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Loop Mediated Isothermal Amplification as a Rapid Molecular Method for *Pseudomonas aeruginosa* T3SS ExoY Gene Septicemia Detection in Beta-Lactamase Species Co-Infections

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Background: *Pseudomonas aeruginosa* is among the most important causative agent of infection in chronically ill patients admitted in hospitals globally. Coupled with its, mixed symptomatology, rapid drug resistance tendency and its causation of severe disease, a fast, reliable and affordable diagnostic technique is required to enable healthcare providers expeditiously mitigate its progression and eventual treatment. The Loop-Mediated Isothermal Amplification (LAMP) technique has the potential to serve as a simple, rapid, specific, sensitive and cost-effective point-of-care diagnostic tool.
Broad Objective: To investigate Loop Mediated Isothermal Amplification as a molecular technique for microbial diagnostic and prognostic predictor.
Study Design: This study was aimed at evaluating LAMP assay against Simple Polymerase chain reaction and Multiplex PCR on the diagnosis of *P. aeruginosa* in mixed clinical samples.
Materials and Methods: This study developed *P. aeruginosa* Loop Mediated Isothermal Amplification (PaLAMP) assay to target the *Exo*Y gene with appropriate primer testing and

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validation procedures. Culture of patient bacterial samples was done on MHA and MHB medium, grown overnight in an Incubator and a incubating shaker at 37°c respectively. Housekeeping gene were identified through online bioinformatics and blasted against known sequences. A set of 6 primers, comprising 2 outer primers (F3 and B3), 2 inner primers (FIP and BIP), and 2 loop primers (FLP and BLP), were designed. Microbial DNA extraction was done followed by PCR amplification as a classical identification using LAMP outer primers 9(F3 and B3). LAMP amplicons were detected by real time turbidimetry (LA-500) at 64°C for 40 minutes as well as under UV light with 1.0 µl of 1/10-diluted original SYBR Green I.

Results: LAMP validation against traditional PCR shows a high limit of detection at 10^{-6}ng/µl compared to 10^{-5}ng/µl for PCR. The findings are consistent with outcomes for real time turbidimetric outcomes. Real time LAMP turbidimetric results was cross validated by direct observation through SYBR fluorescence under UV light for positive *P. aeruginosa* detection through positive amplification.

Conclusion: Thus far, Loop mediated isothermal amplification show significantly high limit of detection comparable to standard PCR, its use in field based diagnosis offers an opportunity for a cheap, reliable and faster method to determine disease trends and therapy approaches. This method can be applied in primary care to enhance accuracy in diagnosis and thereby prompt initiation of mitigation treatment regimens.

Keywords: Loop-mediated isothermal amplification; mitigation treatment; world health organization bioinformatics.

1. INTRODUCTION

According to the World Health Organization (WHO), 2017 report, this bacteria *P. aeruginosa* has been ranked among priority pathogens for research and development of new antibiotics, whereby it is ranked as Priority 1(Critical) multidrug resistant bacterial species including resistance to antibiotics of 'last resort'(β-lactams).

Among the major *P. aeruginosa* virulent factors, the type III secretory system has courted much attention due to its association with severe respiratory system, bloodstream, Wounds, Urinary tract infections [1].

Proper identification of resistant strains can greatly contribute towards preserving already diminishing sensitive drugs by alleviating environmental stress related resistance via broad spectrum administration of therapeutic agents.

The culture methods take ~36 h and in some instances require specific blood-based media and a 5% CO_2 incubator. Moreover, these methods are influenced by administration of prior antibiotic therapy in patients. To address these problems, gene amplification methods, such as the polymerase chain reaction (PCR), were developed to diagnose bacterial infections without requiring culture. However, the equipment required for conventional PCR and real-time PCR assays is relatively expensive, and these techniques are complex to perform in resource-limited laboratory settings in developing countries.

An alternative, rapid, and cost-effective method for gene amplification, the loop-mediated isothermal amplification (LAMP) assay has the potential to overcome the limitations of culture and PCR methods. LAMP employs a DNA polymerase with strand-displacement activity, along with two inner primers (forward inner primer, FIP; backward inner primer, BIP) and outer primers (F3, B3) that recognize six separate regions within a target DNA sequence.

