



SDI Review Form 1.6

Journal Name:	British Microbiology Research Journal
Manuscript Number:	2013_BMRJ_8507
Title of the Manuscript:	Emerging third generation cephalosporin resistance and single RAPD primer for typing clinical Salmonella isolates from Lagos, Nigeria
Type of the Article	

General guideline for Peer Review process:

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound.

To know the complete guideline for Peer Review process, reviewers are requested to visit this link:

(<http://www.sciencedomain.org/page.php?id=sdi-general-editorial-policy#Peer-Review-Guideline>)



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PART 1: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
Compulsory REVISION comments	Title: It is correct to state emergency resistance with results obtained in samples recruited in that period of time? (little bit more than a year) It is interesting to report the existence or appearance of antibiotics resistance, but for declare emergency, probably further follow up would be necessary. Please reconsider modification of the title. Abstract: Line 5. Please review the grammar: to study or to investigate?. The Study design only refers to samples. Type of study? Purpose of the use of samples? How many samples according the source? Malaria and HIV/AIDS are not mentioned here but further in the text. Conclusion does not correspond to the one at the end of the article. Line 14: three hundred patients? Samples? Were evaluated...Line 18: Eliminate the space between PC R method. Line 21: It is not clear if the 28 isolates (from the 63?) had plasmids. Numbers and percentage are mentioned indistinctly. It is recommendable to be consistent with the form to show data along the article to make it comprehensive. Introduction: Line 35 Salmonella (lacks italic): have emerged instead of had emerged. It is necessary to verify some references that do not correspond to the adequate ones or are not related to the text : Eg: 5, 7. Materials and Methods: Line 64: Please add were recruited at the end of the sentence. No info about informed consent, ethical guidelines for human clinical samples use and management, etc. Line 65: Malaria and HIV/AIDS are not mentioned in the Abstract. Which condition in these patients determined that their samples were recruited for Salmonella searching? It is important to clarify how many samples or type, according to the disease, were really studied. 300 patients and 300 samples? 1 sample per patient? How many of each type? Explain the difference in samples according to the patient	



