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Strengthening The Organizing and Reporting of Microbiome Studies (STORMS)

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Abstract

Background: Human microbiome research is interdisciplinary, making concise organizing and reporting of results across the different styles of epidemiology, biology, bioinformatics, and statistics a challenge. Commonly used reporting guidelines for observational or genetic studies lack key aspects specific to microbiome studies.
Methods: A multidisciplinary group of microbiome researchers reviewed elements of available reporting guidelines for observational and genetic studies, and adapted these for application to human microbiome studies. New reporting elements were developed for laboratory, bioinformatic, and statistical analysis specific to microbiome studies, and other parts of these checklists were streamlined to keep reporting manageable.
Results: STORMS is a 18-item checklist for reporting on human microbiome studies, organized into six sections covering all sections of a scientific publication, presented as a table with space for author-provided details and intended for inclusion in supplementary materials.
Conclusions: STORMS provides guidance for authors and standardization for interdisciplinary microbiome studies, facilitating complete and concise reporting.

Availability: STORMS is downloadable as a versioned spreadsheet from storms.waldronlab.io.

Introduction

Reporting the results of human microbiome research is challenging because it often involves approaches from microbiology, genomics, biomedicine, bioinformatics, statistics, and epidemiology. Combined with the novelty of the field, this has resulted in the development and application of a variety of methodological approaches, with inconsistent reporting of methods and results. While some efforts have been made to address reporting standards in microbiome

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studies, (1) no comprehensive standardized guidelines spanning laboratory and epidemiological reporting have been proposed.

Reporting guidelines promote consistency in reporting and, as a consequence, encourage reproducibility and improved study design. Editorial adoption of the CONsolidated Standards Of Reporting Trials (CONSORT) guidelines, for example, has been associated with an increase in trial quality scores.(1,2) Other epidemiological reporting guidelines have seen broad adoption, such as Strengthening the Reporting of OBservational studies in Epidemiology (STROBE) and STrengthening the REporting of Genetic Association Studies (STREGA). The Enhancing the Quality and Transparency of health Research (EQUATOR) website lists over 400 research reporting guidelines, but none as of the time of writing address studies of the human microbiome and health.(3)

Epidemiological studies of the human microbiome face special considerations compared to other epidemiological studies and thus require specific reporting standards. In addition to standard elements of epidemiological study design, microbiome studies involve collection and handling of biological specimens, evolving approaches to laboratory processing with the potential for batch effects, bioinformatic processing, statistical analysis of high-dimensional data, and reporting of results on potentially thousands of microbial measures.(4–6) The field has not reached consensus on many of these aspects, so inconsistencies in reporting inhibit reproducibility and hamper efforts to draw conclusions across similar studies.

For these reasons, we convened a multi-disciplinary working group to develop guidelines for microbiome study reporting. Members of this group include epidemiologists, biostatisticians, bioinformaticians, and microbiologists. The checklist is designed to balance completeness with burden of use, and is applicable to a broad range of human microbiome study designs and analysis. The “Strengthening The Organizing and Reporting of Microbiome Studies (STORMS)” checklist can serve as a tool to organize study planning and manuscript preparation, to improve the clarity of manuscripts, and for reviewers and readers to assess these studies.

Methods

Origin and development

The origins of this project are rooted in a systematic review of papers examining the role of various microbiome sites and disease. The goal of this project is to curate and release a publicly available, standardized database of microbiome study findings to aid future research. This review has revealed a large amount of heterogeneity in reporting, particularly around concepts of epidemiology such as study design, confounding, and sources of bias, but also microbiome-specific issues around statistical approaches to test for and measure relative abundance, and the extent to which potential bias from batch exists are addressed. This heterogeneity highlighted the need for standardized reporting guidelines, similar to those used

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in other fields of study. The curators determined that standardized reporting guidelines would streamline the review process, but would more importantly help researchers throughout the field of microbiome research communicate their findings effectively.

