Author's response to reviews

Title: Nationwide Trends in Molecular Epidemiology of Methicillin-resistant Staphylococcus aureus, Finland, 1997-2004

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Author's response to reviews: see over
Dear Dr Makri,

There is a revised version of our manuscript titled “Nationwide Trends in Molecular Epidemiology of Methicillin-resistant Staphylococcus aureus, Finland, 1997-2004”, which we would like to resubmit for publication in BMC Infectious Diseases in the category of research article. We appreciated the reviewer's comments and have made the requested changes. Detailed responses to all of the reviewers' comments and suggestions are included. We believe that this extensive revision strengthened the paper.

Thank you for considering this paper for BMC Infectious Diseases. Please address correspondence regarding this manuscript at the address below.

Yours sincerely,
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Responses to comments from the Editor
Copy-editing – Our manuscript has been copy-edited by a person who has experience of similar tasks.

Ethics – Based on Finnish legislation, the National Public Health Institute is mandated to conduct infectious disease surveillance and research on this area. Similarly, Finnish clinical microbiology laboratories are mandated to report certain infectious disease agents and cases, including all MRSA isolations, into the National Infectious Disease Register. Thus, no ethics committee approval was required for our investigation.

Responses to Reviewer Comments

Referee: Robert Skov

General comments
It is true that MRSA strains possessing SCCmeC IV are not necessarily community-acquired. We added two new sentences to “Results and Discussion” about the definition of CA-MRSA (page 12, lines 5-7) and transmission of CA-MRSA strains between health-care settings and community (page 12, lines 12-14). Unfortunately, we can not use the information obtained during the questionnaire based survey period to further qualify this statement, as we did not investigate any health-care records during the study (page 11, lines 21-23).

The situation of MRSA in different countries may reflect for the definition of epidemic/outbreak strains, or clusters. We agree that we have many strains which have not caused large epidemic, but we do not consider them as sporadic because more than one person carries it, and thus they are not real sporadic. There are many MRSA outbreak strains in Finland which have caused smaller or larger outbreaks, and many of them have been found only occasionally after or before the outbreak. Many true outbreak strains have caused outbreaks before our present study period, but they appeared only few times during 1997-2004. We consider them outbreak strain, otherwise they would be sporadic isolates in one year, and outbreak isolates in another year. Such “non-sporadic, but non-epidemic” strains are shown in Figure 2, and if we would change the definition of sporadic strains as Skov suggested, total of 17 epidemic strains would disappear from the picture. However, other reviewers were interested in many of the strain types shown in Figure 2, and thus, we believe that it is relevant to show all these strains in Figure 2 and conserve our definition about sporadic strains.

Minor Essential Revisions
The abstract has been shortened and written in a more focused format. We added the year to the sentence of “…constantly increased since 1995“(page 2, line 4) as suggested.

A comment on the slight difference in numbers has been added to page 8, lines 20-23 and page 9, lines 1-2 as suggested.

Discretionary Revisions
“Generally electronically” in parenthesis from page 5, line 7 has been removed as suggested.

Referee: Barbro Olsson-Liljequist

Major Compulsory Revisions

The definition of strains etc. have been simplified and clarified in the manuscript, see abstract, M&M section, page 5, line 22 and page 6, lines 1-17 as suggested. The word “clone” was removed from the text for clarification. The S. aureus PFGE database at KTL is used as the primary basis for naming the isolates (after naming of isolates, they are called strains). Other typing results (MLST, SCCmeC analysis, spa-typing) are important, complementary techniques, but the routine naming of Finnish MRSA isolates is based on PFGE results.

Table 1 and Figure 2 have been revised, and we hope that they are now clearer to follow. We agree that FIN-15 clusters with the FIN-1 group in the dendrogram but if its banding pattern is compared with that of FIN-1, they differ by more than seven bands, which allows us to name them with different FIN names. It is also true that FIN-2e has different characteristics than some other isolates in FIN-2 group. This has been commented in Results and Discussion, page 12, lines 18-22 and page 13, lines 1-2.
The determination of the presence of PVL genes \((lukS-PV - lukF-PV)\) has been added to the manuscript, see page 6, lines 14-15, page 12, lines 7-9, and Figure 2.

**Minor Essential Revisions**

Table 1 was already listed in order of overall prevalence of strains during the study period, but Table 2 was revised accordingly as suggested.

Some description on FIN-15 and FIN-2e isolates has been added to Results and Discussion as suggested, see page 12, lines 1-3 and 16-22, and page 13, lines 1-2.

The sentence “One of the three strains were previously…” was rephrased: “One of the three strains which was previously…”, see page 11, lines 9-10, and the sentence “…more than half of the sporadic strains were found from…” was rephrased: “…more than half of the sporadic isolates were derived from…”, see page 13, line 6, as suggested.

**Referee: Dominique S Blanc**

**Major Comments**

The Results and Discussion sections have been combined as suggested.

There are many things that may have affected the increase of MRSA in 2003 and 2004. The following possible explanations have been added to the Results and Discussion-part:. First, the increase in screening activity is probably one of the reasons (page 14, lines 12-17), although it may not necessarily associate with the number of MRSA cases (page 10, lines 18-20). This was stated in the manuscript already before the revision. Second, the search and destroy policy may be ineffective in some cases, for example if there is lack of resources (page 14, lines 17-20). In addition, some strains may be more fit that others and thus, may survive better on different surfaces or as part of skin flora. Moreover, the carriage of MRSA may become widespread before the first clinical infection is discovered. Furthermore, MRSA isolates may arise de novo, either under the selection pressure of antimicrobials or in the community. Third, the search and destroy policy may slide occasionally. Fourth, the number of invasive (blood and CSF) MRSA isolates increases probably because certain strains have reached the level high enough among the population and thus, it’s proportion among blood or CSF isolates solely reflects this (page 10, lines 1-6). Fifth, some of the strains may be more virulent than others, and this might be seen in association with invasive isolates (page 9, line 20-23, and page 10, line 1).