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	diseases. How many cases 1 patient had more than one disease? Which were those ones? Which types of samples were analysed in those cases? This appeared as a result, in a table, and it is necessary that info in M&M. Line 70: reference for standard methods applied must appeared. Line 71: Cultivation "of the sample" were (instead of are). Line 73: Reference 8 appeared in reference chapter as number 6. Line 75: Only polyvalent antisera? Monovalent antisera were not used for identification?. Line 78: Ref 9 does not correspond to Clinical and Standard... Line 81: Ref 10 does not correspond to previous sentence. Line 90: Ref 11 is not correct Line 92:Ref 12 is not correct Line 95: I would suggest reordering the paragraph. If ESBL bla genes were amplified according to Rotimi et al, please, organize and put the primers sequences, followed by the reference, and then the resting information about the PCR mix. The branch, country of origin of main reagents should appear (along the entire article). Buffer and deionized water was no mentioned as part of the mix. They were not used? No controls appeared in amplification experiments. Ref 13 appeared later as #10. Line 104: Which RAPD-PCR parameters were optimized? Mention it. Which DNA template was used for these optimizing experiments?. It is very important as later on no amplification occurred in most clinical samples, so procedures should be well defined. It seems that the conditions were different for the primers used and the mix conditions were not the same, but it is no well-organized or clear to the reader. Again the origin of reagents is not mentioned nor did controls appear in the reaction. Line 111: Let clear again that 784 and 1254 are RAPD primers. Line 116 and 118. Two different conditions are mentioned for electrophoresis of RAPD products, relative to agarose %, voltage, etc. Pease explain this difference or it was a mistake?. Line 123: ref 14 (Silhavry et al does not appear in References. Line 126: ref 15 is not correct. Yukata et al AND Wong et al are two references in only one cite (15) which is not correct, nor corresponding. It is not explained how Plamid curing method and conjugation experiment were useful for the investigation. Nothing about it in the results.	
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	<u>Results and Discussion:</u> Line 143 and others: Number and percentages are mentioned indistinctly, which make harder the comprehension, If possible please keep a way to show your results. Line 146: Redaction error Lines 146-148: The discussion of the precedent result is not correct. Ref 16 is not correct (a study in Turkey mentioned as if it were in Nigeria). Line 151: Salmonella (italics lacking, please check all the article) Line 153: Some reference would be useful to support that third generation antibiotics are currently the drugs of choice in Nigeria. No other investigation is able to compare the presence (or absence) of resistance to the use of those antibiotics? Line 154-159: Repeated info not comprehensively written. Line 162: Salmonella isolates was (instead of is). Line 163: The same. Ref 18 is not correct Line 169 to 174: Paragraph repeated from a previous one. Lines 176 to 179: If primers 784 and 1254 did not produce amplification for 3 PCR repeats, is obvious that no tying is possible. Please rewrite. The point here is to question why amplification did not occur. IN M&M was stated that conditions of amplification were normalized (using which DNA template?), SO, why isolates DNA did not amplify. This must be discussed. Besides, the author compares this result with that one from Smith et al (19) in terms that are no compatible. Ref 20 does not correspond to Quintaes (or Quintanes?) appeared the two forms. If no amplification was expected why these primers were used, this is not understandable. Ref 21 support that RAPD is lab depending and not very reproducible, then why the authors decided to use it? Explain. Line 187: Eight RAPD patterns were found (referring to 8 profiles? In table appear 7. In order to discriminate the profiles, which reference DNA from reference strains were used, this is no mentioned at all. In general the analysis of RAPD patterns is not clear, how the associations were established, which method was used? The Jaccard similarity coefficient was determined? Which analytical method the authors applied? Probably a dendogram would reflex better the results that the data shown in tables. It is a suggestion to the authors. Line 192: The sentence is not understandable. A reference to a previous work is lacking.	
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	Lines 194 and 195: Redaction must be revised to avoid confusion about the strains origin. RAPD results are no discussed. <u>Tables and Figures:</u> Tables in general without vertical lines nor frame. Table 1. Part of the info is necessary in M&M. In general terms the total of each type of samples per diseases, per patient. Column Organisms should be called Serotypes. Please if possible write in a correct form part of the scientific name of each serotype , even though in the legend appear complete. SPA-S. Parataphi must be changed by Paratyphi. Bac. Bacteremia must be changed by Bacteriemia. In all cases the letter S of the Salmonella genus must be written in italics, along the article also. Please re check. Table 2: Salmonella in italics. Again italics for S. of the genus Salmonella. It is recommendable, for a better comprehension, to put Total/Percentage in the same row. Percentage of resistance? Should be put in the legend or in the table. Table 3: Review the scientific names. Declare briefly in a legend RAPD PCR profiles and RAPD types. In text appeared 8 types, in table 7. It is Correct RARD Types. Plasmid number (0, 1, 2, 3) must be declared, non-understandable. Rage (range?) of plasmid size in Kleb must be explained in a legend. Is no comprehensive that column in relation to the rows. Declare what is ESBL_ve and BlaxCTL (+ve). This table is hard to understand. To better show RAPD results it is recommendable to make a dendogram to see the associations. Fig 1 and 2: Must be ameliorated. No good visualization, no possible to make a correct definition of the differences. Lines and images donot fit. Is necessary to correct all the scientific names of the serotypes, branch and country of origin of the ladder Fig 2 no visible, no informative at all. I will suggest eliminating it. <u>Conclusions:</u> Relating RAPD, the results did not support strongly the conclusions. Only one primer showed discrimination, which is no clearly stated in the article. Truly, this technique is useful and cheaper if reproducibility and discrimination are demonstrated, but the authors do not convince about this topic in their article. <u>References:</u> They are not uniform. Authors should pay attention to be consistent in the references citation. Each reference must be individually checked to correct frequent errors: points, spaces, letters out of place, name of Journals in different forms, scientific names with or without italics, etc. No corresponding between the number of reference in the text and in this chapter. This make, in addition, a little hard to evaluate the discussion, which is already susceptible to be improved	
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Minor REVISION comments		
Optional/General comments	The article shows interesting results derived from the analysis of sample patients from whom Salmonella isolates were obtained. The resistance to 3rd generation antibiotics was investigated and the presence of plasmid and genes ESBL related with the resistance was searched. At the same time, the authors applied RAPD method to type these isolates. The useful research findings must be published and shared with the scientific community, but it is suggested to make a careful revision of the manuscript here analysed, in order to improve its quality for further publishing. I will comment here general remarks on each aspect, and a very detailed analysed is being submitted in Revision comments, if valuable for authors. Abstract: Must be revised. It presents grammatical errors, the study design presented is not adequate, methodology is poor and results incomplete. Conclusions, not entirely funded, will be comment later on. Introduction: Some information is supported on inappropriate references which must be revised. Several info in unnecessary while other important is absent (e.g. Why is important to type Salmonella strains?) Which is the current application of this issue? The purpose of the work, stated at the end of Intro, referred to identifying a faster, easier, cheaper.... This is not well understood, as RAPD was used, apparently, for typing purposes and not for detection from a diagnostic point of view. Materials and Methods: Important information for make them reproducible is lacking. Ethical aspects related to the proper obtain and use of human clinical samples are not described. Some procedures can't be reproduce on the basis of the information given. General references must be added if procedures are not described. RAPD is described only as the amplification procedure, but nothing about the analysis of the results appeared, which later on complicate comprehension. The issues Plasmid DNA curing method and Conjugation experiments are very poorly explained and nothing is mentioned about them on Results. Results and Discussion: It is suggested to be entirely and carefully revised. Some results are repeated in different ways, or not clear or precise enough. Other ones are not mentioned. Figures and tables supporting the results are not always comprehensive. In some cases they do not correspond to the text. Several incorrect or not well cited references appeared. Discussion is poor and must be improved on the bases of more actualized information, by comparing, contrasting or explaining the authors point of view.	



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	Conclusions: It seems to very strong compare to the time of study, the number of isolates truly analysed and the results obtained. Those related to RAPD should be reconsidered by authors. Probably to be only suggestive and careful would be more convenient. References: Must be written in a uniformed format and revised as well. Some of them do not correspond to the work, if possible, more recent literature on the field must be consulted. The manuscript is based on the study of 300 patients, from whom clinical samples were obtained and isolated. At the end of the text the approval of the study by an Ethical Committee is mentioned. However, it is no mentioned in the article that informed consent from patients or relatives were obtained, nor it is known if other ethical guidelines were taken into account to carry on the study, using human clinical samples.	
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Reviewer Details:

Name:	Ana Margarita Montalvo Alvarez
Department, University & Country	Parasitology Department. Institute of Tropical Medicine Pedro Kouri. Havana, Cuba