A multidisciplinary group of bioinformaticians, epidemiologists, biostatisticians, and microbiologists was convened to discuss microbiome reporting standards. The group began by reviewing existing study reporting standards including STROBE,(7) STREGA,(8) MICRO,(4) and STROGAR.(5) The group also reviewed existing articles containing recommendations for microbiome reporting.(9,10) The STROBE and STREGA guidelines were used as a starting point for the STORMS checklist, although inspiration was drawn from the other reporting standards reviewed as well.

Working along the reporting standards development guidelines recommended by EQUATOR, a comprehensive list of potential guideline items was created. From this list, group members added, modified, and removed items based on their expertise. After the first round of edits, the checklist was then applied to a recent microbiome study by group members. Comments, removals, and additions were harmonized after each round. Based on this process, additional changes, simplifications, and clarifications were made with the goal of creating a streamlined and user-friendly checklist. This process was repeated until there was a group consensus that the checklist was ready for use. Outside subject matter experts were invited to review the guidelines and provide feedback and additional revisions were made based on their comments.

Results

Checklist

The latest version of the checklist at time of publication is presented in Table 1. Of the items in the latest version in the STORMS checklist, nine items or subitems were unchanged from STROBE, five were modified from STROBE, one was modified from STREGA, and 27 new guidelines were developed. Rationale for new and modified items are presented below. Documentation of items unmodified from STROBE and STREGA were presented in the publications of those checklists.

Abstract

Along with commonly included abstract materials such as a basic description of the participants and results, authors should report the study design(11)--such as case-control, cohort, or randomized control trial--in the abstract of their article (Item 1.0), as required by other reporting guidelines. Communicating study design in the abstract allows readers to quickly categorize the type of evidence provided.

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Introduction

The introduction should clearly describe the underlying background, evidence, or theory that motivated the current study (Item 2.0). Among other possibilities, this could include pilot study data, previous findings from a similar study or topic, or a biologically plausible mechanism that has been proposed. This clarifies for the reader the motivations for the present study. If the study is exploratory in nature, explain what motivated the current exploration and the goals of the exploratory study.

Methods

Participants

The methods section should contain sufficient information for study replicability. When describing the participants in the study, the population of interest should be described and then how participants were sampled from the target population should be reported (Item 3.0). Because participant characteristics such as environment,(12) demographics,(13) and geography(14) can have important effects on the microbiome, it is essential to include this description. Specific criteria used to assess potential participants for eligibility in the study should also be reported, including both inclusion and exclusion criteria (Item 3.1). This is expanded from STROBE which requires eligibility criteria, but does not specify that both inclusion and exclusion criteria should be reported in detail.

The final analytic sample size should be stated and the reason for any exclusion of participants at any step of the recruitment or laboratory processes (Item 3.2). STROBE suggests using a flow diagram to show when and why participants were removed from the study. If participants were lost to follow-up or did not complete all assessments in a longitudinal study, time-point specific sample sizes should also be reported (Item 3.3). Additionally, studies that matched exposed to unexposed participants should describe what variables were used in matching (Item 3.4).

Laboratory

Describe laboratory methods in sufficient detail to allow replication. The handling of lab samples should be described, including sampling procedure (Item 4.0), storage, handling, processing, and contaminant analysis or negative controls (Item 4.3). Batch effects should be discussed as a potential source of confounding, including steps taken to ensure batch effects do not overlap with exposures or outcomes of interest (Item 4.1).(15) Library preparation, sequencing platform, and references to protocols with versions, should be stated (Item 4.4).

Data sources/measurement

For non-microbiome data (e.g. health outcomes, participant socioeconomic characteristics, environmental variables), the measurement of each variable should be described (Item 5.0). For instance, participant gender and age could be obtained from electronic medical records or from

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a questionnaire distributed to participants; this data source should be described. Limitations of measurement may also be discussed including potential bias due to misclassification or missing data as well as any attempts made to address these measurement issues.