Development of *P. aeruginosa* LAMP assay (*Pa*-LAMP) as well as Multiplex P. aeruginosa LAMP (*Pa*-mLAMP) that targets both housekeeping chromosomal genes as well as acquired virulent genes has the potential of transforming bacterial diagnostics.

LAMP has the ability to detect down to ten copies per reaction within 60 min with sensitivity 1000-fold more than that of conventional PCR [2].

1.1 Antibiotic Exposure and Precision Treatment

A loop-mediated isothermal amplification (LAMP) technique has been used as a novel nucleic acid detection method, whereby the target DNA can be amplified with high specificity and sensitivity

under an isothermal condition using a set of four to six specific primers.

Antibiotics are considered the standard of care for the treatment of most bacterial infections caused by drug-susceptible organisms. However, the worldwide spread of drug-resistant bacterial pathogens has greatly limited the repertoire of antibiotics available to effectively treat patients. As a result, clinicians are becoming increasingly reliant on last-line antimicrobial agents to treat a growing number of common bacterial infections. The efficacy of these agents has also begun to decline in the face of rapidly evolving resistant bacterial populations. Additionally, a growing number of studies are finding that alterations to the community structure of the host commensal microbiota following treatment with traditional antibiotics can have negative effects on long-term host health, especially when administered during childhood.

A recent review on antimicrobial resistance led by the British government has suggested that, without a rapid expansion of our antimicrobial arsenal, "superbugs" resistant to existing antibiotics could kill more than 10 million people a year by 2050. Further, research from the World Bank suggests that the dramatic increase in antibiotic-resistant infections could have dire consequences on the world economy with an estimated $100 trillion being spent to combat these infections by 2050. Together, these findings highlight the antibiotic resistance crisis we currently face and underscore the need to develop treatments that can potentiate or replace broad-spectrum antibiotic therapy in patients with.

Broad-spectrum antibiotic exposure increases the spread and uptake of bacterial genetic elements, including plasmids encoding antibiotic resistance genes, thus contributing to the development and spread of antibiotic resistance while selecting for the growth of bacteria that are resistant to the antibiotic being consumed. Further, by altering the community structure of the microbiota, broad-spectrum antibiotics also disrupt colonization resistance, opening space for pathogens to colonize or for typically low-abundance organisms present in the community to bloom and cause infection within various body sites which can result in long-lasting dysbiosis.

A study by Spaulding, *et al.* [3] showed that the bloom of normally restricted organisms is observed after treatment of mice with a single oral dose of the broad-spectrum antibiotic streptomycin, which produces high levels of intestinal inflammation and enhances colonization by *E. coli* species. The increased fitness of E. coli may be due to several factors, such as disruption of colonization resistance and alterations in the generation of cellular energy. Previous work has found that nitrate, released into the gut lumen as a byproduct of the streptomycin-induced intestinal inflammatory response, can be used by *E. coli* as a terminal electron acceptor for anaerobic respiration, a process not available to many strict anaerobes present in the gut that lack the necessary nitrate/nitrite reductase enzymes. High levels of intestinal inflammation have also been linked to increases in E. coli colonization of the gut of patients with IBD. IBD represents a subset of syndromes that are characterized by constitutively high levels of intestinal inflammation.

Biopsy specimens from patients with Crohn's disease (CD) and Ulcerative Colitis, two IBD syndromes, revealed that these patients have a 3–4 log increase in the levels of Enterobacteriaceae in their intestines compared to healthy controls. The enhanced fitness of E. coli during intestinal inflammation may also increase patients' chances of having bladder infection as several clinical studies have found that IBD patients have a significantly increased risk of recurrent UTI (rUTI) with >80% of patients having rUTI [4].

Therefore, even when used to target susceptible pathogens, treatment with broad-spectrum antibiotics that affect a larger proportion of a community may be detrimental to host health. Therefore, the need to develop highly targeted, precision therapeutics that can specifically kill or eliminate antibiotic resistant pathogens while producing minimal changes to the community structure of the microbiota has gained increased urgency [5].

