Vietnam Journal of Biotechnology **22**(4): 616-629, 2024. DOI: 10.15625/vjbt-21649

DEVELOPMENT OF A LOOP-MEDIATED AMPLIFICATION (LAMP) ASSAY FOR DETECTION OF ENVIRONMENTAL *Pseudomonas aeruginosa*

Thi Phuong Truc Nguyen[1], Nhu Ngoc Nguyen[1,2], Dinh Ky Lim[1,2], Thuc Quyen Huynh[2,3], Thi Thu Hoai Nguyen [1,2,3]✉

[1]*School of Biotechnology, International University, Quarter 6, Linh Trung ward, Thu Duc City, Ho Chi Minh City, Vietnam.*

[2]*Vietnam National University Ho Chi Minh City, Linh Trung ward, Thu Duc City, Ho Chi Minh City, Vietnam.*

[3]*Research Center for Infectious Diseases, International University, Quarter 6, Linh Trung ward, Thu Duc City, Ho Chi Minh City, Vietnam.*

✉*To whom correspondence should be addressed. E-mail: ntthoai@hcmiu.edu.vn*

Received: 03.10.2024
Accepted: 20.11.2024

ABSTRACT

Pseudomonas aeruginosa (*P. aeruginosa*) is a common environmental bacterium found in various habitats, including water and soil. Rapid detection of this microorganism is essential for monitoring environmental contamination and assessing its potential impact on ecosystems and public health. This study aimed to develop a high-efficiency loop-mediated isothermal amplification (LAMP) assay targeting a *P. aeruginosa*-specific gene encoding a hypothetical protein (GenBank ID: 882161). The study involved two main parts: 1) Isolation and identification of *P. aeruginosa* from environmental samples, and 2) Evaluation of the established LAMP assay on these environmental *P. aeruginosa* isolates. A total of 52 samples were collected from various geographical regions in Ho Chi Minh City, with 50 samples (96%) containing *Pseudomonas* species, characterized as rod-shaped, Gram-negative bacteria growing on selective media. Following duplex PCR screening, 14 *P. aeruginosa*-like environmental strains were isolated, and 5 randomly selected isolates were confirmed as *P. aeruginosa* through 16S rRNA sequencing. The LAMP assay was optimized at 60°C, 63°C, and 65°C for 30 and 45 minutes, using specific primers, and tested on all five confirmed *P. aeruginosa* isolates. The results demonstrated that the LAMP assay was highly specific (100%) for detecting environmental *P. aeruginosa*, with a detection limit of 1 pg/µL. In conclusion, *P. aeruginosa* is prevalent in the environment, and the developed LAMP assay shows strong potential for identifying environmental *P. aeruginosa* isolates.

Keywords: duplex-PCR, environmental isolates, LAMP, *P. aeruginosa*

INTRODUCTION

Pseudomonas aeruginosa (*P. aeruginosa*) is a highly adaptable, Gram-negative bacterium (Iglewski, 1996) commonly found in various environments, including soil, water, and hospital settings (Green et al.,

1974) (Gellatly & Hancock, 2013; Nadell et al., 2017; Stover et al., 2000). This bacterium is notorious for its ability to cause a wide range of infections, particularly in vulnerable populations such as burn patients, individuals with cystic fibrosis, and the elderly (Berube et al., 2016) (Li et al., 2016). Its presence in hospitals is particularly concerning, as it accounts for a significant proportion of nosocomial infections, including urinary tract infections, pneumonia, and septicemia. The increasing incidence of multidrug-resistant *P. aeruginosa* infections poses a serious threat to global public health (Sathe et al., 2023).

Traditional diagnostic methods for detecting *P. aeruginosa*, such as bacterial culture and biochemical tests, often fall short in terms of accuracy, processing time, and cost (Jami Al-Ahmadi & Zahmatkesh Roodsari, 2016). Polymerase Chain Reaction (PCR) techniques, though highly sensitive and specific, require expensive equipment and are time-consuming (Mullis et al., 1986). These limitations have driven the development of alternative diagnostic methods, one of which is Loop-mediated Isothermal Amplification (LAMP).

LAMP, a novel technique first introduced in the early 2000s, has gained significant attention due to its simplicity, efficiency, and cost-effectiveness (Notomi, 2000). Unlike PCR, LAMP does not require a thermal cycler, as it amplifies DNA at a constant temperature of around 65°C (Koide et al., 2010; Lim et al., 2013). This technique uses multiple primers to amplify the target gene, resulting in a highly specific and sensitive assay. The amplified products can be easily visualized, making the LAMP assay a practical choice for detecting a wide range of pathogens, including bacteria (Maruyama et al., 2003), viruses (Enomoto et al., 2005), and parasites (Zoheir & Allam, 2010).

The high efficiency of the LAMP process allows for the detection of the amplified DNA through various methods, such as fluorescence signals, turbidity changes, or even visual inspection with the naked eye (Li et al., 2017). Due to its simplicity, rapidity, and high specificity, LAMP has become a valuable tool for detecting various pathogens (Garg et al., 2022).

The study aimed to develop a LAMP assay specifically for detecting *P. aeruginosa* from environmental samples. LAMP was tested on various environmental isolates of *P. aeruginosa*, as well as a laboratory strain, *P. aeruginosa* ATCC 9027. The sensitivity and specificity of the LAMP assay were evaluated to ensure its effectiveness in identifying *P. aeruginosa* in diverse environments.

MATERIALS AND METHODS

Samples collection

Fifty-two environmental *P. aeruginosa*-like samples were collected at various regions of Ho Chi Minh City, Vietnam, particularly from soil and wastewater. These samples were collected using cotton swabs and cultured in cetrimide medium (HiMedia, India), a selective medium for *Pseudomonas* for 2-3 days. All samples were screened based on colony morphology on cetrimide agar and Gram staining under a microscope. The isolates were stored at -80^{o}C in LB broth containing 30% glycerol for further use.

DNA extraction

The DNA was extracted from overnight bacterial culture grown in cetrimide medium

using TE buffer (Tris-EDTA) 1X solution (Kodackattumannil et al., 2023). Briefly, a single bacterial colony was suspended in 300 µL of Tris-EDTA (TE) 1X buffer (pH = 8) and heated to 95 °C for 15 minutes. After heating, the solution is rapidly cooled to 3°C–4°C for 10 minutes. The solution was then subjected to centrifugation at 14,000 rpm for 5 minutes; the supernatant was transferred to a new tube as the DNA stock and stored at −20 °C until use.

