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Green Clinical Microbiology: Sustainable PCR-based Detection of Bacterial Pathogens in Environmental and Clinical Samples

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ABSTRACT: Antimicrobial resistance continues to challenge healthcare systems worldwide. While regularly diagnostic procedures create huge chemical, plastic, and energy waste. This may disagree with the principles of sustainable laboratory management. This study assesses sustainable strategies for molecular detection through the optimization of PCR protocols of *Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli* in clinical and environmental samples. Reduced reaction volumes, non-toxic DNA staining reagents, and energy-efficient thermal cycling conditions, were implemented as green laboratory modifications. The reason for using green laboratory modifications is to minimize resource consumption without compromising analytical performance. The improved protocol demonstrated 95% amplification success rate under the optimized conditions. Interestingly, clinical samples exhibited the highest bacterial detection rate among the tested sample types, followed by water, soil, and surface-swab samples. Significantly, the optimized system reduced reagent consumption by approximately 40%. Single-use plastic tube consumption was also reduced by 33%, from 60 to 40 tubes per run. This led to decreased plastic use and hazardous laboratory waste. Moreover, this can lower energy requirements and maintain reliable results. These results demonstrate that sustainable PCR protocols can effectively integrate environmental responsibility with high-quality molecular diagnostics. The combination of emerging procedures could improve diagnostic efficiency and reduce environmental impact. For example, microfluidic platforms, biodegradable biosensors, and automated low-volume molecular systems. The aim of this study was to assess the effectiveness of green molecular diagnostics in reducing laboratory waste, chemical use, and energy consumption while maintaining accurate pathogen detection.

INTRODUCTION

Infectious diseases remain major challenges for healthcare systems worldwide. Especially with the increase of antimicrobial resistance and the revival of pathogens from environmental sources [1]. As traditional microbiology diagnostic procedures necessary for patient care and disease control. Thus, this requires large amounts of resources and produces chemical waste, disposable plastics, and carbon emissions [2,3]. As well as pollution, climate change, and unsustainable resources further increase environmental pressure. Such as; soil, water, and air can be considered as reservoirs for pathogenic microorganisms and can lead to potential infection risks [4-6].

Classical approaches such as; culture, biochemical testing and PCR are widely used. However, they can require substantial amounts of reagents, energy and plastics resulting in large biomedical waste [7-9]. This environmental burden has led to increased interest in green clinical microbiology. This can also require maintaining diagnostic accuracy and reducing resource consumption and environmental impact simultaneously [7,10].

The application of green microbiology and sustainable technologies has encouraged energy-effective diagnostic approaches. For rapid pathogen detection using paper-based assays, microchip PCR and microfluidic platforms can reduce reagent consumption, plastic use, and energy requirements [11]. These approaches are very important to monitor important microorganisms such as *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans* which are widely spread in environmental and clinical surroundings. Moreover, they may cause high risks to weak populations [12].

During the COVID-19 pandemic, the environmental consequences of diagnostic testing became particularly evident. Because of the large-significant use of disposable plastics and chemical reagents [13]. Accordingly, green materials and safer disinfectants have been considered as sustainable substitutes. These approaches agree with the environmental protection, human health and infectious disease control [14]. Their effective application needs collaboration among microbiologists, environmental scientists and healthcare policymakers [15].

Even with these advances, the prevalent implementation of green diagnostic approaches stays restricted by cost and the absence of standardized guidelines. Thus, this study aims to evaluate green clinical microbiology responsible approaches. To reduce laboratory waste, chemical consumption, and energy use while maintaining accurate pathogen detection.

MATERIALS AND METHODS

Research Design

This study aimed to assess the use of environmentally sustainable approaches in PCR-based detection of environmental and clinical pathogens. The most important focus was on *S. aureus*, *P. aeruginosa*, and *E. coli*. All experiments were handled under Biosafety Level 2 (BSL-2) conditions to ensure safety.

Sample Collection

Environmental and clinical samples were collected between January and June 2026. In terms of Environmental samples, 18 water samples were collected from Al-Diwaniyah General Hospital taps, public fountains, and wastewater treatment facilities. On the other hand, 10 soil samples were collected from Al-Diwaniyah General Hospital gardens, urban parks, and agricultural areas. While 8 surface swabs were taken from frequently touched surfaces such as door handles, medical devices and personal items. However, 30 clinical samples were obtained from the Al-Diwaniyah General Hospital. These samples were obtained as swabs from patients with suspected microbial infections. All swabs were collected in sterile containers (Thermo Fisher Scientific, USA). After that were maintained on ice during transport and managed in 4 hours of collection. As positive controls, reference strains of *S. aureus*, *P. aeruginosa*, and *E. coli* were gained from a certified culture collection (ATCC, USA)

Laboratory Processing

Environmental samples were inoculated onto selective and differential media. For instance, *S. aureus* on Mannitol Salt Agar (Oxoid, UK), *E. coli* on MacConkey Agar (Oxoid, UK) and *P. aeruginosa* on Cetrimide Agar (HiMedia, India). After incubation, colonies were purified using streak plating. Then, confirmation the colonies were done with standard biochemical tests, including catalase (Sigma-Aldrich, USA) and coagulase (Merck, Germany).

