Author's response to reviews

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

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Author's response to reviews: see over
To Editor of BMC Infectious Disease

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Dear Editor

We submit our revised manuscript titled “Serovar distribution, antimicrobial resistance profiles, and PFGE typing of Salmonella enterica strains isolated from 2007-2012 in Guangdong, China” to Editor of BMC Infectious Disease for your consideration.

We appreciate the comments from the reviewers and editor on our manuscript. These comments will definitely improve the quality of our manuscript. According to these suggestions, the manuscript was revised and marked with blue in manuscript. Responses to comments of editor and reviewers are listed as follows.

We would be delighted if you would consider our manuscript.

Sincerely yours

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Authors’ response

Reviewer 1

The paper addresses the evolution of multi drug resistant in 1764 Salmonella isolates, serovar distribution and PFGE typing. The principal focus is the comparison of data from 2006 to 2012. However in 2006 the study counted with just 53 samples and in the following year, 2007, 68 samples; in 2012 this number was almost 13 times higher, 843 samples were included in the study (or 47.7% of the total samples analyzed). The results of the paper look interesting. However, I am not sure about what kind of conclusions we could draft from the few samples obtained in 2006/07. I would like to suggest the inclusion of an epidemiologist in the review process to assure that the authors are using an adequate number of samples.

Authors’ response:
Thanks for the reviewer’s comments. We mainly focus on the molecular characters in this paper, including Serovar distribution, antimicrobial resistance profiles, and PFGE typing. We also realized the isolates number in 2007-2008 was less than 2008-2012. However, that is the total number we can collect in Guangdong Province in 2007-2008. We would like to get further suggestions.

Review 2

e.g. L13 P2 should read susceptibility (singular) and resistance.
Authors’ response: Corrected as the reviewer’s comments.

Pulsotypes need a simpler nomenclature.
Authors’ response: The present study belong to GSS and ESS program which is a global network of laboratories and individuals involved in the surveillance, isolation, identification, and antimicrobial susceptibility testing of Salmonella (http://www.who.int/salmsurv/en/). There is standard nomenclature for pulsotype of each serovar in China, and then could be used to compare with others.

Figures 4C and 3 represent the same data. I would delete Figure 4C.
Authors’ response: Fig 3 give the total distribution of top 5 serovars, while Figure 4C means the distribution top 5 serovars which are multidrug resistant strains. There are different with each other.

Figure 5 does not clarify anything here, and should be deleted, unless a small portion representing a very interesting and particular epidemiologic relevance wants to be highlighted.
Authors’ response: Fig 5 was deleted according to the reviewer’s comments.

I can not understand why a carbapenem is not included in the antimicrobial resistance panel. Please explain.
Authors’ response: Thanks for suggestion. The protocol was arranged by GSS and
ESS program, and they give the antimicrobial resistance panel which used in Salmonella surveillance.

Recently, Salmonella in Africa are highly susceptible to antibiotics (J Infect Dev Ctries. 2013 Oct 15;7(10):691-5.), and rare serotypes have been identified. Are they included in the panel?

Authors’ response: we identified six Salmonella Senftenberg and two Salmonella Stanleyville isolates, no Salmonella Urbana and Salmonella Ouakan were observed. We discuss this finding, and cite this paper in “Discussion” section. See in revised text.

Please include and discuss antibiotic usage and resistance trends in the Guangdong province.

Authors’ response: the antibiotic usage and resistance trends in the Guangdong province were discussed, and compare with other province in China and Africa, see in revised discussion section.

Reviewer 3
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 studies 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.

Authors’ response: Thanks for these comments and the supporting to our paper. We will follow the reviewer’s comments to revise the text, particularly in major points which reviewer suggested, see the following response to reviewer’s comments.

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.

Authors’ response: Thanks for these comments.

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.

Authors’ response: Thanks for these comments. For the RFLP analysis, there are standard procedures according to the ESS global system. The technicians are also accepted the standard training. The quality control will be done using type strain if there are obviously inconsistent data present during the RFLP analysis. Meanwhile, each result of RFLP will be compare to the standard database in our center which assigned by China CDC.

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.

Authors’ response: Thanks for these suggestions. The discussion section was revised according to the reviewer’s comments, such as strict focusing on the obtained data, comparison with available information and with relevant data in western countries, sees in revised text.

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.

Authors’ response: Thanks for these comments.

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.

Authors’ response: Thanks for these comments.

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.

Authors’ response: Thanks for these comments. The whole text was revised according to the reviewer’s comments, and the English was checked and revised. Fig 5 was removed.

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.

