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PCR-based detection of pathogens in improved water sources: a scoping review protocol of the evidence in low-income and middle-income countries

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ABSTRACT

Introduction Occurrence of diverse human enteric bacterial, viral and protozoal pathogens in improved drinking water because of pathogenic microbial contamination is of increasing public health concern, particularly in low-income and middle-income countries (LMICs). Detecting microbial pathogens in water supplies comprehensively and accurately is beneficial to ensure the safety of water in LMICs where water contamination is a major concern. Application of PCR-based methods in detecting the 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 LMICs. This scoping review aims to map the existing evidence on the magnitude and characteristics of diarrhoeagenic pathogens as detected by PCR-based methods in improved water sources within the context of LMICs.

Methods and analysis This study will be undertaken in line with the Joanna Briggs Institute (JBI) methodology for scoping reviews. We will consider the available publications covering PCR-based microbial water quality assessment of improved drinking water sources in LMICs. Searches will be undertaken in PubMed/Medline, Scopus, Web of Science, JBI, Cochrane Library and Google Scholar. A grey literature search will be conducted in Google and ProQuest.

Ethics and dissemination The College of Natural and Computational Science Institution Review Board of Addis Ababa University gave formal ethical approval to this study protocol. The findings of this study will be disseminated to the concerned body through peer-reviewed publications, presentations and summaries.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ Three review authors will work independently to select studies, extract data and assess quality.
⇒ To produce more comprehensive results on pathogen detection using PCR in improved water sources, this study will collect data from a variety of sources.
⇒ Only studies written in English will be included in this scoping review.

INTRODUCTION

Genetic diversity of human enteric bacterial,[1 2] viral[3–6] and protozoal[7] genomes in water is increasing and becoming complicated as a result of contamination of improved water at the sources, collection and home storage that creates increasing public health threats in low-income and middle-income countries (LMICs). Detecting microbial pathogens in water supplies comprehensively and accurately is beneficial to ensure the safety of water in LMICs where water contamination is a major concern.[8] Traditional techniques of using pure cultures grown in labs to characterise microbial water quality have limitations due to false-positive results[9–13] and inability to identify non-culturable microbes since only <1% of natural microorganisms are culturable.[14 15] The causing agent of up to 40% of diarrhoea cases cannot be identified using the culture method.[16] Water-related pathogens were known to cause persistent or acute diarrhoea in humans, dehydration-related diarrhoea in infants and children, traveller's diarrhoea, watery diarrhoea lasting up to a week, and bloody diarrhoea or dysentery.[17] Very limited attention has been paid to the non-culturable but harmful microorganisms[18 19] such as enteric toxin *Escherichia coli*[18] and *Legionella pneumophila*.[20] These are causative agents for diarrhoea and pneumonia which are particularly common in LMICs. A method to characterise microbial quality that provides a relatively unbiased picture on the species type, quantity, distribution and functionality of pathogenic microorganisms in water supply within the context of LMICs is essential. This can help to monitor the progress towards achieving the UN Sustainable

Development Goal target 6.1 (SDG6) such as safely managed drinking water services (SMDWS).[21]

For successful monitoring of SMDWS, it is important to draw on reliable water quality outcomes generated using effective methods of characterising a variety of pathogens in water samples, beyond bacterial presence/absence and colony count.[22] Fresh perspectives on the molecular technique of PCR were first described in 1985 by a scientist named Mullis Kary,[23] followed by the sequence-based molecular method that was first applied for drinking water quality assessment in 2003.[14]

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 methods are powerful molecular techniques that can be used for a range of applications, from detecting up to quantification of bacterial, viral and protozoal pathogens in small quantities (1–2 µL) of water samples.[24] Furthermore, PCR, compared with conventional methods, increases the overall detection frequency by 4% of the bacterial enteropathogens,[25] measures specific gene expressions more rapidly,[26] and detects enteric viruses and enterococci, which can appear in the form of viable but non-culturable pathogens quantitatively and rapidly in water samples.[27] Moreover, some PCR techniques can distinguish viable cells from dead cells.[24]