It is true that clonal complexes can not be associated to a specific pandemic clone. Thus, we rewrote new sentences, see page 3, lines 19-22.

We clarified the sentence “the extensiveness of MRSA strain collection”, see page 4, lines 1-3, and lines 6-8.

The terminology has been clarified (see abstract and M&M, page 5, line 22 and page 6, lines 1-17), but in our opinion, we cannot substitute the term “strain” by “isolate”, because it would confuse the meaning. We have used the word “isolate” if there is an “unknown” MRSA in question, and the word “strain” after we have named the MRSA (FIN-1 – FIN-25). The term “MRSA notification” is used for those that were notified as suggested.
The difference between the number of MRSA isolates and the number of persons behind these isolates has been commented in page 8, lines 20-23 and page 9, lines 1-3 as suggested.

We mentioned 10 epidemic types instead of five, because there are some internationally widespread isolates, such as FIN-6 (UK-EMRSA-16) and FIN-12 (UK-EMRSA-15) (the tenth and the eight most prevalent, respectively) which may be of interest to researchers from overseas. We added the name of the isolates to the text to clarify the message; see page 9, lines 5-9, and we changed the term “prevalent” to “predominant”, see page 9, line 7, as suggested.

The comment on FIN-16 has been added, see page 9, line 23 and page 10, lines 1-6. Statistics were also calculated, see page 7, lines 18-20, page 9, lines 12-14, and 22.

The data on the survey has been included in Table 2, in which a column and a row with totals were also added as suggested.

Statistics were added for FIN-4 (page 11, line 13), FIN-7 (page 11, line 23 and page 13, line 1), FIN-10 (page 12, line 1), FIN-16 (page 9, line 22) and FIN-21 (page 10, line 15-16).

FIN-10 was also mainly found from the screening specimens, and this was added to the page 11, lines 21-23. ST and SCCmec have given for all FIN-types when mentioned first time in the new section.

In Table 2 the lines of “sporadic” and “other strains” were changed by mistake, and it was corrected. It is true, that the total no. of screening specimen from abroad hospital contact is 64, and it was also corrected.

A paragraph on control measures has been included to M&M section, page 6, lines 19-23 and page 7, lines 1-5, as suggested.

It is true that MRSA strains possessing SCC\textit{mec} IV are not necessarily community-acquired. We added two new sentences to the “Results and Discussion” about the definition of CA-MRSA (page 12, lines 5-7) and transmission of “CA-MRSA” strains between health-care settings and community (page 12, lines 12-14).

The legend for Figure 1 has been revised as suggested.

Figure 2:
The figure legend has been revised as suggested.
Subtypes are additionally labelled with letters, if a MRSA epidemic has been caused by a specific subtype. This has been added to page 6, lines 8-9.
Not all related profiles are presented in Figure 2, only representative isolates of a specific type. The number of persons carrying each type has been added to Figure 2 as suggested.
The positions of bands have been removed as suggested.
The descriptions of slv and dlv have been added.
FIN-15 and FIN-1 cluster together in the dendrogram, but still show more than six band difference which allows us to name them with different FIN name.
The comment of different SCC\textit{mec} cassettes within each PFGE clone has been added to Results and Discussion, see page 12, lines 19-22 and page 13, lines 1-2.
The legend of Table 1 has revised: “Ten most common Finnish epidemic MRSA strains analyzed by PFGE (one per person) 1997-2004”.
A row and a column have been added with totals, as suggested.
The ST and SCCmec type have been added as suggested.
Two footnotes have been added for clarifications. Now they are also consistent with Material and Methods (page
MRSA strains include related patterns with a 1-6 band differences.
This represents only FIN-2 subtype

Table 2.
A column and a row have been added with totals, as suggested.
The place of the footnote b was changed to the correct column, as suggested.

Minor comments
NIDR and KTL have been clarified in the text, see page 5, lines 7-8.
Related or possible related types have been removed and the terminology has been clarified, see M&M, page 5, line 22 and page 6, lines 1-17.
The study period has been specified in order to make the difference with the survey period, see page 6, line 10, as suggested.
It is true, that all MRSA strains have been typed, and if persons showed two different strains during the same year, we included all different strain types in our study. This has been added to page 6, line 12.
The sentence of representative isolates has been rewritten: “Representative isolates, one per PFGE type, were further characterized…” as suggested; see page 6, line 13. The types and subtypes have been clarified; see page 5, line 22 and page 6, lines 1-2.
The sentence “During 2001-2003, a more detailed surveillance…” was added to page 7, lines 7-8, as suggested.
The sentence “During 1997-2004, 4026 MRSA notifications…” was revised and added to page 8, line 4.
It is true, that the relative proportion of sporadic MRSA decreased, and we have removed the comment of the decreasing trend on sporadic strains, as suggested, see page 8, lines 19-20.
The sentence “At the beginning of the study period (1997-2001)…” was added for clarification. (The referee suggested “During the first part of studied period…”), see page 9, line 4.
The sentence “after exposure of MRSA” has been clarified; there is a new chapter “Control of MRSA” on page 6, lines 19-23 and page 7, lines 1-5, and it has been removed from its original place, because the survey data has been added into Table 2, as referee earlier suggested.
The sentence “During the survey period (2001-2003)…” was revised and added to page 14, line 12.