Duplex polymerase chain reaction (PCR)

Duplex-PCR using *algD-oprL* was used to screen for *P. aeruginosa*-like isolates and was confirmed to be effective for identifying *P. aeruginosa* (Nguyen et al., 2022). Briefly, the standard 25 µL reaction mixture contained 12.5 µL GoTaq® G2 Hot Start Green master mix (Promega), 10 µM of each primer (PHUSA Biochem Oligo), and 100 ng DNA, nuclease-free water.

The PCR cycle included an initial denaturation of 5 min at 94°C, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, extension at 72°C for 1 min, and final incubation at 72°C for 7 min. The PCR products were then separated by gel electrophoresis at 100V for 45 minutes using a 1.5% agarose gel. *P. aeruginosa* ATCC 9027 was used as the positive control and other bacteria strains, including *E. coli* ATCC 35218, *A. baumannii* ATCC 19606, and *S. aureus* ATCC 29213 were used as negative controls in all experiments.

Table 1: Primers used in the duplex PCR (Nguyen *et al.*, 2022)

Primer		Sequence (5'-3')	Size (bp)	Product size (bp)
algD	Forward	TTCCCTCGCAGAGAAAACATC	21	299
	Reverse	CCTGGTTGATCAGGTCGATCT	21	299
oprL	Forward	CTGTGGGTTGCTCCTCCAAG	20	520
	Reverse	CTCGCCCAGAGCCATATTGT	20	520

***16S rRNA* sequencing**

To validate the results of the duplex PCR and subsequent LAMP assay, the duplex PCR-identified bacterial strains were confirmed using *16S rRNA* sequencing (Nam Khoa Biotek CO., Ltd.). The sequences of *16S rRNA* were then used to construct a phylogenetic tree using Mega 11 (https://www.megasoftware.net). In this process, the sequences were initially aligned using ClustalW (http://www.clustal.org) to ensure accurate homologous region comparison. The Neighbor-Joining method was selected as the optimal evolutionary model based on the dataset. MEGA 11 then generated the phylogenetic tree from the aligned sequences, incorporating bootstrap analysis to assess the statistical robustness of each branch.

Primers used for establishing LAMP to detect *P. aeruginosa*

A *hypothetical protein gene* (GenBank ID: 882161) was used due to its high conservation among *P. aeruginosa* strains, and distinctiveness from other species (Li et al., 2018). Primer design for the LAMP

assay was performed using NEB LAMP Primer Design (https://lamp.neb.com) in conjunction with manual design. The primers were evaluated using the Multiple Primer Analyzer (https://www.thermofisher.com) based on the following criteria for LAMP primers: 1) Tm value: F1c and B1c > F2 and B2 (60-65°C) > F3 and B3; 2) Minimum dimerization ΔG > -4; 3) GC content between 50% and 60%; 4) No secondary structure Primers designed by Li et al. (2018) were compared with those generated using the NEB LAMP Primer Design tool. The comparison revealed that Li et al.'s primers exhibited cross-primer dimers between *B3* and *FIP*, whereas the primers designed in this study did not show such dimer formation.

All sets of primers were synthesized commercially (PHUSA Biochem Oligo) and are listed in Table 2.

Table 2. Loop-mediated isothermal amplification primers used in the study

Name	Primer	Sequence (5'-3')	Size (bp)	Product size (bp)
Set 2 (this study)	*F3*	GACCTCGACTACTCCAGC	18	217
	B3	AGCCGCGGACGAAGTC	16	
	FIP	TGCGGCATTCCCTCTTGCATTTTCAATTCCC CGAGGGCTTC	41	
	BIP	ATCTTCGAGACCTACGACCACCTTTTTTCGC GCCCAGGATG	41	

PCR to detect a hypothetical protein gene of P. aeruginosa using F3 and B3 primers

Purified DNA of *P. aeruginosa* and non - *P. aeruginosa* samples underwent polymerase chain reaction (PCR) to amplify the *hypothetical protein* gene for *P. aeruginosa* identification. Each 25 µL PCR reaction includes 12.5 µL GoTaq® G2 Hot Start Green master mix (Promega), 1 µL of each primer designed (10 µM) (PHUSA Biochem Oligo), 100 ng DNA, and nuclease-free water. The PCR program is set up with an initial denaturation at 95°C for 5 min, followed by 32 cycles of denaturation at 95°C for 30 sec, annealing at 57°C for 30 sec, extension at 72°C for 30 sec, and incubation for 7 min at 72°C. The PCR products were analyzed by gel electrophoresis at 100V for 45 minutes using 1X SB buffer and a 1.5% agarose gel.

Loop-mediated isothermal amplification (LAMP) assay

LAMP was performed using WarmStart® Fluorescent LAMP/RT-LAMP Kit (with UDG) (New England Biolabs Ltd.). The reaction mixture consisted of 12.5 µLWarmStart Multi-Purpose LAMP/RT-LAMP 2X Master Mix (with UDG) (New England Biolabs Inc.), 1.6 µM primers *FIP* and *BIP*, 0.2 µM each of the outer primers *F3* and *B3*, 100 ng DNA template, and nuclease-free water to a total volume of 25 µL. Isothermal amplification was conducted at 60°C, 63°C, and 65°C for 30 min and 45 min to select the optimal temperature. The

reaction was then terminated by heating at 80°C for 2 minutes. The products were visualized by adding 0.5 µL SYBR-Green I to each reaction tube for fluorescence detection or gel electrophoresis using 1.5% agarose gel (45 min, 100 V, 1X SB buffer).

Specificity and sensitivity analysis

Specificity testing of the developed LAMP assay was performed using environmental *P. aeruginosa*, and non - *P. aeruginosa* isolates, including *coli* ATCC 35218, *A. baumannii* ATCC 19606, and *S. aureus* ATCC 29213. The specificity of the LAMP assay was evaluated and compared with the PCR results using *F3-3* and *B3-3* primers.

The specificity was calculated using the following formula (Parikh et al., 2008):

$$\text{Sensitivity} = \frac{True\ negative}{True\ negative + false\ positive}$$

(Probability of being tested positive when the gene presents)

The analytical sensitivity of the LAMP reaction was tested using serially diluted DNA of *P. aeruginosa* with concentrations ranging from 100 ng/µL to 10^{-5} ng/µL. The LAMP products were analyzed by 1.5% agarose gel electrophoresis.