Research design considerations for causal inference

Observational data is often used to test associations that aim to infer cause and effect. Methods include, for example, the use of multivariable analysis or matching to adjust for confounding variables that lie on a common causal path between a hypothesized exposure (such as abundance of a microbial taxon) and the disease or condition under study. If variables are adjusted for in the analysis, theoretical justification for inclusion of these variables should be provided (Item 6.0). Consider including a directed acyclic graph showing the hypothesized causal relationships of interest.(16) Additionally discuss the potential for selection or survival bias (Item 6.1). For example, such bias may occur due to loss-to-follow-up (in longitudinal studies) or due to participants not being included in the study due to the condition itself (e.g. participants who have died of aggressive forms of colorectal cancer have not survived to be in a hypothetical study of colorectal cancer microbiomes).(17)

Bioinformatics and Statistical Methods

Adequate description of bioinformatic and statistical methods is essential to producing a rigorous and reproducible research report. Transformations of quantitative variables (such as normalization, rarefaction, and percentages) should be described (Item 7.0). All statistical methods used to analyze the data should be stated, (Item 7.1) including how results of interest were selected (e.g. using a p-value or other threshold) (Item 7.6). In the interest of reproducibility, all software, packages, databases, and libraries used for the analysis of the data should be described and cited including version numbers (Item 7.7).

Results

Outcome Data

The main outcomes of the study should be detailed including descriptive information, findings of interest, and the results of any additional analyses. Descriptive microbiome analysis (for instance, dimension reduction such as Principal Coordinates Analysis, measures of diversity, gross taxonomic composition) should be reported for each group and each time point (Item 9.0). This contextualizes the results of differential abundance analysis for readers. When reporting differential abundance test results, the magnitude and direction of differential abundance should be clearly stated (Item 9.1) for each identifiable standardized taxonomic unit (Item 9.2). Some results (e.g. non-significant results) can be included in supplements, but should not be excluded entirely. Although the problem has been known for decades,(18) journals across many fields are recognizing the issue of publication bias and therefore the issue of non-reporting of null results.(19) Including such results in publications will help to reduce the severity of this bias and improve future systematic reviews and meta-analyses.

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Discussion

Most recommendations for the Discussion section remain unchanged from STROBE. One additional recommendation is made: discuss the potential for bias and how they would influence the study findings (Item 12.1). Many forms of bias such as measurement bias or selection bias(20) could affect the interpretation of the results of the study and it is important to acknowledge potential sources of bias when discussing the results.(21) If different forms of bias were not assessed or assumed to be negligible, this should be stated.

Other Information

Reproducible research practices serve as quality checks in the process of publication and further transparency and knowledge sharing.(18) Journals are increasingly implementing reproducible research standards that include the publishing of data and code and those guidelines should be followed when possible.(23,24) STORMS itemizes the accessibility of data and code (Items 18.0 through 18.4). If data or code are not made publicly available, an explanation should be given.

Discussion

The STORMS guidelines can improve the quality and communication of studies of the human microbiome, by introducing a shared grammar of study reporting for the field. They encourage reproducibility and open data sharing, and ease barriers to conducting systematic reviews and meta-analyses. The development of STORMS is an ongoing process and new versions of the checklist will be released to reflect evolving standards and technological processes. We invite interested readers to join the working group by contacting the corresponding author or by visiting the working group website for more information (storms.waldronlab.io). We also encourage journals to include the STORMS checklist in their instructions to authors and advise peer reviewers to consult the checklist when reviewing submissions.

There are some limitations to the STORMS checklist. The checklist was not created to assess study or methodological rigor. It is meant to make it easy for readers to assess how a study was conducted and analyzed. Conclusions about the quality of studies should not be made based on their adherence to STORMS guidelines, although it is our hope that the reporting guidelines will help readers review studies critically. It does not encourage, discourage, or assume the use of null hypothesis significance testing(22) or methods of compositional data analysis,(23) topics of some controversy in the field. In general the checklist avoids reference to or guidance on specific statistical methodological decisions.

The working group believes that the STORMS checklist is sufficiently flexible and “user-friendly” to support widespread adoption and contribution to microbiome study standards.

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Its adoption will encourage thoughtful study design, study reproducibility, collaboration, and open knowledge sharing between research groups as they explore the human microbiome.