The advances in pathogenic microbial testing methods improves our understanding about newly emerging waterborne diarrhoeal pathogens. For example, reports showed an increasing number of different microbial genotypes[28] that can cause diseases. Water quality results obtained through PCR methods would give a better picture in providing sensitive and rapid results[29] and can be applied as a microbial source-tracking tool for generating more reliable information, which in turn contributes to addressing the SDG target for water supply in LMICs. Despite its importance, the use of PCR in the identification and quantification of water pathogens has not been taken up by the LMICs. On the other hand, the disadvantages of PCR-based water quality methods for environmental water samples, which may not be the case with samples from improved sources, are: (1) Need to isolate the microorganisms due to the dilution effect (low concentration of microorganisms per volume) or require a culture step to increase the number of microorganisms in the sample. (2) Pollutants can accumulate along with the target microorganisms for environmental water, which may not be the case for water samples from improved water. (3) Membrane clogging during filtration of environmental water samples, which is not the case with water samples from improved water sources. (4) Loss of target cells and/or nucleic acids during the various water sample preparation steps (concentration, extraction, purification), which can result in the inefficient concentration of the target microorganisms to be detected and consequent false-negative results. (5) Lengthy and complex procedures; and (6) Inhibitors are difficult to remove and are not eliminated (some have the same solubility properties as nucleic acids).[30]

The different types of waterborne pathogens detected using PCR methods, as well as their magnitude in terms of rates or concentrations in various developed countries have been outlined. In the USA, *E. coli* O157:H7 was found in drinking water at a concentration of 30–40 cells per 100 mL.[26] In another study conducted in Florida, USA, *Cryptosporidium* rates were 45%, poliovirus rates were 55% and *Giardia* rates were 41%.[7] *E. coli* cells were detected at a mean concentration of 310 cells per 100 mL isolated from rainwater tanks in Australia.[31]

This review will discuss current and emerging advanced techniques for comprehensively and accurately assessing pathogenic microbial water quality that authorities in LMICs can use. The purpose of this scoping review, therefore, is to map the existing evidence on the magnitude and characteristics of diarrhoeagenic pathogens detected by PCR-based methods in improved water sources in the context of LMICs.

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 LMICs through the PCR method?'

METHODS AND ANALYSIS

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 Joanna Briggs Institute (JBI) methodology.[32] Briefly, the first step in the scoping review is defining the review question using the Participant (Drinking Water Sources), Concept (PCR-based Microbial Quality), and Context (LMICs) or the Population, Concept and Context (PCC) framework. The PCC inclusion criteria which are discussed in detail below will be used for structuring the search strategy. After selecting the relevant studies to be included in the review, data will be extracted using a prepared data extraction excel spreadsheet tool standardised and presented in a tabular/diagrammatic form and a narrative summary in line with the scope of the review.

Inclusion criteria

Types of water sources

For this review, we will consider studies that included PCR-based microbial water quality assessment of improved drinking water sources. We will use the standard definition of 'improved drinking water source' as it is defined by the WHO/UNICEF Joint Monitoring Programme, as a water source that, by nature of its construction, is adequately protected from outside contamination, particularly faecal matter.[33] 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 about 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 categorised as 'un-improved' in the above definition, bottled water will be considered in this review as the reason for its categorisation as 'un-improved' (ie, 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 the microbial quality of drinking water determined by PCR-based methods. Accordingly, this review will consider studies that identified, quantified and characterised pathogens in drinking water using PCR techniques such as digital PCR and real-time quantitative PCR (qPCR). Employed PCR detection methods and detection capacity, type of detected pathogens, detected gene numbers/copies, types and associated waterborne diseases will be explored.

Context

This review will include studies conducted within the context of LMICs. The World Bank classification of economies according to 2019 gross national income per capita will be used to identify these countries.[34]

Types of studies

All peer-reviewed studies that characterise diarrhoeagenic pathogens in improved water sources using PCR techniques and conducted in LMICs will be included in this review. These may include quantitative and/or mixed-method studies, cross-sectional studies, and intervention/experimental studies. Conference items/abstracts will not be considered in this review to avoid potential duplication.