RESULTS

***Pseudomonas aeruginosa*-like isolates from the environment samples**

After isolating 52 samples, 50 out of 52 isolates (96%) were Gram-negative, rod-shaped under the light microscope at 100X and were capable of growing on cetrimide agar after 18–24 hours of incubation. The colonies obtained were single, with morphology and purity resembling *P. aeruginosa* (Figure 1).

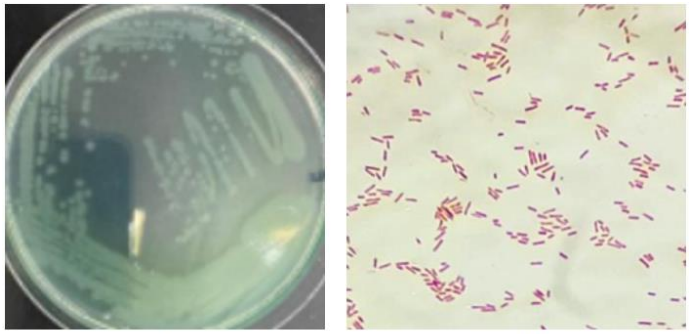


Figure 1. Representative of environmental *P. aeruginosa*-like isolates. (A) Green pigment produced by a *P. aeruginosa* strain on Cetrimide agar. (B) Gram stain of the isolate observed under a 100X magnification light microscope.

Screening the presence of *P. aeruginosa* using duplex PCR

Among the fifty samples, fourteen (14/50, 28%) environmental *P. aeruginosa*-like samples tested positive for both *oprL* and *algD*, twenty-six samples (26/50, 52%) tested positive for *oprL* only, and the remaining ten samples did not show any product on gel electrophoresis (Figure 2; detailed in Supplementary Data Figure S1).

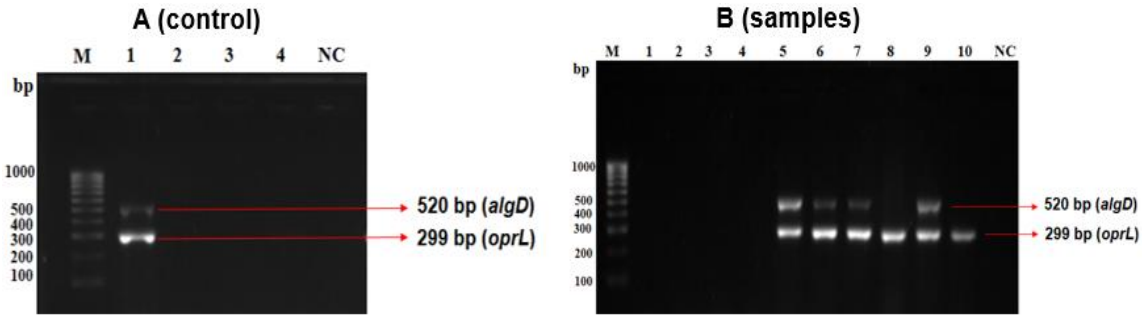


Figure 2. Representative duplex PCR products of the *oprL-algD* gene. (A) Laboratory bacteria. Lane 1: *P. aeruginosa* ATCC 9027 (positive control); lane 2-4: *E. coli* ATCC 35218; *A. baumannii* ATCC 19606; *S. aureus* ATCC 29213 (negative control). (B) Environmental *P. aeruginosa*-like isolates. Lane 1 – 10: *P. aeruginosa*-like isolate M: 100bp DNA ladder; NC: Negative control (no DNA).

Confirmation of the duplex PCR identified strains with *16S rRNA* sequencing

Five samples that were positive for both *algD* and *oprL* were randomly selected for *16S rRNA* sequencing and were confirmed as *P. aeruginosa*, suggesting the screening using duplex PCR was effective. The results were further used for a phylogenetic tree analysis, where the *16S rRNA* gene sequences of these five samples clustered closely with known *Pseudomonas aeruginosa* strains, confirming their identity (Supplementary data, Figure S2). This finding validates the duplex PCR as an effective screening method for detecting *P. aeruginosa* in environmental samples.

Specificity of *F3* and *B3* primers

Four laboratory bacterial strains, including *P. aeruginosa* ATCC 9027, *E. coli* ATCC 35218, *A. baumannii* ATCC 19606 and *S. aureus* ATCC 29213 were used to test the specificity of *F3* and *B3* primers. The PCR product appeared as a single band at approximately 217 bp for *P. aeruginosa* strains, while no bands were observed for the negative control strains (Figure 3). This result confirmed the BLASTN findings that our LAMP primer sequences were specific to only *P. aeruginosa*.

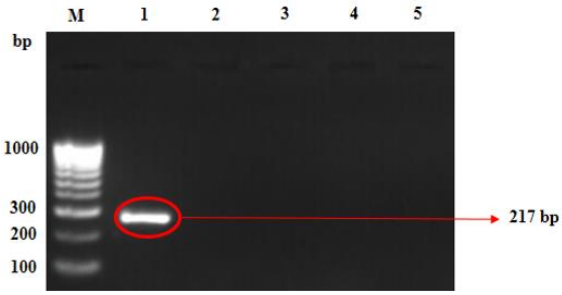


Figure 3. Agarose gel electrophoresis of PCR products with *F3-3* and *B3-3* primers. M: 100bp DNA ladder; Lane 1: *P. aeruginosa* ATCC 9027; Lane 2: *E. coli* ATCC 35218; Lane 3: *A. baumannii* ATCC 19606; Lane 4: *S. aureus* ATCC 29213; Lane 5: Negative control (no DNA).

Detecting environmental *P. aeruginosa* isolates using LAMP assay

For detecting *P.aeruginosa* isolates, primer set 2 was used, and the products were analyzed using gel electrophoresis for a better illustration of the method efficacy. LAMP products were observed as the pattern of DNA laddering at 65°C for 45 minutes. As detailed below, *P. aeruginosa* ATCC 9027 was positive, while *E. coli* ATCC 35218, *A. baumannii* ATCC 19606 and *S. aureus* ATCC 29213 were negative. Therefore, this temperature and running time were selected for specificity and sensitivity analysis on environmental *P. aeruginosa* isolates.