Authors’ response: Thanks for these comments. The paper was check by native speaker, and the essential revision (sampling plan) were done according to the reviewer’s comments, see the following response to reviewer.

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.

Authors’ response: Thanks for these comments.

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 Guangdong, China”

Authors’ response: the reviewer’s recommendation was accepted, the title was changed according to the suggestion.

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.

Authors’ response: Continuous line number was added to the whole text.

11. Minor essential revisions
Abstract (p. 2):
Line 6: Please remove “further”.
Authors’ response: Corrected as the reviewer’s recommendation.

Line 7: Please add “tests” instead of test
Authors’ response: Corrected as the reviewer’s recommendation.

Line 12: Please remove “the”
Authors’ response: Corrected as the reviewer’s recommendation.

Line 13: Please replace “resistant” by “resistance”
Authors’ response: Corrected as the reviewer’s recommendation.

Line 14: “20 – 30% increasing percentage”. This is not comprehensible. Please clarify and modify the text accordingly.
Authors’ response: this sentence was revised to be “a 20%-30% increase in the number of isolates resistant to ciprofloxacin (n=142, 8.05%) and third-generation cephalosporins (n=88, 4.99%) from 2007-2012”

Line 17: What is “severe” MDR? How is it quantified? I would recommend to omit this word in the text.
Authors’ response: “severe” was omit, “A high burden of MDR” was used to instead of “severe” to highlight the situation of MDR.

Line 19: Please use plural for “cephalosporin” and “quinolone
Authors’ response: Corrected as the reviewer’s recommendation.

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.
Authors’ response: Corrected as the reviewer’s recommendation.

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.
Authors’ response: The patterns of MDR were analyzed and arranged again according to the available publication, the present styles were also changed. The new abbreviations were explained in main text, see in revised text.

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.
Authors’ response: Thanks for this suggestions. We added this background information in the first part of Introduction section, see in revised text.

Line 7: Non-typhoidal Salmonella serovars: Please add some important
representatives as examples (e.g. S. Enteritidis, Typhimurium)
Authors' response: Corrected as the reviewer’s recommendation.

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.
Authors’ response: Corrected as the reviewer’s recommendation. The sentence revised to be “normally cause self-limiting diarrhea or gastroenteritis, which results in a significant disease burden, with an estimated 2.8 million cases of diarrhea and 93.8 million cases of gastroenteritis, worldwide, and 155,000 deaths each year [6].”

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.
Authors’ response: Corrected as the reviewer’s recommendation.

Line 14: The fatality rate of 216,000 is the number of global cases in 2000 – not restricted to Asia. Please correct it accordingly.
Authors’ response: Corrected as the reviewer’s recommendation.

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.
Authors’ response: Corrected as the reviewer’s recommendation.

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).
Authors’ response: Corrected as the reviewer’s recommendation.

Line 18: “…is usually high”. Where? In China, Africa, Asia or worldwide? Please indicate in the text.
Authors’ response: Corrected as the reviewer’s recommendation. The area was added in sentence.

Line 21: Please replace “NTS infection” by “the rate of infections by NTS”.
Authors’ response: Corrected as the reviewer’s recommendation.

Page 5:

Line 3: Please be aware to use the correct tense: please replace “still is” by “was”
Authors’ response: Corrected as the reviewer’s recommendation.

Line 4: Please replace “while the non-susceptibilities” by “strains non-susceptible”
Authors’ response: Corrected as the reviewer’s recommendation.

Line 5: increased
Authors’ response: Corrected as the reviewer’s recommendation.

Line 6: increased
Authors’ response: Corrected as the reviewer’s recommendation.

Line 7: Please replace “2006-2007 isolates” by “isolates collected during 2006 and 2007”
“Are” by “were”
Authors’ response: Corrected as the reviewer’s recommendation.

Line 11: “are found” by “were”
Authors’ response: Corrected as the reviewer’s recommendation.
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 ....”

Authors’ response: Corrected as the reviewer’s recommendation.

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.”

Authors’ response: Corrected as the reviewer’s recommendation.

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 Common place. It does not add any useful information.

Authors’ response: Corrected as the reviewer’s recommendation.

Line 9: Please explain why you have selected exactly those 15 cities? Why have some districts (like Yunfu, Quing yuan etc…) not submitted samples for analysis?

Authors’ response: According to the GSS system, all the cities marked in Fig 1 were on the list to submit the samples for analysis, while not all of them following the system. We did not get response from those cities, e.g. Yunfu, Qingyuan… that is why no sample from those cities. We explained that in the material and methods section. See in revised text.

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”.

Authors’ response: This sentence was rewritten; see in material and method section. The Guangdong Province was marked in Fig 1. The first sentence was also revised as reviewer suggested.