Search strategy

The literature search strategy consists of a three-stage process and will be structured using the PCC framework in line with the JBI scoping review methodology.[35] In the first stage, text words contained in the titles and abstracts of the articles will be reviewed. In the second stage, a fully comprehensive search will be undertaken using the following search terms: (/Improved drinking water sources) AND (/PCR-based microbial quality) AND (/Detection OR Identification OR Characterisation) AND (/Pathogenic microbes) (/Low- and middle-income countries) (see online additional file 1) for PubMed. A full comprehensive search will be undertaken in the following databases: PubMed/Medline, Scopus, Web of Science, JBI, Cochrane Library and Google Scholar. Relevant index terms will be identified and a search grid will be developed for each database with guidance of a research librarian. Only peer-reviewed full-text studies conducted in LMICs (ie, the Context) and published in English will be included. Studies that are not peer-reviewed and literature reviews will be excluded. In the third and final stage, the reference lists of the included studies will be examined to identify additional sources.

Study selection

All identified citations will be exported to Mendeley Desktop reference management software V.1.19.4 (Mendeley, Elsevier, the Netherlands). After removing duplicates, the titles and abstracts of the identified studies will be reviewed by three independent reviewers (STG, NES and YMT). The independent reviewers will create a single online Mendeley software reference management account for data extractors to share articles. The documents without abstracts will be screened at the full-text level. Then, the full texts 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 reviewer. For the screening of articles at full-text level, rejection of an article will be decided by the review team on the 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 flow diagram.[36]

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. The spreadsheet will be prepared, piloted for feasibility and modified based on the results of the pilot test. For each study, authors' name, place and year of publication, study period, date of search, 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, the result of included studies: detected type of pathogen/s, amount, detection capacity and associated waterborne diseases will be extracted. We will also include the indirect or direct viability data comprising culturable faecal indicator microbes such as *E. coli, enterococci* or other effects on free chlorine and total chlorine levels to understand the potential risk of positive detections. Viable but non-cultivable microorganisms that may be present as a result of environmental stresses or water treatment processes are not detected by culture-based methods and can therefore lead to false-negative assessments of *E. coli* in water samples.[30] SRG will reconcile the extracted data to avoid missing important information or including irrelevant information. The data extraction form will be pretested and revised as necessary during the review process. We will apply the dMIQE2020 guideline

with a simplified and modified minimum information for publication of quantitative digital PCR experiments (dMIQE) table to determine the quality of the data reported in the studies to be collected. The application of this method helps in assessing the number of nucleic acids, including molecular weight and calculations when using mass.[37] Similarly, we will use the minimum information for publication of quantitative Real-Time PCR experiment (MIQE) checklist for the real-time qPCR data.[38] The reviewers will contact the authors of primary studies for missing data and clarifications if required; such inclusions will be reported in the final scoping review. A particular study will be excluded if there is no response from the author(s).

Collecting, analysing and reporting the results

Following data extraction by independent reviewers, the studies will be classified based on the type of waterborne pathogen detected by PCR in improved water sources, detection methods and the level of contamination investigated, the setting, and the study method, as well as significant findings (see online additional file 2). We will create a useful summary that identifies and maps important evidence on the magnitude and characteristics of pathogens identified by PCR in improved water sources by combining all relevant findings from multiple sources. The data will be managed using a narrative synthesis approach, with the aim of summarising and explaining the findings of these studies. This will include a numerical summary of all of the review's findings, thematic analysis of qualitative data and summary statistics of participatory studies to summarise outcomes.

Patient and public involvement

It was not appropriate or possible to involve patients or the public in the design, or conduct, or reporting, or dissemination plans of our research.

Ethics and dissemination

The College of Natural and Computational Science Institution Review Board (CNS-IRB) of Addis Ababa University gave formal ethical approval to this study protocol. As one of the 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 CNS-IRB, Addis Ababa University Review Board (award/grant number=CNSDO/729/10/2018) dated 24 July 2018. The findings of this study will be disseminated to the concerned body through a peer-reviewed publication, presentations, and summaries, email and social media.

Contributors STG deliberated on the subject of the study, participated in its design and coordination, was involved in data extraction, critically appraised all papers selected for inclusion in the review, drafted the protocol, and mainstreamed and included feedback from other team members. NES and YMT developed the research methods and supported the first drafting of the manuscript. AFD and SRG conducted the review, and read and shaped the manuscript. All authors contributed to the drafting of the protocol, and have read, reviewed and approved the final manuscript.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

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