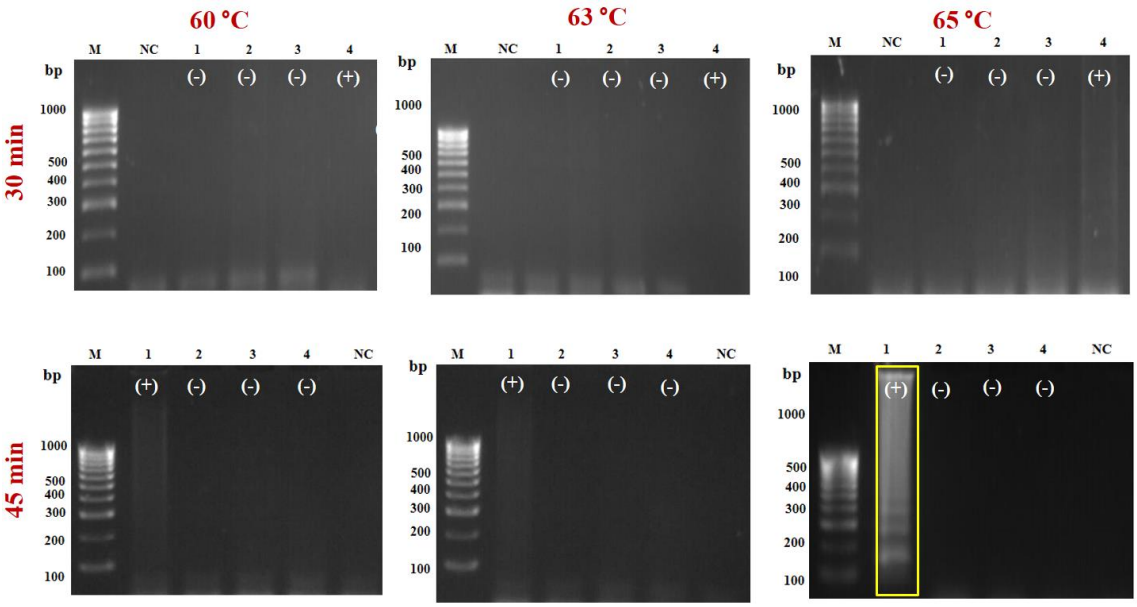


Figure 4. LAMP optimization period (A) 30 min and temperatures 60, 63 and 65 °C. (B) 45 min and temperatures 60, 63 and 65 °C. M: 100bp DNA ladder; NC: Negative control (no DNA); (+): *P. aeruginosa* ATCC 9027, (-): *E. coli* ATCC 35218; *A. baumannii* ATCC 19606; *S. aureus* ATCC 29213.

Specificity and sensitivity analysis

In terms of specificity evaluation, all environmental *P. aeruginosa* were correctly identified. The PCR product appeared as a single band at approximately 217 bp, while the LAMP product appeared to show multiple bands of various sizes. The negative control showed no band on gel electrophoresis. These findings indicated that LAMP results were consistent with PCR, demonstrating high specificity (Figure 5, detailed in Supplementary data, Figure S3-4).

For sensitivity evaluation, 10-fold serial dilutions (100 ng/µL to 10^{-5} ng/µL) of DNA from *P. aeruginosa* were tested by PCR and LAMP. Agarose gel electrophoresis results showed a limit of detection of 10^{-3} ng/µL, which corresponds to around 192 genome copies per reaction (according to the genome size of *P. aeruginosa*) (Tümmler et al., 2011). This ensures that even at low concentrations, the assay retains its ability to detect the presence of *P. aeruginosa* DNA with high accuracy. The sensitivity of the LAMP assay was consistent with that of

conventional PCR, further supporting its reliability as an efficient detection tool.

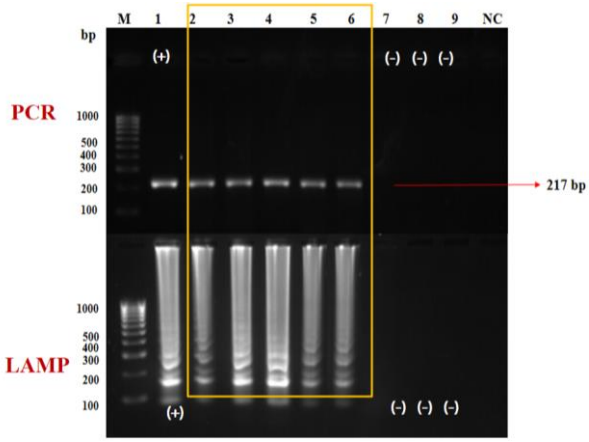


Figure 5. The detection of environmental *P. aeruginosa* by LAMP compared with PCR. M: 100bp DNA ladder; control (+): *P. aeruginosa* ATCC 9027; (-): *E. coli* ATCC 35218; *A. baumannii* ATCC 19606; *S. aureus* ATCC 29213; Lane 2-6: environment *P. aeruginosa*. NC: Negative control (no DNA).

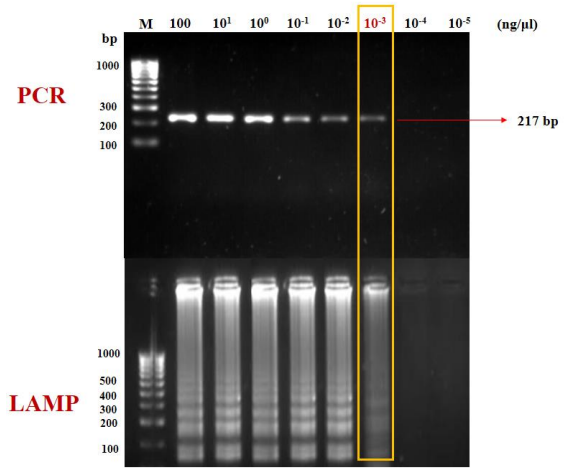


Figure 6. Comparison of the lowest detectable concentration of environmental *P. aeruginosa* by LAMP and PCR by 10-fold serial dilution (100 ng/µL to 10^{-5} ng/µL). M: 100 bp DNA ladder; NC: Negative control (no DNA).

DISCUSSION

Environmental *P. aeruginosa* is found in areas closely associated with human activity and can also be present in the human body (Crone et al., 2020). Therefore, controlling this bacterium in related infections is crucial, and an effective detection method is needed.