During periods of dysbiosis, as occurs during antibiotic exposure, normal microbiota can infect the normally sterile body sites. Developing precision antimicrobials that target species in the specific locations may enhance treatment efficacy. However, targeting these organisms at the site of infection with small molecules may be limited by the ability of the compounds to penetrate biofilms. Yet, treating patients with precision antimicrobials may permit clinicians to reduce the population of these organisms in the

host reservoir and thus prevent the re-seeding. Identifying genes that promote the establishment of microbiota could potentially identify targets against which small molecule antagonists could be developed.

A significant pathogen in the development of pneumonia is the Gram-negative bacterium Pseudomonas aeruginosa. Cystic fibrosis patients are particularly vulnerable to acquiring a lung infection of this type. The global prevalence of multidrug-resistant P. aeruginosa is quickly rising, increasing the urgency for the development of alternative treatment strategies. While P. aeruginosa infections are more prevalent in immunocompromised individuals, this organism is also a common cause of skin and soft tissue infections in patients with burns or serious wounds. It is also known to regularly colonize medical devices such as catheters, and is a frequent cause of CAUTI. Multiple diverse therapeutic strategies have been proposed and pursued which target P. aeruginosa, including novel antibiotics and antimicrobials targeting key bacterial virulence factors [6].

1.2 Resistance

Antibiotic resistance, prompted by the overuse of antimicrobial agents, may arise from a variety of mechanisms, particularly horizontal gene transfer of virulence and antibiotic resistance genes, which is often facilitated by biofilm formation. The importance of phenotypic changes seen in a biofilm, which lead to genotypic alterations, cannot be overstated. Irrespective of if the biofilm is single microbe or polymicrobial, bacteria, protected within a biofilm from the external environment, communicate through signal transduction pathways (e.g., quorum sensing or two-component systems), leading to global changes in gene expression, enhancing virulence, and expediting the acquisition of antibiotic resistance. Thus, one must examine a genetic change in virulence and resistance not only in the context of the biofilm but also as inextricably linked pathologies.

Observationally, it is clear that increased virulence and the advent of antibiotic resistance often arise almost simultaneously; however, their genetic connection has been relatively ignored. Although the complexities of genetic regulation in a multispecies community may obscure a causative relationship, uncovering key genetic interactions between virulence and resistance in biofilm bacteria is essential to identifying new druggable targets, ultimately providing a drug discovery and development pathway to improve treatment options for chronic and recurring infection [7].

1.3 Horizontal Gene Transfer (HGT)

The majority of acquired antibiotic resistance is propagated through horizontal or lateral gene transfer between bacteria often due to the polymicrobial nature of infections and proximity of pathogens. Lateral gene transfer can occur either through uptake of environmental DNA, infection by bacteriophages or recombination/ exchange of plasmids. However, such a transfer of genetic material does not come without an evolutionary cost. To minimize this evolutionary cost bacteria have developed several genetic strategies such as plasmids, transposons, gene clusters, and operons.

While all of these strategies represent complexity when treating antibiotic resistance, a combination of an operon or cluster surrounded by mobile genetic elements is perhaps the most ominous, allowing genetic elements that confer antibiotic resistance to move as an unaltered block. The ability to shuffle genetic material between species may explain not only the transfer of antibiotic resistance but also the expansion of resistance beyond a single drug. High- level resistance often produces low- level resistance to an antibiotic in the same class as a byproduct

1.4 Bacterial Beta-Lactamases

Bacterial beta-lactamases conferred resistance on bacteria against penicillin (Papp-Wallace *et al.*, 2011). This seemingly ugly scenario led scientists to embark on a massive search for beta-lactamase inhibitors. Beta-lactamase inhibitors Carbapenems occupy a very important place in our fight against bacterial infections. This is because they are able to resist the hydrolytic action of beta-lactamase enzyme. Among the several hundreds of known beta-lactams, carbapenems possess the broadest spectrum of activity and greatest potency against Gram-positive and Gram-negative bacteria. As a result, they are often called “antibiotics of last resort” and are administered when patients with infections become gravely ill or are suspected of harboring resistant bacteria (Torres *et al.*, 2007). Examples of carbapenem are: Imipenem, meropenem, ertapenem (Brink *et al.*, 2004). Sadly, emergence of bacterial pathogens resistant to this life saving class of antibiotics has been reported. More worrisome is the fact that bacterial resistance to carbapenems is on the increase globally (Livermore *et al.*, 2011; Patel

and Bonomo, 2011) and is fast becoming an international concern (Papp-Wallace *et al*., 2011).

