

PCR-based Detection of Diarrheagenic Pathogens in Improved Water Sources: a Scoping Review Protocol of the Evidence in Low- and Middle-income Countries

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Study protocol

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Abstract

Background: Occurrence of diverse human enteric bacterial, viral and protozoal pathogens in drinking water samples because of fecal contamination is of increasing public health concern, particularly in developing countries. Application of Polymerase Chain Reaction (PCR) based methods in detecting microbial quality of water provides more accurate, sensitive and rapid outcomes over conventional methods of microbial identification and quantification. Therefore, exploring water quality outcomes generated through PCR based methods is important to better understand the status and monitor progress towards internationally set goals for low- and middle-income countries. The aim of this scoping review is to map the existing evidence on the magnitude and characteristics of diarrheagenic pathogens as detected by PCR-based methods in improved water sources within the context of low- and middle-income countries.

Methods: This scoping review will be undertaken in line with the JBI methodology for scoping reviews. We will consider studies from 2003 onwards that included PCR-based microbial water quality assessment of improved drinking water sources in low- and middle-income countries. The searches will be undertaken in PubMed/Medline, Scopus, Web of Science, JBI, Cochrane Library, and Google Scholar. A gray literature search will be conducted in Google and ProQuest.

Discussion: This review will systematically discover and integrate the evidence available on the detection of diarrheagenic pathogens through the application of PCR based methods for water quality determination. In this review, information about the applied PCR method, detected diarrheagenic pathogens, water sample size, effectiveness in terms of time and cost, will be gathered and summarized. Diagrammatic and/or tabular presentation supported with a narrative synthesis will be used. The review will be reported according to a reporting guideline for scoping reviews: PRISMA extension for scoping reviews (PRISMA ScR).

Background

Genetic diversity of human enteric bacterial [1, 2], viral [3–6] and protozoal [7] genomes in water samples are becoming increasing and complicated as a result of contamination of improved water at the sources, collection and storage at home that creates increasing public health threats in low- and middle-income countries. The detection of human enteric viruses in drinking water confirms the quality limits for indicator bacteria that warrants an investigation of the potential transmission risk of infection constituted by different waterborne genomes of viruses [8, 9] and protozoans [10]. Microbial identification based on traditionally pure cultures grown in laboratory has limitations in which most microorganisms cannot grow [11] and only < 1% of natural microorganisms are culturable [12]. For long time these nonculturable but harmful microorganisms are ignored in the microbiology science. Therefore, a method that provides a relatively unbiased picture on the species type, quantity, distribution and functionality of pathogenic microorganisms in water supply within the context of low and middle income countries is essential. This can help inform the Sustainable Development Goal (SDG) monitoring of drinking water in terms of safety related requirements of the indicator: safely managed drinking water services (SMDWS).

SMDWS refers to mean water from improved source, free from contamination, accessible on premises and available when needed [13]. For successful monitoring of SMDWS, reliable water quality outcomes generated using effective methods of characterizing variety of pathogens in water samples, beyond bacterial presence/absence and colony count, is important [14]. Studies showed that false positive results by different techniques of pathogenic microbes' detection methods [15–19] in water samples mislead the actions to be taken to improve the water quality. Thus, fresh perspectives on molecular technique based on polymerase chain reaction (PCR) was applied on drinking water since 2003 followed by identification of the microbiome in human gut in 2006 [11].

PCR is a simple chain reaction method applied for genome-wide analysis through a three-step procedure involving information coding of nucleic acids (DNA/RNA, mRNA) and protein. The steps are: (1) Denaturing: which is the -breakage of the helical structure using heat to separate the defined substrate (DS) or the DNA. (2) Annealing: which is the strengthening of primers to single stranded (SS) DNA. (3) Elongation, which is the movement of ribosome from the 5'- end (phosphate group) to the 3'- end (hydroxyl group) of the mRNA, which will be used as a template to make new DNA [20, 21]. The methods can be used for a range of applications, from detecting up to quantification of bacterial, viral and protozoal pathogens in small quantities (1–2 microliters) of water samples [22].

PCR methods are powerful molecular biology techniques for the detection of target DNA in various samples and for the detection of many kinds of pathogens. PCR, compared to conventional methods, increases the overall detection frequency of the bacterial enteropathogens, measures specific gene expressions, and detects enteric viruses and viable but nonculturable (VBNC) pathogens in water samples [23, 24], more rapidly [25]. Moreover, there are PCR techniques such as real-time PCR that can distinguish the viable cells from dead cells even though it requires technical expertise and cost for master mixes and targeted primers [22].