This research aimed to develop a qualitative LAMP assay for detecting environmental *P. aeruginosa* to address certain limitations of traditional methods, including culturing and conventional PCR (Jami Al-Ahmadi & Zahmatkesh Roodsari, 2016).

The highlight of this study lies in both the selection of the target gene and the design of

the primers. In contrast to previous studies that primarily focused on genes like *oprL* (Dong et al., 2021), this assay targeted a highly conserved hypothetical protein gene (GenBank ID: 882161), which is unique to *P. aeruginosa*. While this gene has been utilized in LAMP assays for clinical *P. aeruginosa*, it has not been applied to environmental isolates until now. Furthermore, the primers were carefully designed to avoid cross-primer dimerization, a limitation seen in earlier LAMP assays. These advancements improved the assay's specificity and reduced the risk of false positives, setting this study apart from previous efforts to detect *P. aeruginosa*.

In this study, there was a high prevalence of *P. aeruginosa*-like isolates in the environmental samples, accounting for 96% of total samples. Duplex PCR targeting *oprL* and *algD* revealed that 28% (14/50) of the *P. aeruginosa*-like isolates, or 27% (14/52) of all environmental samples, were positive for both genes. Additionally, 52% (26/50) of *P. aeruginosa*-like isolates tested positive for *oprL*. Previous studies have reported that *oprL* primers have 70% specificity for clinical *P. aeruginosa* isolates (49 confirmed *P. aeruginosa* out of 70 oprL-positive isolates (Rashno Taee et al., 2014) . However, in our study, the detection rate using *oprL* for environmental isolates was lower, at only 35% (14/40). These results suggested that while the *oprL* primers also detected non-*P. aeruginosa* alongside with *P. aeruginosa* isolates, the duplex PCR targeting *oprL* and *algD* was accurate for identifying *P. aeruginosa*.

For the LAMP assay, our designed primers effectively detected environmental *P. aeruginosa* isolates and we employed agarose gel electrophoresis to confirm the LAMP results. Although this additional step may increase the time and cost of the assay, it provides a more definitive validation of the amplification products. Gel electrophoresis allows for more precise differentiation of specific amplification patterns, thereby reducing the potential for false positives. This does not significantly detract from the overall efficiency of the LAMP assay but rather enhances the reliability of the results in a research context, especially when high specificity is essential.

The specificity of LAMP assay *for the five* identified *P. aeruginosa* isolated was 100%, and its sensitivity was 1 pg/µL (10^{-3} ng/µL), which is comparable to conventional PCR. This sensitivity aligns with previous studies evaluating the LAMP method. Valladares and Vazquez (2016) reported a limit of detection of 10^{-3} ng/µL for a LAMP assay detecting *hepatica*, which was also achieved with standard PCR using the outer primers.

In this study, the LAMP products were analyzed by gel electrophoresis, and bands were visualized under UV light for an efficient illustration of the obtained results. Other visualization methods, such as turbidity (Wang et al., 2015), Calcein dye (Yuan et al., 2019), or Hydroxynaphthol blue (HBN) dye (Yang et al., 2014) which have also been used to optimize detection efficiency and these would be applied to generate a rapid kit in our future work.

LAMP is highly susceptible to contamination due to its high amplification efficiency and the presence of multiple inverted repeats in the amplification products (Longo et al., 1990). High concentrations of amplification products can easily become airborne, potentially contaminating the environment, reagents, pipettes, and the operator's gloves and clothing. To mitigate this risk, we conducted

LAMP and gel electrophoresis in separate rooms using dedicated equipment to reduce carryover contamination. While this approach effectively controls the spread of amplification products, it cannot completely eliminate contamination. Hsieh et al. (2014) reported that dUTP and UDG enzyme treatment could prevent carryover contamination between subsequent reactions. Thus, using a LAMP kit with UDG can be effective for identifying environmental *P. aeruginosa.*

One limitation of this study is the relatively small sample size of 52 environmental isolates, which may restrict the generalizability of the findings. Although the LAMP assay demonstrated high specificity and sensitivity for detecting *P. aeruginosa*, further validation with a larger and more diverse sample set is necessary. Expanding the study to include a wider range of environmental samples from different geographic locations and conditions would help to assess the robustness of the assay and its applicability across various settings. Moreover, expanding the sample size and incorporating clinical isolates would provide a more thorough assessment of the assay's effectiveness in real-world conditions, thereby enhancing its credibility as a dependable diagnostic tool.

Recently, many LAMP-based assays have been developed to detect various pathogens, including *Staphylococci* (Xu et al., 2012), *Salmonella* (Zhao et al., 2010), and *E. coli* (Zhao et al., 2009). In the future, maintaining consistent conditions will be crucial for improving detection capabilities

CONCLUSION

The LAMP assay for detecting *P. aeruginosa* proved to be a viable option, with specificity and sensitivity comparable to conventional PCR. The LAMP assay presented here is a simple and sensitive tool for identifying environmental *P. aeruginosa.*

ACKNOWLEDGEMENTS

This study was supported by the International University, Vietnam National University Ho Chi Minh City. The authors are grateful for the valuable resources and support provided throughout the research process.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

Berube, B. J., Rangel, S. M., & Hauser, A. R. (2016). *Pseudomonas aeruginosa*: breaking down barriers. *Curr Genet*, *62*(1), 109-113. https://doi.org/10.1007/s00294-015-0522-x

Crone, S., Vives-Florez, M., Kvich, L., Saunders, A. M., Malone, M., Nicolaisen, M. H., . . . Bjarnsholt, T. (2020). The environmental occurrence of *Pseudomonas aeruginosa*. *APMIS*, *128*(3), 220-231. https://doi.org/10.1111/apm.13010

Dong, K., Kang, Z., Ji, X., Zhang, X., Cheng, P., & Sun, B. (2021). A Loop-mediated Isothermal Amplification With a Nanoparticle-Based Lateral Flow Biosensor Assay to Detect *Pseudomonas aeruginosa* in Endophthalmitis. *Translational Vision Science & Technology*, *10*(14). https://doi.org/10.1167/tvst.10.14.26

Enomoto, Y., Yoshikawa, T., Ihira, M., Akimoto, S., Miyake, F., Usui, C., . . . Asano, Y. (2005). Rapid Diagnosis of Herpes Simplex Virus Infection by a Loop-Mediated Isothermal Amplification Method. *Journal of Clinical Microbiology*, *43*(2), 951-955. https://doi.org/10.1128/jcm.43.2.951-955.2005