DNA Extraction

The method of basic boiling lysis was utilized to reduce reagent consumption and maintain DNA. Each colony was suspended in 50 µL of sterile nuclease-free water (Qiagen, Germany). Then, it was heated at 95°C for 10 minutes. After that, colonies immediately cooled on ice. Then, by microcentrifuge (Eppendorf, Germany) the samples centrifuged at 12,000 × g for 5 minutes. And finally, using PCR amplification to assess the genomic DNA that was obtained from the supernatant.

PCR Amplification

Specific genes were chosen to confirm the gene of *nuc* for *S. aureus* (270 bp), *toxA* for *P. aeruginosa* (396 bp) and *blaTEM* for *E. coli* (516 bp). PCR was accomplished in final volume of 15 µL to reduce reagent use. Each reaction of PCR included 7.5 µL Master Mix (Promega, USA), 0.5 µL of forward primer (10 µM, Macrogen, Korea), 0.5 µL of reverse primer (10 µM, Macrogen, Korea), 4 µL of DNA template, and 2.5 µL of nuclease-free water (Qiagen, Germany). The cycling of PCR conditions consisted of denaturation at 95°C for 3 min. Following by 35 cycles of denaturation at 95°C for 30 s. Each gene has specific annealing, *nuc* at 55°C for 30 s, *toxA* 58°C for 30 s, and for *blaTEM* 60°C for 30 s. Then, followed by extension at 72°C for 45 s and finally the extension continued for 5 min at 72°C.

Amplicon Detection

PCR mixture was run on 1.5% agarose gels (Bio-Rad, USA) prepared with safe stain of DNA (GelRed, Biotium, USA). DNA ladder of 100 bp (Thermo Fisher Scientific, USA) was involved for each run. Bands were detected under blue-light transilluminator (Cleaver Scientific, UK) to prevent hazardous UV exposure. For subsequent analysis, Gel images were captured by using high-resolution gel documentation system (Bio-Rad, USA).

Quantitative Data Collection

Each stage of the PCR workflow was recorded such as the amount of reagents for DNA extraction and PCR, as well as the usage of plastics (Axygen, USA) and energy consumed. For each type of sample, the total number of samples that examen were recorded as well as to determine pathogen detection percentages**.** This detection was based on the ratio of PCR-positive samples among the total samples tested such as water, clinical, soil and surface-swab samples.

Data Analysis

To check workflow feasibility, laboratory observations were reviewed. For instance, practical challenges and the benefits of the optimized PCR procedure. Statistical analysis was used to summarize isolate counts, amplification rates, and resource consumption. This includes means, standard deviations, and frequencies. The chi-square test was used to categorize variables Differences between conventional and optimized PCR protocols. Also, *t*-test was used for continuous variables, as appropriate by using GraphPad Prism version 10 (GraphPad Software, USA)

Ethical Considerations

All experiments were controlled using BSL-2 conditions. Used materials and culture waste were sterilized by autoclave. Each experiment was repeated in triplicate as well as data records were checked for accuracy. Moreover, positive and negative controls were included to reduce bias and ensure reliability.

RESULTS

PCR Detection of Environmental and Clinical Pathogens

A total of 66 samples were detected. Interestingly, PCR results were highest among clinical samples (24/30, 80%). Followed by water samples (13/18, 72.2%), soil samples (6/10, 60%) **(Table 1& Figure 1).** While surface swabs were the least detected samples (3/8, 37.5%). PCR detected types of bacteria like *S. aureus*, *P. aeruginosa*, and *E. coli* in the analyzed samples. The expected amplicon sizes were observed. For *S. aureus nuc* gene (270 bp), *P. aeruginosa toxA* (396 bp), and for and *E. coli blaTEM* (516 bp). To confirm successful amplification using the optimized PCR protocol **(Figure 2).**

Table 1. Distribution of samples and PCR detection rates by sample type

Sample type	Total samples (N)	PCR-positive (n)	Detection rate (%)
Clinical	30	24	80.0
Water	18	13	72.2
Soil	10	6	60.0
Surface swabs	8	3	37.5
Total	**66**	**46**	—