1.5 Isothermal Amplification Methods

Current advancement in PCR has led to the development of isothermal amplification methods, including loop-mediated isothermal amplification (LAMP). Loop-mediated isothermal amplification (LAMP) is increasingly used in molecular diagnostics as an alternative to PCR based methods. There are numerous reported techniques to detect the LAMP amplification including turbidity, bioluminescence and intercalating fluorescent dyes.

The isothermal amplification method requires only basic inexpensive equipment (i.e. standard heat block) with minimal operator training (Diaz and Winchell, 2016) or in some cases a simple turbid meter both of which are capable of providing reliable results within one hour or less. This method is useful for clinical screening, quickly especially under lack of resources or for point of care testing. LAMP is a unique nucleic acid amplification technique that amplifies few copies of DNA into billion copies within an hour under isothermal conditions with greater specificity. (Notomi *et al.,* 2000).

Furthermore, LAMP enables direct detection from clinical patient plasma without the requirement for DNA purification (Yang *et al*., 2016). The disadvantages of LAMP are proper primer designing required and LAMP multiplexing approach is less developed compared to PCR. [8].

LAMP method offers significant advantages for screening patients on a population basis and for diagnosis in clinical settings. Method utilizes a unique priming mechanism that yields specific DNA products in a shorter time than PCR. By using additional primers (i.e., the loop primers: loop primer forward, LF; loop primer backward, LB) designed to anneal the loop structure in LAMP, the LAMP reaction can be accelerated, resulting in enhanced sensitivity.

Highly desirable characteristics of LAMP includes high sensitivity and specificity with rapid reaction times and there is also evidence that LAMP amplification will proceed in the presence of PCR inhibitors permitting less stringent DNA extraction procedures.

CF *P. aeruginosa* isolates show higher numbers of colony variants by culture and more-pronounced between-patient and within-patient MIC heterogeneity. Examining antibiotic susceptibilities, a higher proportion of co-isolates from the CF cohort had discordant susceptibilities with respect to at least one antimicrobial agent than among those from the non-CF cohort.

Antimicrobial MIC heterogeneity is more characteristic of CF P. aeruginosa than of non-CF P. aeruginosa. The average number of P. aeruginosa coisolates per culture and the average number of agents with discordant susceptibilities between coisolates are higher in the CF cohort than in the non-CF cohort. CF cohort P. aeruginosa isolates presented antimicrobial MICs that were more heterogeneous than those seen with non-CF isolates, in the overall analyses as well as in the within-patient and between-patient analyses. The characteristic phenomenon of the CF *P. aeruginosa* heterogeneous antimicrobial MIC distribution in clonally related isolates is strongly supported by the genomic evidence of a high degree of core genome relatedness as well as unshared coding changes.

However, heterogeneous susceptibilities generated from colony variants of the same culture have not received elevated recognition. These have begun to address susceptibility characteristics that are beyond the clear-cut “S,” “I,” and “R” categories. The term “heteroresistance” may represent just one specific segment on the continuum of heterogeneous MICs.

The finding of disparate intraclonal antimicrobial MICs in vitro may be explained by bacterial functional divergence with increased interdependence of growth and resistance during niche specialization. With the advances in treatment of congenital or acquired defects and many other complex diseases, there is a growing patient population receiving frequent and long term antibiotic therapy that may be at increased risk of developing chronic focal infections. [9].

Toward this end, loop-mediated isothermal amplification (LAMP) assay has emerged as an attractive technique and has been recommended by the WHO for diagnosis of tuberculosis (WHO, 2016). The LAMP assay takes about an hour to give results, which can be visualized with naked eye. However, evidence remains limited for Microbial infection cases.