Garg, N., Ahmad, F. J., & Kar, S. (2022). Recent advances in loop-mediated isothermal amplification (LAMP) for rapid and efficient detection of pathogens. *Current Research in Microbial Sciences*, *3*. https://doi.org/10.1016/j.crmicr.2022.100120

Gellatly, S. L., & Hancock, R. E. (2013). *Pseudomonas aeruginosa*: new insights into pathogenesis and host defenses. *Pathog Dis*, *67*(3), 159-173. https://doi.org/10.1111/2049-632X.12033

Green, S. K., Schroth, M. N., Cho, J. J., Kominos, S. K., & Vitanza-jack, V. B. (1974). Agricultural plants and soil as a reservoir for *Pseudomonas aeruginosa*. *Appl Microbiol*, *28*(6), 987-991. https://doi.org/10.1128/am.28.6.987-991.1974

Iglewski, B. H. (1996). Pseudomonas. In th & S. Baron (Eds.), *Medical Microbiology*. https://www.ncbi.nlm.nih.gov/pubmed/21413324

Jami Al-Ahmadi, G., & Zahmatkesh Roodsari, R. (2016). Fast and specific detection of *Pseudomonas aeruginosa* from other pseudomonas species by PCR. *Ann Burns Fire Disasters*, *29*(4), 264-267. https://www.ncbi.nlm.nih.gov/pubmed/28289359

Kodackattumannil, P., Sasi, S., Krishnan, S., Lekshmi, G., Kottackal, M., & Amiri, K. (2023). Protocol for the High-quality Plasmid Isolation from Different Recalcitrant Bacterial Species: *Agrobacterium spp.* , *Rhizobium sp.*, and *Bacillus thuringiensis*. *Bio-Protocol*, *13*(15). https://doi.org/10.21769/BioProtoc.4788

Koide, Y., Maeda, H., Yamabe, K., Naruishi, K., Yamamoto, T., Kokeguchi, S., & Takashiba, S. (2010). Rapid detection of *mecA* and *spa* by the loop-mediated isothermal amplification (LAMP) method. *Letters in Applied Microbiology*, *50*(4), 386-392. https://doi.org/10.1111/j.1472-765X.2010.02806.x

Li, C., Shi, Y., Yang, G., Xia, X. S., Mao, X., Fang, Y., . . . Song, Y. (2018). Establishment of loop-mediated isothermal amplification for rapid detection of *Pseudomonas aeruginosa*. *Experimental and Therapeutic Medicine*. https://doi.org/10.3892/etm.2018.6910

Li, J., Zou, M., Dou, Q., Hu, Y., Wang, H., Yan, Q., & Liu, W. E. (2016). Characterization of clinical extensively drug-resistant *Pseudomonas aeruginosa* in the Hunan province of China. *Ann Clin Microbiol Antimicrob*, *15*(1), 35. https://doi.org/10.1186/s12941-016-0148-y

Li, Y., Fan, P., Zhou, S., & Zhang, L. (2017). Loop-mediated isothermal amplification (LAMP): A novel rapid detection platform for pathogens. *Microbial Pathogenesis*, *107*, 54-61. https://doi.org/10.1016/j.micpath.2017.03.016

Lim, K. T., Teh, C. S. J., & Thong, K. L. (2013). Loop-Mediated Isothermal Amplification Assay for the Rapid Detection of *Staphylococcus aureus*. *BioMed Research International*, *2013*, 1-5. https://doi.org/10.1155/2013/895816

Longo, M. C., Berninger, M. S., & Hartley, J. L. (1990). Use of uracil DNA glycosylase to control carry-over contamination in polymerase chain reactions. *Gene*, *93*(1), 125-128. https://doi.org/10.1016/0378-1119(90)90145-h

Maruyama, F., Kenzaka, T., Yamaguchi, N., Tani, K., & Nasu, M. (2003). Detection of Bacteria Carrying the stx2 Gene by In Situ Loop-Mediated Isothermal Amplification. *Applied and Environmental Microbiology*, *69*(8), 5023-5028. https://doi.org/10.1128/aem.69.8.5023-5028.2003

Mullis, K., Faloona, F., Scharf, S., Saiki, R., Horn, G., & Erlich, H. (1986). Specific Enzymatic Amplification of DNA In Vitro: The Polymerase Chain Reaction. *Cold Spring Harbor Symposia on Quantitative Biology*, *51*(0), 263-273. https://doi.org/10.1101/sqb.1986.051.01.032

Nadell, C. D., Ricaurte, D., Yan, J., Drescher, K., & Bassler, B. L. (2017). Flow environment and matrix structure interact to determine spatial

competition in *Pseudomonas aeruginosa* biofilms. *Elife*, *6*. https://doi.org/10.7554/eLife.21855

Nguyen, T. T. H., Nguyen, V. D., Nguyen, N. M. H., Nguyen, L. Q. A., Vo, N. H. G., Pham, T. T. V., . . . Huynh, T. Q. (2022). Identification of commensal *Pseudomonas aeruginosa* isolates using duplex PCR targeting the *oprL* and *algD* genes. *Science and Technology Development Journal*, *25*(3), 2481-2488. https://doi.org/10.32508/stdj.v25i3.3961

Notomi, T. (2000). Loop-mediated isothermal amplification of DNA. *Nucleic Acids Research*, *28*(12), 63e-63. https://doi.org/10.1093/nar/28.12.e63

Parikh, R., Mathai, A., Parikh, S., Chandra Sekhar, G., & Thomas, R. (2008). Understanding and using sensitivity, specificity and predictive values. *Indian Journal of Ophthalmology*, *56*(1). https://doi.org/10.4103/0301-4738.37595

Rashno Taee, S., Khansarinejad, B., Abtahi, H., Najafimosleh, M., & Ghaznavi-Rad, E. (2014). Detection of *algD*, *oprL* and *exoA* Genes by New Specific Primers as an Efficient, Rapid and Accurate Procedure for Direct Diagnosis of *Pseudomonas aeruginosa* Strains in Clinical Samples. *Jundishapur J Microbiol*, *7*(10), e13583. https://doi.org/10.5812/jjm.13583