However, this development in method of identification and quantification of pathogens in water samples was not up taken by the low- and middle-income countries as required despite molecular assays are needed for broad access in the developing world [26] to assist in comprehensive and accurate assessments of water quality [27]. Because of the advances in pathogenic microbial testing methods, better

understanding about newly emerging waterborne diarrheal pathogens is happening. For example, reports showed increasing number of different microbial genotypes [28]. Water quality results obtained through PCR methods would give better picture in providing sensitive and rapid results [29], which in turn contributes in addressing the SDG target for water supply. PCR method can be applied as microbial source-tracking tool (MST) for generating more credible information that can inform monitoring the progress towards meeting SDG targets in low- and middle-income countries. These multiple initiated this work, which primarily focuses on the available evidence regarding the magnitude of diarrheagenic pathogenic microbes in water by application of PCR method/s in low- and middle-income countries.

We performed an initial search on selected databases with the aim to identify previously published or ongoing systematic reviews or scoping reviews on a similar topic. The databases include the Joanna Briggs Institute (JBI) Database of Systematic Reviews and Implementation Reports, MEDLINE (PubMed), the Cochrane Database of Systematic Reviews, Google and Google Scholar. No relevant reviews addressing the magnitude and characteristics of diarrheagenic pathogens in improved water samples using PCR detection and enumeration techniques in low- and middle-income countries were identified. This scoping review will follow the JBI approach for conducting scoping reviews [30].

The purpose of this scoping review, therefore, is to map the existing evidence on the magnitude and characteristics of diarrheagenic pathogens as detected by PCR-based methods in improved water sources within the context of low- and middle-income countries.

Review question

The scoping review will be guided by the question, 'What are the types, magnitude and concentrations of waterborne pathogens detected in improved water samples from low- and middle-income countries through PCR method?'

Methods

Study design and protocol

The research question is best addressed through a Scoping review, as it is broad and aims to map all the available evidence on the topic. This scoping review will be undertaken in line with the JBI methodology for scoping reviews.

Inclusion criteria

Types of water sources

For the purpose of this review, we will consider studies that included PCR-based microbial water quality assessment of improved drinking water sources. We will use standard definition of 'improved drinking water source' which is defined, by the WHO/UNICEF joint monitoring program, as a water source that, by nature of its construction, is adequately protected from outside contamination, particularly faecal matter [31]. This definition includes piped water in a dwelling, plot or yard, and other improved sources such as public taps or standpipes, tube wells or boreholes, protected dug wells, protected springs and rainwater collection. This review will not include water quality data in relation to the following 'un-improved' water sources: unprotected dug well, unprotected spring, cart with small tank/drum, tanker truck, and surface water (river, dam, lake, pond, stream, canal, irrigation channels). Although it is categorized as 'un-improved' in the above definition, bottled water will be considered in this review because the reason for its categorization as 'un-improved' (i.e., in relation to 'water quantity') is not relevant for the focus of this review which is on 'water quality'.

Concept

The overarching concept of interest for this scoping review is microbial quality of drinking water in developing countries as determined by PCR-based methods. The PCR is a technique in molecular genetics that permits the analysis of any short sequence of DNA (or RNA) in samples containing only minute quantities of DNA or RNA. PCR is used to reproduce (amplify) selected sections of DNA or RNA for analysis [20]. Accordingly, this review will consider studies that identified, quantified and characterized diarrheagenic pathogens in drinking water using PCR techniques. Employed PCR detection methods and detection capacity, type of detected pathogens, detected gene numbers/copies, types and associated water borne diseases, will be explored.

Comparator

The comparators could be related to detection methodology (e.g. across different PCR tools) or other contributing factors such types of water sources (e.g. between piped water and other improved sources) or water treatment methods (between treated and untreated or across different water treatment techniques) or proximity to known contamination sources. Other comparators that may be identified along the review process will also be considered for the reporting.

Context

This review will include studies conducted within the context of low- and middle-income countries. The World Bank classification of economies according to 2019 gross national income (GNI) per capita will be used to identify this countries [32].

Types of studies

All studies that characterize diarrheagenic pathogens in improved water sources using PCR techniques and conducted in low- and middle-income countries since 2003 will be included in this review. These may include quantitative, and /or mixed method studies, cross-sectional studies, and intervention/experimental studies. Other relevant sources such as systematic reviews, theses, dissertations, unpublished and gray literature will also be included in this review. Conference items/abstracts will not be considered in this review to avoid potential duplication.

Search strategy

The literature search strategy consists of a 3 stage process, as recommended by the JBI [33]. In the first stage, an initial search will be undertaken in two databases (PubMed and SCOPUS). The initial search strategy for PubMed with the identified keywords is detailed in Table 1. In the second stage, titles and abstracts of the first stage articles will be reviewed to identify search terms. A full comprehensive search will be undertaken in the following databases: PubMed/Medline, Scopus, Web of Science, JBI, Cochrane Library, and Google Scholar. A gray literature search will be undertaken for unpublished studies in Google and ProQuest. In the third stage, the reference lists of the included sources literatures will be examined to identify additional sources. The search will be limited to English language.

Table 1
Search terms to be used for the preliminary search in Pubmed.

