Author’s response to reviews

Title: Diagnosis of central-venous catheter colonizations using cultivation-dependent and -independent techniques

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Author’s response to reviews: see over
Dear Editor

We are grateful for the suggestions for improvements from the two reviewers and hope the significantly revised manuscript will be accepted for publication. Below please find our responses to the reviewer’s comments. Figure 1 has been revised in addition. A native English speaking colleague has read and corrected the paper according to your advice.

Sincerely,

Trine Rolighed Thomsen

Response to reviewers, MS: 1962346585188520
Diagnosis of central-venous catheter colonizations using cultivation-dependent and-independent techniques by Mette KS Larsen, Trine R Thomsen, Claus Moser, Niels Høiby and Per H Nielsen

REVIEWER 1
Major Compulsory Revisions
1) This paper deals with the suitability of molecular techniques (rRNA gene sequencing, DGGE and FISH) compared to the conventional culture-dependent procedures (Maki semi-quantitative method) in the laboratory diagnosis of CVC-related infections. However, according to most microbiologists and clinicians expert in this field, a realistic approach for routine diagnosis of CVC-associated infections is based on the combined use of semiquantitative catheter tip culture and conventional peripheral blood cultures, since these procedures are considered to be reliable, easy to be performed and cost effective. On the other hand, a Gram staining of skin swabs collected at the CVC insertion site and at the inner surface of the hub could provide a rapid and inexpensive method for the diagnosis of CRBSI. This method that have high negative predictive value, may be coupled with other techniques such as the measurement of the differential time to positivity between hub blood (taken from the catheter port) and peripheral blood cultures, thus facilitating in situ diagnosis. Further, Gram staining and the acridine-orange leucocyte cytospin test on through-catheter blood culture have been proposed for rapid diagnosis of CRBSI without catheter removal. In conclusion, today there is a large consent on the use of the above listed culture-dependent procedures that authors of the manuscript have not mentioned and/or discussed at all.

These topics have now been included in the paper- both in the introduction and discussion.

2) It’s surprisingly scarce the list of microbial species isolated and identified by culture-dependent procedures and these are not described in details in M&M. In particular, it’s unclear why species easy to cultivate as Enterococcus spp., Serratia spp., Acinetobacter spp., Stenotrophomonas spp., Micrococcus spp., etc. were only detected by molecular methods. Were all these bacterial species only colonizing the inner lumen? Such a result, according to the literature data, could appear quite unusual.

Table 2 provides the cultivation data and these were the microorganisms identified. We have also included some blood cultivation data in Table 1 and the list of bacteria detected by cultivation is
now increased. The results are discussed further and for instance this has been included: Importantly, the cultivation approach could have been more intensive in this study, using e.g. sonication to remove cells or anaerobic cultivation techniques, which would probably have resulted in a higher diversity in the detected microorganisms.

3) Although the paper provides useful informations on the large variety of microbial species that can be possibly involved in catheter colonization and associated infections, its weakness is also represented by the poor acceptance that the above mentioned molecular techniques could find in clinical microbiology labs. Thus, the authors statement (page 3,lines 47-49) “ The results presented in this study supports (?) molecular detection (?) should be included in international diagnostic on removed catheters if a central catheter-related infection is suspected” is certainly unappropriate.

This has been changed to: The results show that diagnosis based on molecular methods improves the detection of microorganisms involved in central catheter-related infections. The importance of these microorganisms needs to be investigated and this information can be used for development of fast and more reliable diagnostic tools, which can be used in combination with traditional methods.

REVIEWER 2
Major Compulsory Revisions:
1. Abstract: The authors state: “…many other bacteria … were also found stressing that only a minor part of the species present was found by cultivation.” Although this is likely to be true, what evidence do the investigators have that establish that the organisms were not introduced through inadvertent contamination following catheter removal.

Contamination has generally been avoided but can not be excluded as a possibility. We have integrated the following in the paper in different sections: “All catheters sent for microbiological investigation were generally handled under aseptic conditions”......And: “Negative controls including water and PCR mix were included for every five samples and were always negative indicating there was no contamination of the reagents. Stringent procedures were generally employed to avoid contamination e.g. using a PCR cabinet with UV light and all DNA handling was done with aerosol filter pipette tips to avoid cross contamination”

Minor Essential Revisions:
2. Methods: Because other investigators have noted significant differences in technique for doing catheter tip cultures, it would be important to note if the cultures were always performed by the same individual or if training on the appropriate technique was performed.

The culture work was always performed by the same trained personal, and this has now been integrated in the manuscript according to the reviewer’s suggestion.

3. Methods: There appears to be a typographical error on lines 119-120: “More than 15 cfu were denoted with + and less than 15 cfu were denoted with (+).”

This is the standard way of writing culture results at Copenhagen University Hospital: Many cfu is denoted with a plus and less than 15 cfu is written with a plus in parentheses. We can change this if necessary.
4. The “molecular methods” and “clonal libraries” portions of the Results section includes data that belong in the Methods section. This has been removed and/or moved to the correct section.

Discretionary Revisions:
5. Introduction: The introduction should be more concise.
We have tried to make the introduction more concise according to the reviewer’s suggestion.

6. Small sample size with significant differences among patients for the indication for catheter tip analysis (positive blood cultures vs. “routine”)
Yes, the sample size is small and this study just indicates a large microbial diversity seems to be present on the investigated catheters. This needs to be investigated further.

7. The Results section should be more concise.
We have tried to make the result section more concise according to the reviewer’s suggestion.