Sathe, N., Beech, P., Croft, L., Suphioglu, C., Kapat, A., & Athan, E. (2023). *Pseudomonas aeruginosa*: Infections and novel approaches to treatment “Knowing the enemy” the threat of *Pseudomonas aeruginosa* and exploring novel approaches to treatment. *Infectious Medicine*, *2*(3), 178-194. https://doi.org/10.1016/j.imj.2023.05.003

Stover, C. K., Pham, X. Q., Erwin, A. L., Mizoguchi, S. D., Warrener, P., Hickey, M. J., . . . Olson, M. V. (2000). Complete genome sequence of *Pseudomonas aeruginosa* PAO1, an opportunistic pathogen. *Nature*, *406*(6799), 959-964. https://doi.org/10.1038/35023079

Tümmler, B., Davenport, C. F., Wiehlmann, L., Cramer, N., & Klockgether, J. (2011). *Pseudomonas aeruginosa* Genomic Structure and Diversity. *Frontiers in Microbiology*, *2*. https://doi.org/10.3389/fmicb.2011.00150

Wang, X. R., Wu, L. F., Wang, Y., Ma, Y. Y., Chen, F. H., & Ou, H. L. (2015). Rapid detection of *Staphylococcus aureus* by loop-mediated isothermal amplification. *Appl Biochem Biotechnol*, *175*(2), 882-891. https://doi.org/10.1007/s12010-014-1328-x

Xu, Z., Li, L., Chu, J., Peters, B. M., Harris, M. L., Li, B., . . . Shirtliff, M. E. (2012). Development and application of loop-mediated isothermal amplification assays on rapid detection of various types of staphylococci strains. *Food Res Int*, *47*(2), 166-173. https://doi.org/10.1016/j.foodres.2011.04.042

Yang, B. Y., Liu, X. L., Wei, Y. M., Wang, J. Q., He, X. Q., Jin, Y., & Wang, Z. J. (2014). Rapid and sensitive detection of human astrovirus in water samples by loop-mediated isothermal amplification with hydroxynaphthol blue dye. *BMC Microbiol*, *14*, 38. https://doi.org/10.1186/1471-2180-14-38

Yuan, X. Y., Wang, Y. L., Meng, K., Zhang, Y. X., Xu, H. Y., & Ai, W. (2019). LAMP real-time turbidity detection for fowl adenovirus. *BMC Vet Res*, *15*(1), 256. https://doi.org/10.1186/s12917-019-2015-5

Zhao, X., Li, Y., Wang, L., You, L., Xu, Z., Li, L., . . . Yang, L. (2009). Development and application of a loop-mediated isothermal amplification method on rapid detection *Escherichia coli* O157 strains from food samples. *Molecular Biology Reports*, *37*(5), 2183-2188. https://doi.org/10.1007/s11033-009-9700-6

Zhao, X., Wang, L., Chu, J., Li, Y., Li, Y., Xu, Z., . . . Yang, L. (2010). Development and application of a rapid and simple loop-mediated isothermal amplification method for food-borne Salmonella detection. *Food Science and Biotechnology*, *19*(6), 1655-1659. https://doi.org/10.1007/s10068-010-0234-4

Zoheir, K. M. A., & Allam, A. A. (2010). A rapid method for sexing the bovine embryo. *Animal Reproduction Science*, *119*(1-2), 92-96. https://doi.org/10.1016/j.anireprosci.2009.12.013

SUPPLEMENTARY DATA

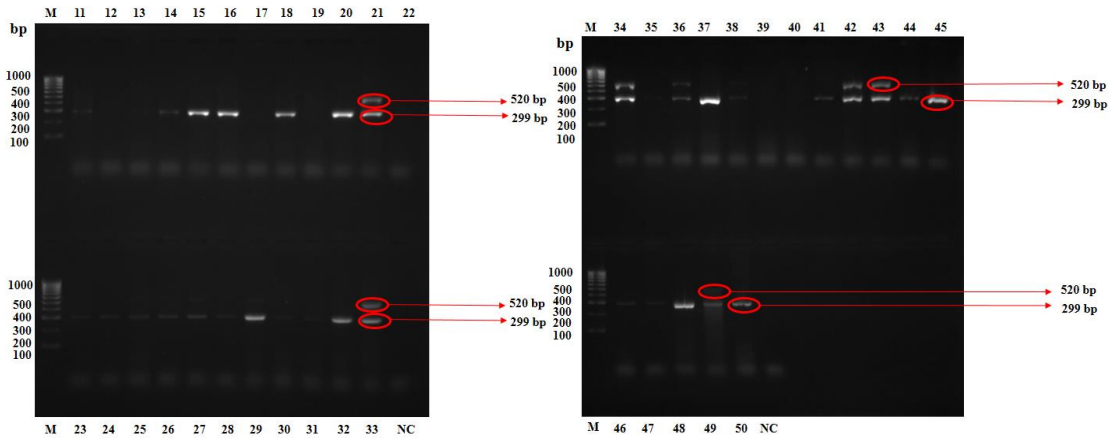


Figure S1. Detection of *P. aeruginosa* like-isolates using duplex PCR. M: 100bp DNA ladder; lane 12,13,17,19,22, 39, 40: non-*P. aeruginosa*; lane 21, 33, 29, 27, 34, 36, 42, 43, 49: environmental *P. aeruginosa* detected by *oprL-algD*; lane 11, 14-16, 18, 21, 37, 38, 41, 44, 45, 46-48, 50: environmental *P. aeruginosa* only detected by *oprL*.

