Please provide evidence for your assumption either with references or by experimental data.

This is not only “interesting” but of crucial importance. Genotype data would substantially enhance the quality of this paper. However, it is clear that providing these data would be extremely costly and time consuming

Please remove. This is no conclusion but a simple statement. Start with the following sentence: “In conclusion, Salmonella Typhimurium and Salmonella 4,5,12:i:- were the most common serovars isolated during the observation period from 2007 to 2012 in Guangdong causing predominantly salmonellosis in infants, whereas Salmonella Enteritidis mainly caused salmonellosis in adults. In 2008 a serovar shift from Salmonella Enteritidis to Salmonella Typhimurium became apparent. A high burden of multidrug resistant strains and an increasing incidence of quinolone and third generation cephalosporin resistance could be observed. Close surveillance ... is therefore indicated to prevent outbreaks of disease, track potential transmission pathways and rationalize antimicrobial therapy of salmonellosis.

Figure 1: Please add province name (Guangdong) either in the figure or in the legend.

Figure 2: Y-axis: unit description is missing. Or explain in the legend.

Figure 5: The association between serovar and colour is hard to discern. Please
provide a clear colour scheme (e.g. S. Stanley: blue; S. Derby: yellow etc…) and indicate by arrow in the figure.

12. Major compulsory revisions

Abstract (p. 2)

Background: Line 3: Please indicate whether these 1764 Salmonella isolates have been either i) representatively selected for the Guandong province or ii) if they have been randomly chosen or iii) if they have been selected on a first in – first out basis of the participating laboratories and/or iv) if these strains cover actually all collected Salmonella isolates of the province during the study period. This information is necessary for the reader to be able to assess the relevance of the presented data.

Background (p. 4)

Line 6: Typhoid fever is not only restricted to immunocompromised hosts and infants but essentially dependent on the bacterial inoculum/infectious dose. Please revise the wording of the sentence.

Material and methods (p. 6)

Sampling, bacterial culture and identification

Line 9: Please explain the sampling plan. Have the strains been isolated statistically representative for the Guandong province or have they been sampled randomly (compare with the reviewer’s comment on the respective part in the abstract)? This is crucial information necessary for evaluation of the relevance of the results.

Please provide prevalence information on infections caused by Salmonella in the study area (= Guandong province)

Line 13: Please provide information about “drop outs”: How many stains have been collected, how many strains could be re-cultivated, how many strains did not grow in the laboratory and, thus, were unavailable for further investigations.

Line 18: How did you determine that all strains were epidemiologically unrelated? Did you infer this because of the large spatial distances between the collection centers or only due to the fact that public health officials did not notify an outbreak of disease officially? Did you pay attention to avoid isolation of several specimens from the same patient or did you collect multiple specimens from the same patients at different time points?

Results (p. 8):

Page 9:

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Not suitable for publication unless extensively edited

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interests.