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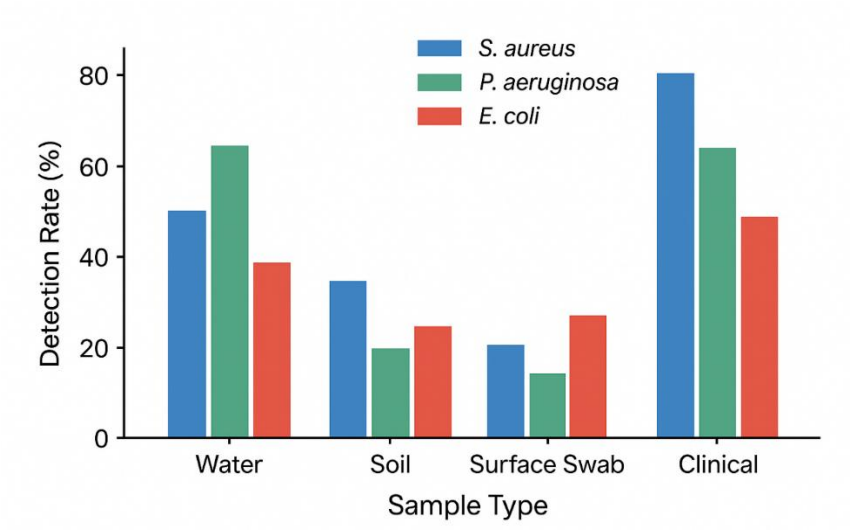

Figure 1. Comparison of pathogen-specific detection rates in environmental and clinical samples.

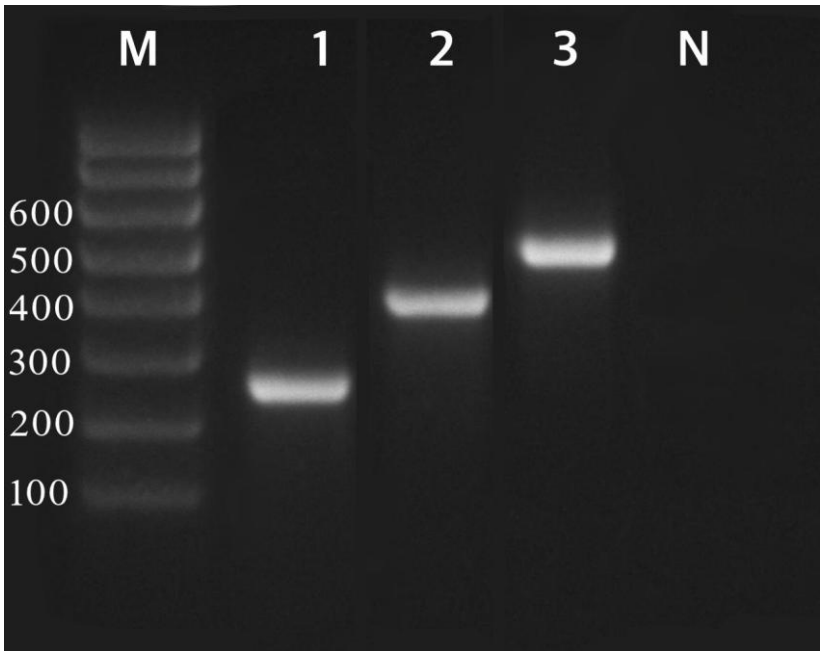

Figure 2. Agarose gel electrophoresis of PCR products. Lane M: molecular weight marker; Lane 1: (*nuc* gene, 270 bp) for *S. aureus*; Lanes 2: (*toxA*, 396 bp) for *P. aeruginosa*; Lanes 3: (*blaTEM,* 516 bp) for and *E. coli*; Lane N: negative control.

Laboratory Resource Efficiency

PCR Optimization system reduced resource consumption and maintained effective amplification. The volume of reactions was reduced from 25 µL to 15 µL, this about 40% reduction in reagent volume per reaction. The number of disposable plastic tubes for each run decreased from 60 to 40. This is about 33% reduction in tube use **(Table 2).** The optimized system also included safer DNA staining method (GelRed). Besides blue-light visualization was used instead of UV-based detection to reduce the effect of hazardous UV exposure**.**

Table 2. Comparison of Conventional and Optimized PCR Workflows

Parameter	Conventional PCR	Optimized PCR	Reduction (%)
Reaction Volume (µL)	25	15	40
Plastic Tubes per Run	60	40	33
Hazardous Waste Generated	High	Low	–

Relationship Between Resource Use and Diagnostic Efficiency

The adjusted reduced-volume PCR protocol accomplished 95% amplification rate. However, the reaction volume was reduced from 25 µL to 15 µL. This is about 40% reduction in reagent volume. On the other hand, Plastic tube use was also reduced from 60 to 40 tubes per run. This is approximately 33% reduction of plastic usage. These outcomes declared that the adjusted procedure reduced the consumption of resources. While maintaining effective PCR procedure under the experienced conditions **(Figure 3).**