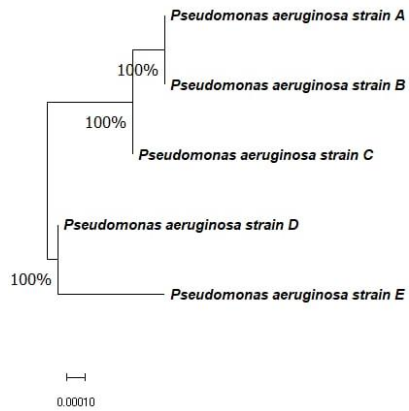
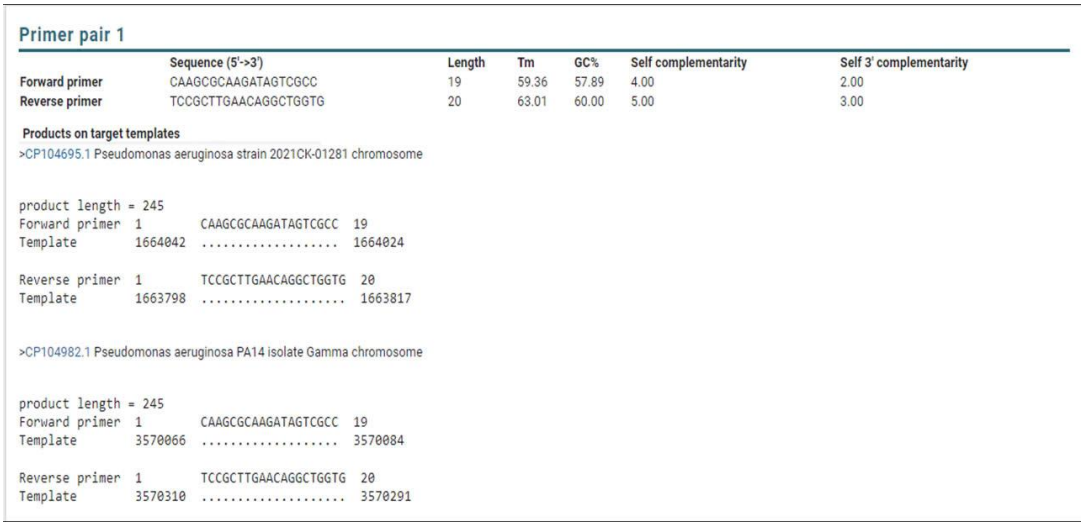


Figure S2. The phylogenetic tree of 5 *Pseudomonas* strains based on the phylogenetic analysis of 16S rRNA using Mega 11. Distance matrices were calculated by the Jukes-Cantor method. Dendrograms were generated by Neighbor-Joining. The bar indicates sequence divergence.



Primer pair 1

	Sequence (5'->3')	Length	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	CAAGCGCAAGATAGTCGCC	19	59.36	57.89	4.00	2.00
Reverse primer	TCCGCTTGAACAGGCTGGTG	20	63.01	60.00	5.00	3.00

Products on target templates

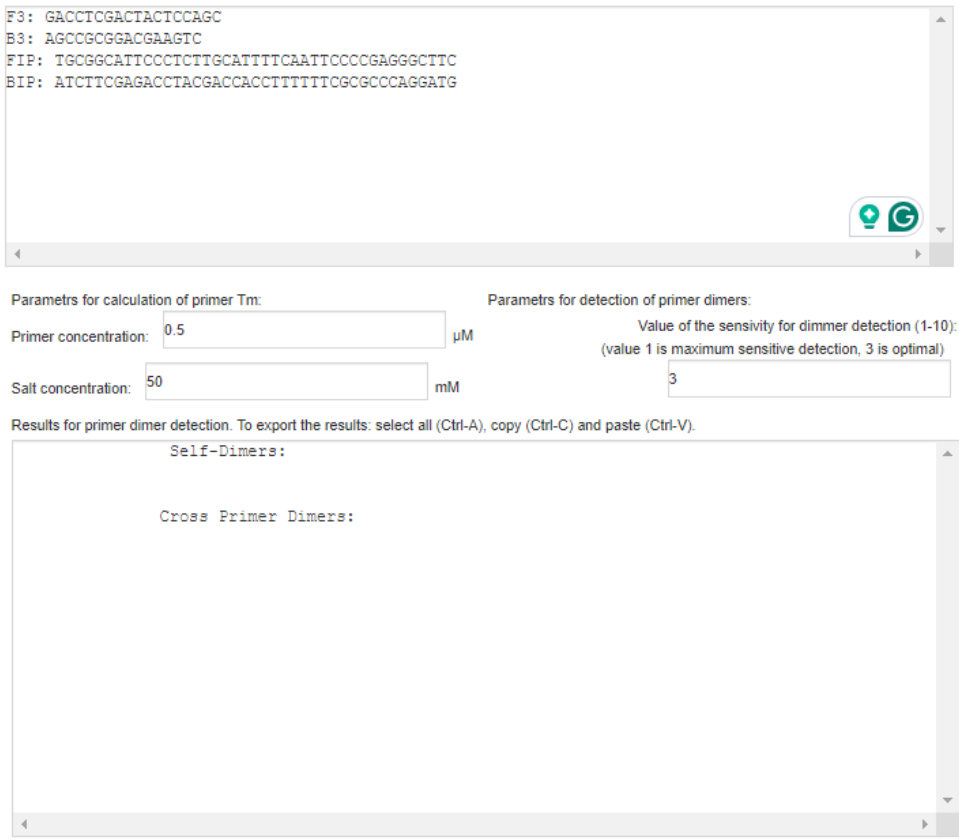
>CP104695.1 Pseudomonas aeruginosa strain 2021CK-01281 chromosome

```
product length = 245
Forward primer  1        CAAGCGCAAGATAGTCGCC  19
Template        1664042  ...................  1664024

Reverse primer  1        TCCGCTTGAACAGGCTGGTG  20
Template        1663798  ...................  1663817
```

>CP104982.1 Pseudomonas aeruginosa PA14 isolate Gamma chromosome

```
product length = 245
Forward primer  1        CAAGCGCAAGATAGTCGCC  19
Template        3570066  ...................  3570084

Reverse primer  1        TCCGCTTGAACAGGCTGGTG  20
Template        3570310  ...................  3570291
```

Figure S3. *In silico* PCR analysis of *F3* and *B3* primers for detection of *P. aeruginosa*



```
F3: GACCTCGACTACTCCAGC
B3: AGCCGCGGACGAAGTC
FIP: TGCGGCATTCCCTCTTGCATTTTCAATTCCCCGAGGGCTTC
BIP: ATCTTCGAGACCTACGACCACCTTTTTTCGCGCCCAGGATG
```

Parametrs for calculation of primer Tm:

Primer concentration: 0.5 µM

Salt concentration: 50 mM

Parametrs for detection of primer dimers:

Value of the sensivity for dimmer detection (1-10):
(value 1 is maximum sensitive detection, 3 is optimal)

3

Results for primer dimer detection. To export the results: select all (Ctrl-A), copy (Ctrl-C) and paste (Ctrl-V).

```
Self-Dimers:


Cross Primer Dimers:
```

Figure S4. *In silico* LAMP analysis of outer primers (*F3*, *B3*) and inner primers (*FIP*, *BIP*) for detection of *P. aeruginosa*