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.

Authors’ response: the medium for transfer sample is “semi-solid Rappaport Vassiliadis Medium (OXOID, France)”, see in revised sampling section.

Line 12: Where is the Guangdong Provincial Center for Disease Control and Prevention located exactly? You never mention the location.

Authors’ response: The location of Guangdong Provincial CDC was added in sampling plan section.
Line 15: Please provide supplier name for Kligler Agar
Authors’ response: the supplier of Kligler Agar was added as “(Huankai, Guangzhou, China).”

Authors’ response: Corrected as the reviewer’s recommendation.

Antimicrobial susceptibility
Line 5: Please put the antimicrobial compounds in brackets after “agents” and use the plural e.g. “…agents (aminoglycosides, cephalosporins, penicillins….)
Authors’ response: Corrected as the reviewer’s recommendation.

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.
Authors’ response: Here we use the agar diffusion method to test the susceptibility of each strain to twelve antibiotics. The classification of resistance of strains is based on the diameter of inhibition zone according to the the guidelines of the Clinical and Laboratory Standards Institute (CLSI).

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.
Authors’ response: Corrected as the reviewer’s recommendation.

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…”
Authors’ response: Corrected as the reviewer’s recommendation.

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.
Authors’ response: Corrected as the reviewer’s recommendation.

Authors’ response: this sentence was revised to be “major causative serovar in diarrhea outpatients”

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).”

Authors’ response: this sentence was revised to be “From that point, Salmonella Typhimurium and Salmonella 4,5,12:i:- were the predominant 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 …”

Authors’ response: Corrected as the reviewer’s recommendation.

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

Authors’ response: Corrected as the reviewer’s recommendation.

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

Authors’ response: Corrected as the reviewer’s recommendation.

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.

Authors’ response: Corrected as the reviewer’s recommendation. The disease which cephalosporins and fluoroquinolones are first line antibiotics were explained in discussion section.

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

Authors’ response: Corrected as reviewer’s recommendation.

Line 15: Please explain the “few exceptions”.

Authors’ response: this sentence was revised to be “The incidence of Salmonella infections caused by the top 11 NTS serovars (n=1401, 79.42%) showed an increasing trend from 2007 to 2012, except for Salmonella Typhimurium in 2010 (Table 1).”

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)”

Authors’ response: Yes, the correction has been corrected as the reviewer’s recommendation.

Page 11:

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

Authors’ response: Corrected as reviewer’s recommendation

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

Authors’ response: 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.
Authors’ response: we arranged the MDR pattern according to the published paper, and explain the MDR pattern abbreviation in main text. See in revised text.

Discussion (p. 12):
Line 9: Please be aware that it is common to use the plural for cephalosporins.
Authors’ response: Corrected as reviewer’s recommendation

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.
Authors’ response: Guangdong joined in the GSS project in 2007. After training of technician, the sampling was start from April, 2007. However, on September, 2009, new training program was launched. That is probably reasons the samples increased significantly from 2009. We revised this part and discuss it in both material and discussion section.

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.
Authors’ response: Corrected as reviewer’s recommendation

Line 6 - 7: Please remove “instead of Salmonella Typhimurium and Salmonella 4,5,12:i:-.”
Authors’ response: Corrected as reviewer’s recommendation

Line 7: Different eating habits of infants and adults or special host adaptations may be the reason for the observed distribution pattern.
Authors’ response: Corrected as reviewer’s recommendation

Line 15: “Europe”
Authors’ response: Corrected as reviewer’s recommendation

Line 16: Please remove “could be able”
Authors’ response: Corrected as reviewer’s recommendation

Line 17: “in a large surface”: What does this mean? Please explain.
Authors’ response: this sentence was revised to be “The antimicrobial susceptibility analyses cover antimicrobial resistance patterns in 15 of 21 cities in Guangdong where the Salmonella situation had been poorly characterized.”

Page 14
Line 1: A similar increase of resistance to antibiotics was observed in fish and chicken in Guangdong
Authors’ response: Corrected as reviewer’s recommendation

Line 4: “differing from higher ciprofloxacin…”. Please explain, what do you want to tell the reader exactly?
Authors’ response: this sentence was revised to be “The major difference is the
higher ciprofloxacin resistance observed in Henan than Guangdong (54% vs 8.05%).
Meanwhile we found resistance to both ciprofloxacin and cephalosporins in
Salmonella Typhimurium, Salmonella 4,5,12:i:- and Salmonella Enteritidis.”

Line 9: WHO reference is missing. Please provide.
Authors’ response: the reference was added.

Line 10: Reference is missing. Please provide.
Authors’ response: the reference was added.