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Number	Item	Recommendation	Source	Additional Guidance	Yes/No/NA	Comments	Relevant Text from article
Abstract							
1.0	Study Design	Study design is stated in the abstract.	STORMS	Examples: case-control (participants with condition of interest are matched to controls), cross-sectional (data collected at one time point, no matching done between cases and controls), randomized controlled trial (condition is randomly assigned to participants), time series (participants are followed over time to observe changes), cohort (participants are followed over time to see who develops the condition of interest and who does not). For more information about common study designs see: https://doi.org/10.1592/phco.30.10.973			
Introduction							
2.0	Background and Rationale	State underlying background, scientific evidence, or theory driving the current hypothesis. If the study is exploratory, state what drives the current exploration.	STORMS				
Methods							
3.0	Participants	State what the population of interest is, and the method by which participants are sampled from that population.	STORMS	Examples of the population of interest could be: adults with no chronic health conditions, adults with type II diabetes, newborns, etc. This is the total population to whom the study is hoped to be generalizable to. The sampling method describes how potential participants were selected from that population. This includes the geographic region where participants were sampled from.			
3.1	Eligibility criteria	List any criteria for inclusion and exclusion of recruited participants.	Modified STROBE	Among potential recruited participants, how were some chosen and others not?			
3.2	Analytic sample size	Explain how the final analytic sample size was calculated, including the number of cases and controls if relevant, and reasons for dropout at each stage of the study. This should include the number of individuals in whom microbiome sequencing was attempted and the number in whom microbiome sequencing was successful.	STORMS	Consider use of a flow diagram (from STROBE).			
3.3	Loss to follow-up	For longitudinal studies, describe sample size at follow-up by group or condition and discuss any loss to follow-up.	STORMS	If there is loss to follow-up, discuss the likelihood that drop-out is associated with exposures, treatments, or outcomes of interest.			
3.4	Matching	For matched studies, give matching criteria.	Modified STROBE	"Matched" refers to matching between comparable study participants as cases and controls or exposed / unexposed.			
4.0	Laboratory	State the body site sampled from and how samples were collected.	STORMS				
4.1	Batch effects	Discuss any likely sources of batch effects, if known. Detail any blocking or randomization used in study design to avoid confounding of batches with exposures or outcomes.	STORMS	Batch effects are unavoidable in all but the smallest genomic studies, and can be introduced by subtle differences in sample collection, storage, library preparation, and sequencing.			
4.2	Storage	Storage. State the laboratory/center where laboratory work was done. Describe how the laboratory stored samples, including time between collection and storage as well as any preservation buffers used.	STORMS	State where each procedure or lot of samples was done if not all in the same place.			
4.3	Laboratory methods	Describe laboratory methods including (where relevant): collection, shipping, extraction (including kit and version), human DNA removal (if applicable), amplification, primer selection, and contaminant analysis/negative controls.	STORMS	For amplicon sequencing (for example, 16S variable region), state the region selected. If enrichment is performed (e.g. for viromes), include here. Note any modifications of lab protocols and the reason for protocol modifications.			
4.4	Sequencing	Describe sequencing methods and platforms as well as any profiling software used (name and version).	STORMS				
4.5	Controls	Describe positive and negative controls used in extraction, sequencing, preprocessing, and/or analysis.	STORMS				
5.0	Data sources/ measurement	For each non-microbiome variable, including the health condition of interest, state how it was measured or collected.	STORMS	State any sources of potential bias in measurements, for example multiple interviewers or measurement instruments, and whether these potential biases were assessed or accounted for in study design.			