S. No	Query
1.	"polymerase chain reaction"[MeSH] OR Polymerase chain reaction[tw] OR PCR(tw)
2.	Detection (tw) OR Identification (tw) OR Characterization(tw)
3.	"diarrhea"[MeSH] OR "diarrhea"[tw] OR "diarrheas"[tw] OR "diarrhoea"[tw] OR "diarrhoeas"[tw]
4.	Pathogenic microbes (tw) OR "bacteria"[MeSH] OR Bacteria[Text Word] OR " <i>escherichia coli</i> "[MeSH s] OR <i>E. coli</i> [tw]OR "viruses"[MeSH] OR Virus[tw]OR Protozoa (tw)
5.	Improved water (tw) OR "drinking water"[MeSH] OR Drinking water[tw]
6.	Low- and middle-income countries (tw) OR Africa (tw) OR Asia (tw) OR Caribbean Countries (tw)
7.	1 AND 2 AND 3 AND 4 AND 5 AND 6
8.	Limit 7 to English
MeSH = Medical subject headings, tw = text word	

Study selection

All identified citations will be exported to Mendeley Desktop reference management software version 1.19.4 (Mendeley Ltd., Elsevier, Netherlands). After removing duplicates, the titles and abstracts of the identified studies will be reviewed by three independent reviewers (STG, NES and YMT). The documents without abstracts will be screened at the full text level. Then, the full text of selected studies will be retrieved and assessed in detail against the inclusion criteria by the same reviewers. Any disagreement between the three reviewers during the screening stage will be resolved through discussion or by the involvement of a fourth and fifth reviewer. For the screening of articles at full text level, rejection of an article will be decided by the review team upon suggestion of the first reader. At each stage, the number of studies excluded and

the reasons for exclusion will be archived and reported in the final scoping review. The results of the search strategy and screening process will be reported in full in the final scoping review report and presented in a Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) flow diagram [34].

Data extraction

Data will be extracted by three independent reviewers (STG, NES and YMT) from studies included in the review using a prepared data extraction excel spreadsheet tool. For each study, authors' name, place and year of publication, study period, date of search, and the country/region of the study, type of water source, water treatment technique, if any, sample type, data on sample size, types of PCR techniques used, result of included studies: detected type of pathogen/s, amount, detection capacity and associated water borne diseases will be extracted. The data extraction form will be pretested and revised as necessary during the review process. The reviewers will contact the authors for missing data and clarification of primary studies if required; such inclusions will be reported in the final scoping review. The particular study will be excluded if there is no response from the author/s.

Discussion

This review will systematically explore the evidence available on the detection of diarrheagenic pathogens in improved water samples by the application of the PCR methods in low- and middle-income countries. In this review, information about the types of PCR techniques used, result of included studies: detected type of pathogen/s, amount, detection capacity and health impact will be gathered and summarized. Diagrammatic and/or tabular presentation supported with a narrative synthesis will be used. The review will be reported according to a reporting guideline for scoping reviews: PRISMA extension for scoping reviews (PRISMA ScR) [35]. The findings from this study will provide directions for future research and improve water quality monitoring with an understanding of the importance and challenges of PCR application in informing the magnitude of pathogens in low- and middle-income countries.

Abbreviations

DNA: Deoxyribonucleic acid; GNI: Gross National Income; JBI: Joanna Briggs Institute; MST: Microbial Source-tracking Tool; PCR: Polymerase chain reaction; PRISMA: Preferred Reporting Items for Systematic review and Meta-Analyses; PRISMA-ScR: Preferred Reporting Items for Systematic review and Meta-Analyses extension for scoping reviews; RNA: Ribonucleic acid; SDG: Sustainable Development Goal; SMDWS: Safely Managed Drinking Water Services; UNICEF: United Nations Children Fund; WHO: World Health Organization.

Declarations

Ethics approval and consent to participate

As one objective of the other objectives in the fulfilment of the PhD title 'Identification and characterization of major diarrheagenic bacteria in improved water supply systems and consuming community in South Wollo, Ethiopia', this study protocol was approved by the College of Natural and Computational Science Institution (CNS-IRB), Addis Ababa University Review Board (CNSDO/729/10/2018) dated July 24, 2018.

Consent for publication

Not applicable

Availability of data and materials

Not applicable

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

Shibabaw Tadesse Gemeda (STG) deliberated of the study, and participated in its design and coordination. Involved in data extraction, critical appraisal conducting using all papers selected for inclusion in the review, drafted the protocol and included feedback from other team members. Negasa Eshete Soboksa (NES) and Yonatal Mesfin Tefera (YMT) developed the research methods and supported in the first drafting of the manuscript. Adey Feleke Desta (AFD), and Sirak Robele Gari (SRG) conducted the review, reading and shaping of the manuscript. All authors contributed to the drafting of the protocol, read, reviewed and approved the final manuscript.

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