Lines 12 – 14: 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.
Authors’ response: Corrected as reviewer’s recommendation
Line 14: reduced susceptibility to ciprofloxacin
Authors’ response: Corrected as reviewer’s recommendation
Line 18: implicating
Authors’ response: Corrected as reviewer’s recommendation
Line 19: “unrelated epidemiologic background”: Please provide evidence for this
statement.
Authors’ response: “unrelated epidemiologic background” was explained in the
sampling section. See in text.
Line 20 – 21: Do you mean “few dominant PFGE patterns in past and recent isolates
of Salmonella Typhimurium …”?
Authors’ response: Corrected as reviewer’s recommendation

Page 15
Line 3: “PFGE patterns perhaps …”. Please provide evidence for your assumption
either with references or by experimental data.
Authors’ response: one reference was added.

Line 9: 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
Authors’ response: Thanks for this comments, we would like to focus on the
genotyping study in future, and perhaps could be used to track the asymptomatic
carriers and identify the circulation of major clones in Guangdong Province.
“Interesting” was replaced by “crucial importance”.
Lines 12 – 13: 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 Guandong 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.

Authors’ response: Thanks for these revisions of “in conclusion” section, it was improved definitely. These revision were completely accepted, see in revised text.

Figure 1: Please add province name (Guangdong) either in the figure or in the legend.
Authors’ response: Corrected as reviewer’s recommendation, we add the province name in legend of Fig 1.

Figure 2: Y-axis: unit description is missing. Or explain in the legend.
Authors’ response: Corrected as reviewer’s recommendation, “Y-axis” was added in legend of Fig 2.

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.
Authors’ response: Fig 5 was deleted according to the reviewers 2 and 3 due to limited information presented.

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.
Authors’ response: These 1764 Salmonella isolates were collected from 15 hospitals in Guangdong Province. They are actually all collected Salmonella isolates of the province during the study period except outbreak isolates. The outbreak isolates were epidemiology related and collected in a separate system. Therefore, that is why we state that “All the strains are epidemiologically unrelated”. We revised and explain this part in both abstract and sampling section. See in revised text.

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.
Authors’ response: this sentence was revised to be “Typhoid fever, caused by Salmonella Typhi and Salmonella Paratyphi, is a bacteremic illness which occurs in strong, healthy hosts in addition to immunocompromised hosts and infants and is essentially dependent on the bacterial inoculum/infectious dose [5]”

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)

The World Health Organization Global Salm-Surv (WHO GSS) program launched in January 2000 is a global network of laboratories and individuals involved in the surveillance, isolation, identification, and antimicrobial susceptibility testing of Salmonella (http://www.who.int/salmsurv/en/). In 2005, the WHO GSS launched the Enhanced Salmonella Surveillance Project in China. Guangdong province was included in this Project in 2007. In 2009, this project was upgraded as ESS program. There is standard procedure for the sampling. The recruitment of patients followed specific guidelines. Patients who had two of the following three symptoms were chosen for the study: 1) diarrhea more than three times within 24 hours, with watery stools; 2) fever>38°C, headache, chills, malaise; 3) diarrhea with vomiting, abdominal pain, and watery stools.

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.
Authors’ response: we check our records again. We found that the total stool samples are 42,899. After re-culture, we got 1764 Salmonella isolates from 2007-2012. The drop outs data about “how many strains could be re-cultivated”, “how many strains did not grow in the laboratory” were missing.

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?
Authors’ response: According the standard procedure of GSS and ESS program, the patient’s personal information was recorded. We will sample again if the first time culture is not success, and we keep each isolate for each patient. However, we cannot make sure that they are not come from same outbreak which is not reported. All the patients’ information could be tracked in system. We would like to get further comments about this item.


Authors’ response: these sentence were revised to be “The predominant NTS consisted of Salmonella Typhimurium (n=523, 29.65%), Salmonella Enteritidis (n=257, 14.57%), Salmonella 4,5,12:i:- (n=244, 13.83%), Salmonella Stanley (n=167, 9.47%), Salmonella Derby (n=46, 2.61%), Salmonella Rissen (n=36, 2.04%), Salmonella Weltevreden (n=35, 1.98%), Salmonella Infantis (n=28, 1.58%), Salmonella Agona
(n=24, 1.36%), *Salmonella* Albany (n=22, 1.25%) and *Salmonella* Newport (n=19, 1.08%) (Table 1). The incidence of *Salmonella* infections caused by the top 11 NTS serovars (n=1401, 79.42%) showed an increasing trend from 2007 to 2012, except for *Salmonella* Typhimurium in 2010 (Table 1).”