2. MATERIALS AND METHODS

2.1 Bacterial Strain

Clinical and Standard bacterial strains were obtained for use in this LAMP evaluation. Three samples of *Klebsiella pneumonia, Escherichia coli* and *Pseudomonas aeruginosa* standard strains were provided in triplicates by the 1st Hospital of Harbin medical university repository, Harbin Medical University (Harbin, HB, China). *P. aeruginosa* Standard strains were validated with the 16s rRNA sequencing using the laid down protocols as reference strains in accordance with the manufacturer's instructions and the sequence deposited with the Gene Bank (Gene Bank, MT048531.1 Accession number; *P. aeruginosa* strain HMU2019).

2.2 *P. aeruginosa* Clinical Strains

The clinical samples for *P. aeruginosa* were provided by 1st Hospital of Harbin Medical University and Daqin university Hospitals respectively.

2.3 Study Design

Patient samples suspected of *P. aeruginosa* infections were isolated. Sample processing and microbiological confirmation of *P. aeruginosa* was carried out, of which a total of 100 *P. aeruginosa* positive samples were identified and cultured in MHA in the Harbin Medical University Laboratory repository. The samples were then transferred to the laboratory of Department of Microbiology, Harbin Medical University for further processing.

2.4 Bacterial Strains and Culture Conditions

Hospital Bacterial cultures were subcultures by streaking on commercial Muller Hinton Agar in triplicates and incubated at 37 degrees Celsius for 24 hours. MHA sub-cultured colonies were transferred MHA broth under sterile conditions for an extra 24 hours incubation in an automatic shaker. The resultant bacteria was identified/confirmed through standard microbiological identification criteria (Isenberg HD. Essential procedures for clinical microbiology. Washington, D.C.: ASM Press; 1998.).

2.5 DNA Extraction

Genomic DNA from the samples was extracted from 71 strains by the Genomic DNA kit as per the manufacturer's instructions (MagJET Genomic DNA Kit). For detection limit analysis, genomic DNAs from *P. aeruginosa* serogroups was obtained and the concentrations were determined using spectrophotometric analysis (Pulton NanoPhotometer, P200/P200+; Pulton). Working dilutions of DNA were thereby prepared and stored at -20° C until further use.

To ascertain the detection limit of the LAMP assay, serial tenfold dilutions of genomic DNA was amplified, and the results contrasted with that obtained using conventional PCR. For the detection limit, LAMP testing was performed using tenfold dilutions of genomic DNA. The supernatant of a pooled *P. aeruginosa*-negative specimens was used, in which serial tenfold dilutions of genomic DNA was amplified, and the results of LAMP and conventional PCR compared.

2.6 *P. aeruginosa* 16s rRNA Sequencing

Positive P. aeruginosa controls were taken through ribosomal RNA sequencing by restriction digestion in Illumina Miniseq platform for species level identification and classification of *P. aeruginosa* for positive controls [10].

2.7 Gene Selection and Primer Design

After alignment analysis, two LAMP primer sets of six oligonucleotide primers targeting the identified specific gene *Exo*Y (Gen- Bank) and *Tox*A (Gen- Bank) conserved genes were identified by Primer Explorer 5, (http://primerexplorer.jp/lampv5/index.html). The identified primers were aligned to BLAST and analyzed for sequence similarity and specificity. Each LAMP primer set included two outer primers (F3 and B3), an FIP (Forward Inner Primer), BIP (Back Inner Primer), and a two loop primer (LF or LB). *Exo*Y gene was selected as the primer of choice given its specificity and sensitivity both in BLAST and in subsequent pilot assay for specificity (Table. 1).

2.8 *P. aeruginosa* PCR

Conventional PCR for ExoY genes. The isolated bacterial DNA shall be subjected to conventional polymerase chain reactions for detection of this gene. The reaction mixture contained 50 ng of sample DNA, 2.5mM of PCR buffer, 300 μM dNTP mix, 50 ng of each primer (forward and reverse), 1U of Taq polymerase in a 25 μl of final volume. The reaction conditions were optimized accordingly.

