Author's response to reviews

Title: Rapid and robust phylotyping of spa t003, a dominant MRSA clone in Luxembourg and other European countries.

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Author's response to reviews: see over
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BMC Infectious Diseases Editors,

Thank you for the opportunity to submit a revised version of our manuscript titled: “Rapid and robust phylotyping of spa t003, a dominant MRSA clone in Luxembourg and other European countries” (MS#2139535009892165). Our revisions are all based on the reviewers suggested edits or are in direct response to any questions raised. Our responses to the reviewers’ comments as follows:

Reviewer #1
1. More information on time, costs, and utility related to both the real-time PCR assays and the WGST methods in the Conclusions section.
2. PFGE was not included in the analysis, as this methodology is not in use for *Staphylococcus* subtyping at the Luxembourg Laboratoire National de Santé. This older typing methodology, while still extremely useful for a number of pathogens, is used less and less for *Staphylococcus*.
3. The MLVA data is included for comparison only. This was previously collected data for the isolates analyzed with the PCR assays and WGST. A brief mention of its utility remains in the Conclusions section. While MLVA is an important tool, and invented by some of the authors on this paper, its high resolving capabilities are constrained by lack of phylogenetic relationship information on a larger level.
4. No additional data was presented on WGST for other spa types as this analysis was only performed for the strains described (primarily spa type t003).

Reviewer #2
1. We agree with the reviewer that subtyping strategies and approaches should be tied to the local epidemiology and subtype populations. A reflection of the understanding of the dominant *Staphylococcus* populations and epidemiology in Luxembourg is currently in the Background and Conclusions Sections. An additional statement to this general point has been added to the Conclusions Section.
2. Again, we agree with the reviewer in regards to the importance of the local epidemiology of the strains being subtyped. This study was conducted primarily to develop improved rapid genotyping tools based on the dominant strains seen at Luxembourg’s national health lab, and the assays were compared to other typing results from several strain collections. Additionally, for demonstration purposes we analyzed a set of isolates from a previously studied Long-Term Care Facility investigation (Mossong et al. 2012. *Epidemiol Infection*). We therefore did not apply the assays to any ongoing epidemiologic investigations. It is our hope that the assays will be used for prospective outbreak analyses and their epidemiologic performance can be better assessed.
3. The last paragraph has been modified to separate the respective conclusions on the utility of the PCR assays and the whole genome analysis.
4. In reference to the question regarding details on SNP identification and selection, more details have been added to the Methods section; in short, the only phylogenetically informative orthologous SNPs were used, regardless of coding versus non-coding status. The in house script (SolSNP) is referenced and a link is provided to the most recent version of the tool. Further assistance with usage of SolSNP or any other tools in the described analysis pipeline is available upon request, as now stated in the SolSNP reference. In addition, the authors are part of a consortium to generate a complete and comprehensive WGST pipeline, including a version of the SolSNP script, which will also be published upon completion.

5. The Reviewer suggests re-ordering the isolates listed in either Figure 1, or the corresponding Supplemental Tables 1 and 5. The order of strains in the phylogenetic tree in Figure 1 cannot be re-ordered, as the order and grouping are determined by phylogenetic relationships. The strains in Supp Table 1 are listed in order of selection and with their corresponding “match” as described in the methods. We believe reordering these isolates to match their placement on the tree will de-link the pairs and cause reader confusion and as such would prefer to keep the order of isolates in the tree and the corresponding tables as is. Additionally, genomes from Supp Table 5 are also included in Figure 1. If a change in the isolate order is important to the editor, we will re-order the isolates in Supp Table 1. Lastly, any missing data for the MLST sequence type, was due to those isolates being untypeable in our lab. The Tables now reflect this.

Please let me know if I can provide any further information. Thank you for your consideration.

Sincerely,

[Signature]
David M. Engelthaler
Director, Programs and Operations