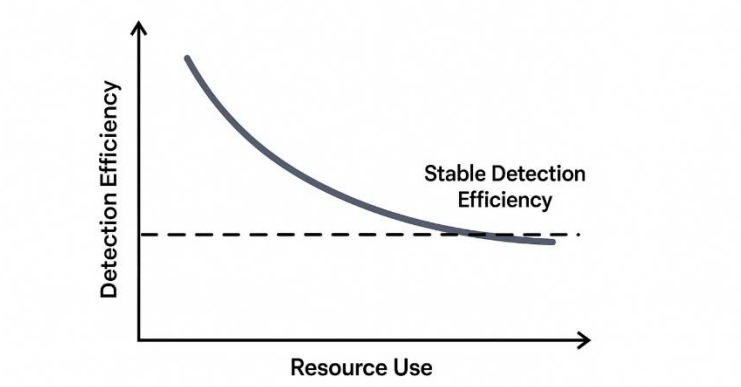

Figure 3. Relationship Between Resource Use and PCR Diagnostic Efficiency

DISCUSSION

This study demonstrated that the PCR workflow for detecting environmental and clinical pathogens can be optimized to reduce laboratory resource consumption while maintaining successful amplification. Reducing the reaction volume from 25 µL to 15 µL resulted in a 40% reduction in reagent volume per reaction, while the number of single-use tubes decreased from 60 to 40 per run, representing a 33% reduction. These outcomes agreed with previous studies that show the reduced-volume molecular protocols can be decreased reagent. Also, it can be reduced consumable requirements with maintaining reliable amplification [16–18]. Moreover, the cost of laboratories equipment may be reduced with limited resources.

The successful detection of both environmental and clinical specimens by the optimized PCR, the analyzed samples demonstrate that *S. aureus*, *P. aeruginosa*, and *E. coli*. The expected amplicons of 270 bp for *nuc*, 396 bp for *toxA*, and 516 bp for *blaTEM* confirmed successful genes of the selected targets respectively. The highest detection rate was observed among clinical samples (80%). followed by water (72.2%) then, soil (60%), and surface-swab samples (37.5%). This variation in detection rates with these types of sample could reflect differences in microbial contamination and exposure conditions. These results emphasize the value of molecular monitoring across both clinical and environmental settings [19,20].

The optimized protocol achieved an overall amplification success rate of 95%, indicating that the reduction in reaction volume did not adversely affect amplification performance under the tested conditions. This finding is important because reducing reagent volumes can provide a practical approach for decreasing laboratory resource requirements without necessarily compromising the generation of the expected PCR products [21,22]. However, the present findings demonstrate successful amplification rather than formal diagnostic sensitivity or specificity.

The basic of this study is the sustainability of the workflow. By supportively using GelRed as alternative DNA stain and blue-light visualization instead of UV-based detection. Thus, these modifications can reduce laboratory exposure to hazardous chemicals and UV radiation while maintaining visualization of PCR products. Incorporating safer detection practices therefore provides an additional occupational-safety benefit alongside resource conservation [23-25].

Despite these improvements, several limitations should be considered. This study estimated resource reductions primarily through reaction volume and single-use tube consumption. However, direct electricity consumption and the total mass of laboratory waste were not quantitatively determined. Further studies with larger sample and standardized resource-consumption measurements are needed to regulate the reproducibility of sustainable PCR workflows.

CONCLUSION

This work exhibited that the PCR procedure for detecting *S. aureus*, *P. aeruginosa*, and *E. coli* can be optimized. The reason for that was to reduce resource consumption and maintain valuable amplification at the same time. Decreasing the volume of reaction from 25 μL to 15 μL reduced the volume of reagent by 40%. While the consumption of single-use tube was lowered by 33%. The efficiency of this protocol accomplished 95% amplification rate. Furthermore, the target genes that mentioned are produced with the use of blue-light visualization to provide safer DNA detection. These findings confirm the viability of engagement resource-efficient with safer practices in routine molecular diagnostics. Further studies should be evaluated by using more samples. Also, standardized measurements of laboratory waste and energy consumption are needed to assess its reproducibility and extensive applicability.

CONFLICT OF INTEREST

The authors affirm that no conflicts of interest are associated with this publication. There were no financial, personal, or institutional factors that could have influenced the outcomes or interpretations presented in this work.

ETHICAL APROVAL

This research was based on the use of rejected clinical samples that were no longer needed for diagnostic purposes. As such, no direct patient intervention was involved, and according to institutional guidelines, formal ethical clearance was not required. full respect of ethical standards.

INFORMED CONSENT

Environmental and discarded clinical samples were examined in this work, with no participation from patients or volunteers. Thus, informed consent was not applicable.

AUTHOR'S CONTRIBUTIONS

Conception, data gathering, laboratory experiments, and result clarification were shared equally among the authors. The manuscript was reviewed and approved collectively before submission.

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