Table 1. *Exo Y* lamp primer sequence

Target genes	Primers	Amp. Temp	Sequence (5'– 3')		No. of bases
Exo Y gene	*ExoY*_F3	63	AGCCCGGACCGCTACT	PCR-F	16
	*ExoY*_B3	63	TCACCGAGAAGCCCTTGG	PCR-R	18
	*ExoY*_FIP	63	ATGTTCGACGGGAACCCCCAC-GCGGATATGCAGGCACGG		39
	*ExoY*_BIP	63	CGTCTTTGGCATTCGCCCGG-CGGGAAACCCTCTTCGATCA		40
	ExoY-_FL	63	CTGCGCATCGAACAGGTCCTG		21
	*ExoY*_BL		TTGAAAGAATCGTCACCACGC		21

2.9 LAMP reaction

Two separate *P. aeruginosa* LAMP DNA product detection techniques were used. The Real time Loop Mediated Isothermal Amplification performed on LA-500 LAMP real time turbidimeter at 63ºc for 40 minutes reaction optimization limit and the End point detection with SYBR Green under UV light. The LAMP assay reaction mixture constituting, 1.5 µM for each of the FIP and BIP, 0.5 µM of each of the LF and LB, 0.2µM of each of the F3 and B3, 9.0 U of Bst polymerase, 6.0 mM Mg2+, 1.4mM of deoxynucleoside triphosphate, 10mM KCl, 10Mm$(NH_4)SO_4$, 2.0 µL template DNA topped up to 25µL of final volume was set-up as per the manufacturer's instructions followed by incubation at 65°C for 40 min. Positive and negative controls were included in each run of the assay and the experiment done in triplicate. Non-*P. aeruginosa* pathogens were included in each run and the results were interpreted appropriately.

2.10 Statistical Analysis

Efficiency of LAMP assay was compared to Composite Reference Standard, which included culture, PCR among different specimen types using Chi-square test. A p-value ≤.05 was considered statistically significant for all the analyses. The clinical sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of conventional PCR testing was compared to those of LAMP with PCR as the gold standard.

3. RESULTS

3.1 Standard strains: *P. aeruginosa, E. coli, K. pneumonia*

P. aeruginosa standard strain samples were validated through sequencing of standard strain 16s rRNA sequencing (Sequence accession numbers: NCBI's Gen Bank: MT048531.1; *P. aeruginosa* strain HMU2019). Species specificity of standard strains was also tested against the *P. aeruginosa ExoY* LAMP primer set against other bacterial strains. Products for *P. aeruginosa* were observed within 40 Minutes in the turbidimetric real time LAMP amplification and no reaction was observed in other selected Beta-lactamase bacterial species (Fig.3). With this outcome it was demonstrated that the amplification products belong to the sequence target for the bacterial of interest using *Exo*Y-F3 and *Exo*Y – B3 primers.

3.2 P. aeruginosa Clinical Specimens

A set of 70 MHA overnight culture confirmed *P. aeruginosa* clinical specimens from Harbin Medical University First Hospital and Daqin University Medical Hospital were selected - 40 Samples from patients attending Harbin Medical University 1st Hospital and 30 Samples from Daqin Hospital - were obtained for *B*-Lactam *P. aeruginosa* species specific LAMP testing. From the outcome LAMP was able to detect 99% of the positive specimens analyzed while PCR identified 96% of the positive clinical specimens (Fig .1).

3.3 Specificity between LAMP and PCR

3.3.1 PCR specificity

With PCR as the gold molecular testing technique, all the specimens were validated using F3 and B3 primers with 25nl reaction mixture PCR. The outcome showed all the 70 hospital specimens were positive for *P. aeruginosa* while the non *P. aeruginosa* specimens were negative respectively (Fig.2).