Number	Item	Recommendation	Source	Additional Guidance	Yes/No/NA	Comments	Relevant Text from article
6.0	Research design for causal	State which variables are controlled and state the rationale for controlling for them within your hypothesized causal framework.	STORMS	For example, hypothesized confounders may be controlled for by multivariate adjustment, but colliders or mediators should not be. Consider using a Directed Acyclic Graph (DAG) to sumarize hypothesized causal pathways.			
6.1	Selection bias	Discuss potential for selection or survival bias.	STORMS	Selection bias can occur when some members of the target study population are more likely to be selected for the study than others. Some examples include survival bias (where part of the target study population is more likely to die before they can be studied), convenience sampling (where members of the target study population are not selected at random), and loss to follow-up (when probability of dropping out is related to one of the things being studied).			
7.0	Bioinformatic and Statistical Methods	Describe any transformations to quantitative variables used in analyses (e.g. use of percentages instead of counts, normalization, rarefaction, categorization).	STORMS				
7.1	Statistical methods	Describe all statistical methods.	Modified STROBE				
7.2	Subgroup analysis	Describe any methods used to examine subgroups and interactions.	STROBE				
7.3	Missing data	Explain how missing data were addressed.	STROBE				
7.4	Methods for sampling	If applicable, describe methods taking into account of sampling strategy/survey design.	Modified STROBE	This could include using methods that adjust for stratification, clustering, or sample weighting.			
7.5	Sensitivity analyses	Describe any sensitivity analyses.	STROBE				
7.6	Findings	State criteria used to select findings for reporting.	STORMS	For example, false discovery rate with total number of tests, effect size threshold, significance threshold, microbes of interest.			
7.7	Software	Cite all software (including read mapping software) and databases (including any used for annotating amplicons, if applicable) used. Include version numbers.	Modified STREGA	For R, installed package versions should be stated and cited in addition to the version of R used.			
Results							
8.0	Descriptive Data	Give characteristics of study participants (e.g. dietary, demographic, clinical, social) and information on exposures and potential confounders.	STROBE	Typically reported in Table 1. Indicate number of participants with missing data for each variable of interest This includes environmental and lifestyle factors that may be important to the relationship between the microbiome and the condition of interest. Participant diet and medication use should be summarized, if known. At the very minimum, age and sex of all participants should be reported.			
9.0	Outcome Data	Report descriptive findings for microbiome analyses by group and (if applicable) by time.	STORMS				
9.1	Differential abundance	Report results of differential abundance analysis by group and (if applicable) by time, clearly indicating the direction of change.	STORMS				
9.2	Taxonomy	Identify taxonomy using standardized taxon classifications that are sufficient to uniquely identify taxa.	STORMS	If not using full taxonomic hierarchy, make sure it is clear whether names stated are species, genera, family, etc. Italicize genus/species pairs.			
10.0	Other analyses	Report other analyses done—e.g. analyses of subgroups and interactions, and sensitivity analyses.	STROBE				
Discussion							
11.0	Key results	Summarise key results with reference to study objectives	STROBE				
12.0	Limitations	Discuss limitations of the study, taking into account sources of potential bias or imprecision.	STROBE				
12.1		Discuss any potential for bias to influence study findings.	STORMS				
13.0	Interpretation	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	STROBE	Define or clarify any subjective terms such as "dominant," "dysbiosis," and similar words used in interpretation of results.			
14.0	Generalizability	Discuss the generalisability (external validity) of the study results	STROBE				
Other information							

Number	Item	Recommendation	Source	Additional Guidance	Yes/No/NA	Comments	Relevant Text from article
15.0	Funding	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	STROBE				
16.0	Supplements	Indicate where supplements may be accessed and what materials they contain	STORMS				
17.0	Supplementary data	Provide a supplementary spreadsheet of results with for all taxa and all outcome variables analyzed. Indicate the taxonomic level of all taxa.	STORMS	Depending on the analysis performed, examples of the supplemental results included could be mean relative abundance, differential abundance, raw p-value, MHT-adjusted p-values, and standard error. All discussed taxa should include the taxonomic level (e.g. class, order, genus)			
18.0	Reproducible research	(a) State where raw data may be accessed including demultiplexing information.	STORMS				
18.1	Processed data access	(b) State where processed data may be accessed.	STORMS				
18.2	Participant data access	(c) State where participant data may be accessed.	STORMS				
18.3	Source code access	(d) State where code may be accessed.	STORMS				
18.4	Full results	(e) Provide full results of all analyses, in computer-readable format, in supplementary materials.	STORMS	For example, any fold-changes, p-values, or FDR values calculated.			