Review of: "A Rapid and Robust DNA Extraction Method for PCR-Based Diagnosis of V. cholerae"

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Potential competing interests: No potential competing interests to declare.

I really appreciate this study. The relevance addresses a significant problem in the field of diagnostic microbiology by proposing a rapid and effective method for the extraction of Vibrio cholerae DNA for PCR-based diagnosis. Rapid detection is crucial in cases of cholera outbreaks, which makes the contribution of the study highly relevant to public health.

The proposed DNA extraction method, which uses only sterile distilled water for bacterial lysis, offers a simple, rapid, and low-cost alternative compared to traditional methods that require culture media and expensive chemicals. This is a significant advance, especially for laboratories with limited resources.

The study validates the proposed method on a considerable number of V. cholerae strains (44 strains), including different variants (El Tor and Haitian variants), which strengthens the reliability of the results. The demonstration of the efficacy of the method under laboratory conditions and the direct comparison with the traditional method reinforce the applicability of the proposed method in real situations, such as cholera outbreaks.

In my opinion, the study does not compare the proposed method with other rapid DNA extraction methods that may be available on the market. This limits the assessment of the relative performance of the proposed method. Because of this, I suggest comparing it with the gold standard.

Although the study validated the method in V. cholerae strains, strains of other pathogens were not explored, which could demonstrate the versatility and applicability of the method for other relevant bacteria. The method was validated in cultured strains and in simple clinical samples (rectal swab samples), but more complex clinical samples, such as contaminated water or food samples, which may present additional challenges for DNA extraction, were not tested. The sensitivity of the method, $1.5x10^3$ CFU per assay, while acceptable, is lower compared to other molecular techniques that can detect lower bacterial loads. This may limit the usefulness of the method in cases of infections with low bacterial load.

Evaluation of Methods, Results, and Data Interpretation:

* Methods: The proposed DNA extraction method is well described and easy to replicate, which is a strength. However, the absence of a comparison with other rapid DNA extraction methods is an important omission that would have strengthened the study. In addition, it would have been interesting to include a more detailed cost-benefit analysis to demonstrate the economic advantages of the method in different scenarios.

* Results: The results presented demonstrate that the proposed method is effective for DNA extraction from V. cholerae, with successful amplification of the target genes in all PCR assays. The specificity of the method was also confirmed, since there was no cross-amplification with other bacterial species. However, the interpretation of the results could have been strengthened by a more in-depth discussion of the potential limitations of the method in more varied clinical samples.
* Data Interpretation: The interpretation of the data is generally coherent and well-founded. The authors correctly highlight the applicability of the method in outbreak situations and in laboratories with limited infrastructure. However, a more detailed discussion of the potential limitations of the method, especially in terms of sensitivity and applicability to other bacteria, would have been useful.

In summary (in my opinion), this study presents a promising methodology for the rapid and efficient extraction of V. cholerae DNA for PCR-based diagnostics, with potential for implementation in public health settings, especially in resource-limited settings. However, for the method to be widely adopted, it would be necessary to expand the validation to include other clinical samples (like cross-reaction presentation, or other infectious parasitic diseases) and compare it with other available rapid DNA extraction methods (One well-known rapid DNA extraction method available on the market is the “Chelex 100” method, which is commonly used for extracting DNA in a quick and efficient manner, and a Qiagen QIAprep Spin Miniprep Kit). Furthermore, exploring the applicability of the method to other relevant pathogens could significantly increase the impact of this work.