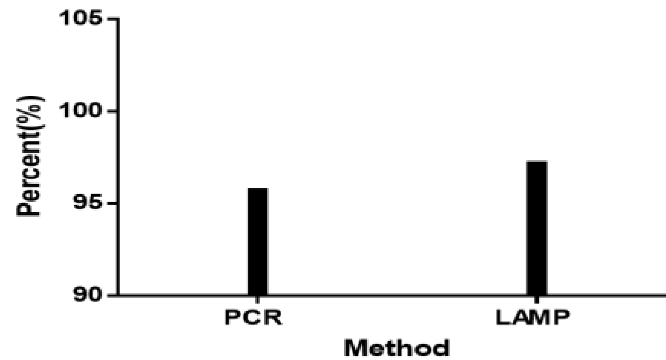

Fig. 1. Graph showing *P. aeruginosa* ExoY gene detection between PCR and LAMP

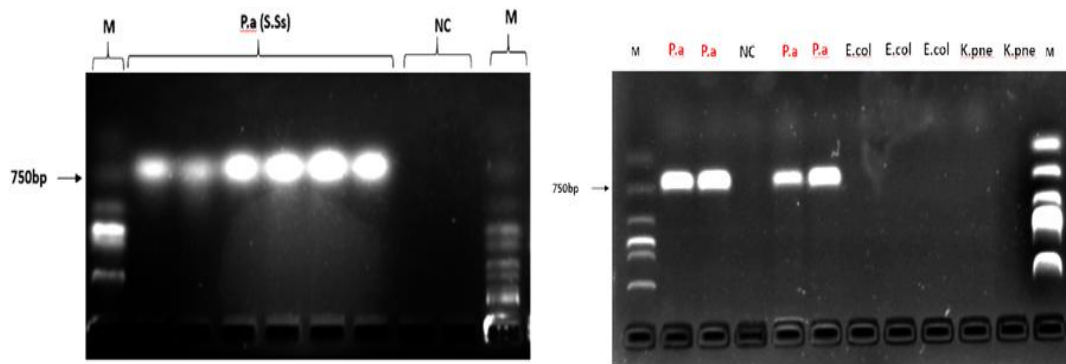

Fig. 2 a & b. Showing LAMP primer specificity using forward and reverse primer PCR
KEY: P.a - Pseudomonas aeruginosa; NC – Negative control; E. coli – E. coli Standard strain; K. pne – K. pneumonia standard strain; M – Marker

3.3.1.1 Concentrations

Fig. 2b. : Lane 1&2: 100 ng/µl, Lane 3&4: 250 ng/µl, Lane 5&6: 500 ng/µl, Lane 7: Negative Control, Lane 8: Negative Control, M: Marker.

3.3.2 LAMP specificity

LAMP showed high specificity to *P. aeruginosa.*

3.3.3 LAMP sensitivity

Using 25 µl reaction mixture for testing, the sensitivity assay was carried out in quadruplets. For LAMP sensitivity the outcome showed that LAMP would detect above 10^{-6} of dilution (Table 2) while PCR was limited at 10^{-4} and 10^{-5} (Table 2). On calorimetric inspection the detection limit was equally above 10^{-6} serial dilution. (Table 2).

3.3.4 Real-time turbidimetry reading (Absorbance over time)

Real time readings on LAMP turbidimetry showed consistency in absorbance with concentration gradient over time at concentration gradient of 10ng/µl and 10^{-6} ng/µl (*Fig.4*).

Table 2. Table showing LAMP against PCR sensitivity/ detection limits (LOD)

Sensitivity comparisons (LAMP vs PCR detection limit)							
Assay	1	10	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
LAMP (Real Time tubidimeter)	+	+	+	+	+	+	+
PCR	+	+	+	+	+	±	-
Calorimetric dye inspection	+	+	+	+	±	±	±

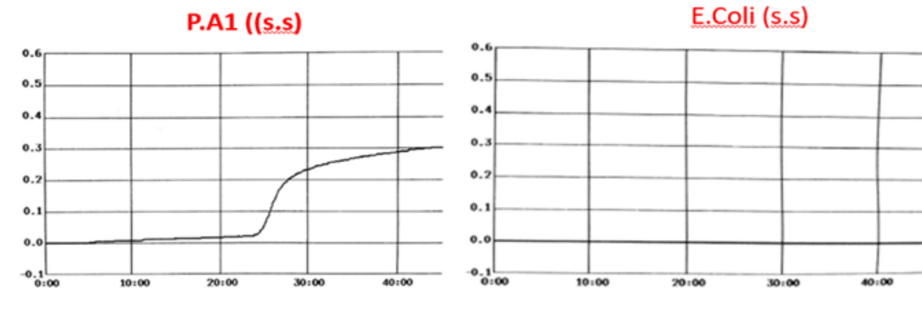

Fig. 3. Real time LAMP specificity reading by graphical turbidimetric changes

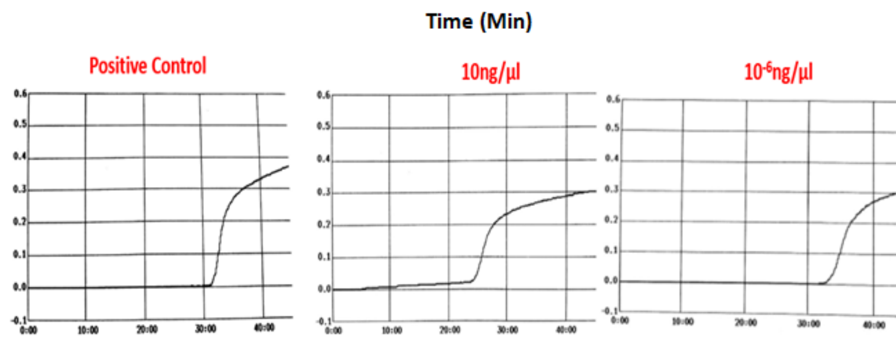

Fig. 4. Real time LAMP turbidimetry with concentration gradient

3.4 SYBR Dye Mediated Monitoring

The LAMP primer amplification with end point testing using nuclear dye, SYBR Green 1 showed consistent results to real time turbidimetric analysis with colour change from colourless to light green fluorescence visual change depicting a positive reaction to *P. aeruginosa.* It showed the colour changes across the concentration gradient as well as colour change for P. aeruginosa positive and negative samples and controls**.** These findings showed consistence with the outcomes in LAMP turbidimetric change as well as the PCR outcome.

4. DISCUSSION AND CONCLUSION

Beta Lactamases poses an eminent threat to treatment of bacterial infections, *P. aeruginosa* presents a classical bacteria in this group and yet a species that is top on the list of the priority bacterial species in this category for early detection and elimination. Quick, accurate and affordable filed based method provides a good chance for expeditious treatment as well as control of severe disease which has been as a result of delayed diagnosis and unreliable diagnostic criterion. Culturing technique is one other oldest methods of bacterial detection and identification albeit it has been occasioned with inaccuracies, time consuming and laborious. PCR which is a reliable and accurate molecular diagnostic technique has been a method of choice but the prohibitive cost and the need for expertise and material cost has made it inaccessible to low and middle income medical establishments.

LAMP provides viable alternative to conventional PCR given its applicability in both hospital and field setups with no need for tedious DNA purification required in PCR. The isothermal conditions eliminates the complexity of alternating temperatures and therefore eliminate need for sophisticated equipment.

Among the bacterial infections of major concern, Beta Lactamases pose a greater risk of drug selection and rapid disease progression. Among the Beta Lactams, Pseudomonas aeruginosa is a central pathogen that has been categorized as a priority infectious microbe by the World health organization. Its prompt detection and appropriate targeted therapy provides a mitigation towards severe disease. For proper mitigation, the diagnosis has to be accurate, thereby the need for use of a molecular

technique which is the most accurate diagnostic method. Loop Mediated Isothermal Amplification provides this opportunity and is likely to provide a viable alternative to the methods currently in use.

In this study *ExoY, a 378* amino acid adenylate cyclase housekeeping gene for *P. aeruginosa*, which is a part of the T3SS genes which the bacteria uses to achieve translocation apparatus that facilitate movement of effector proteins into the host for antibiotic resistance was identified as the gene of interest for identification given its role in drug selection, consistent positive result, primer specificity and lack of cross reactivity.

With the increased risk of future antibiotic resistance among Beta lactam strains among other infections, LAMP diagnosis can form a basis for future advancements in mixed infections through multiplexed gene identification LAMP to include all major infections of human interest.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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