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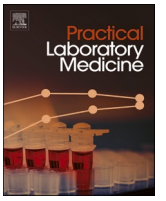

Registered Report Stage II

The value of loop-mediated isothermal amplification in diagnosing lower respiratory tract infections in children

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ABSTRACT

Objective: To evaluate the diagnostic value of loop-mediated isothermal amplification(LAMP) chip method (hereinafter referred to as "LAMP") in the detection of pathogens in children with lower respiratory tract infections(LRTIs).

Methods: Sputum samples from 1723 children with LRTIs hospitalized from April 2020 to April 2021 were collected. Pathogen detection was performed using both LAMP and sputum culture method(SCM).Detection rates and consistency between the two methods were analyzed using the Chi-square test and Kappa analysis.

Results: The positive detection rates of the LAMP and the SCM were 58.97 %(1016/1723) and 43.64 %(752/1723), respectively($P < 0.001$). The detection rates of *Streptococcus pneumoniae* (Spn)(24.26 %/13.52 %), *Staphylococcus aureus*(Sau)(13.12 %/10.39 %), *Acinetobacter baumannii* (Aba)(1.33 %/0.48 %), *Stenotrophomonas maltophilia* (Sma)(0.58 %/0.12 %), and *Haemophilus influenzae*(Hin)(31.05 %/16.19 %) were significantly higher with the LAMP than with the SCM ($P < 0.05$). Both methods showed that single infections were predominant among children, with positive rates of 65.06 % and 87.23 %, respectively, with Hin(49.92 %/33.69 %) being the most common pathogen.In mixed infections, the positive rates were 34.94 % and 12.77 %, respectively, with mixed infections of Hin and Spn being the most common, accounting for 48.89 % and 32.29 % of cases, respectively. There were significant differences in the detection rates of Spn, Sau, *Klebsiella pneumoniae*(Kpn), Sma, Hin, and *Escherichia coli*(Eco) between single and mixed infections($P < 0.05$). The detection results of Spn, Sau, Kpn, Hin, and Eco exhibited high consistency between the two methods, while the consistency for *Pseudomonas aeruginosa*(Pae), Aba, and Sma was lower.

Conclusion: The LAMP is simpler, faster, more sensitive and specific than SCM, offering a reliable laboratory diagnostic basis for clinical management of LRTIs in children.

1. Introduction

Lower respiratory infections (LRTIs) are common infectious diseases in children and represent the leading cause of death in children under five years old in developing countries, posing a severe threat to children's health and well-being [1]. The etiology of pediatric LRTIs is complex and varied, and the therapeutic approach differs according to the specific pathogen. Therefore, identifying the causative pathogen is essential for early, rational antibiotic use and to mitigate the increasing issue of antimicrobial resistance

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(AMR) [2], which, in turn, helps control the progression of the disease more effectively. While bacterial culture remains a widely used traditional detection method, it has notable drawbacks, such as complicated procedures, long detection cycles, poor timeliness, and low positive detection rates. Moreover, it has limitations in detecting certain pathogens. Currently, various PCR-based detection techniques are widely employed in pathogen identification. Among them, Loop-mediated isothermal amplification (LAMP) [3], a PCR-based LAMP, has been increasingly applied in pathogen detection due to its simplicity, speed, accuracy, high sensitivity, and specificity. However, no reports have addressed the diagnostic value of the LAMP in detecting pediatric LRTIs in this region, necessitating further clarification.

This study utilized the LAMP to simultaneously detect 13 common LRTIs pathogens: *Streptococcus pneumoniae*(Spn), *Staphylococcus aureus*(Sau), *Klebsiella pneumoniae*(Kpn), *Pseudomonas aeruginosa*(Pae), *Acinetobacter baumannii*(Aba), *Stenotrophomonas maltophilia* (Sma), *Haemophilus influenzae*(Hin), methicillin-resistant *Staphylococcus* (MRS), *Escherichia coli*(Eco), *Legionella pneumophila* (Lpn), *Mycoplasma pneumoniae* (Mpn), *Chlamydia pneumoniae* (Cpn), and *Mycobacterium tuberculosis* complex (MTBC). The results were then compared with those from SCM to explore the application value of the LAMP in the early diagnosis of pediatric LRTIs pathogens.

2. Materials and methods

2.1. Study subjects

This study selected 1723 pediatric inpatients diagnosed with LRTIs at our hospital from April 2020 to April 2021. The diagnostic criteria for LRTIs referred to the "Diagnostic Criteria for Hospital-acquired Infections (Trial)" [4]. Among the patients, 1083 were male, and 640 were female; 1554 patients were ≤3 years old, 94 were 3–6 years old, and 75 were between 6 and 14 years old (excluding 6-year-olds).

2.2. Methods

2.2.1. Reagents and Instruments

Bacterial genome DNA extraction kits, respiratory pathogen nucleic acid detection kits, Mini Smart Centrifuge 15054750 microfluidic chip centrifuge, and RTisochip™-A isothermal amplification microfluidic chip nucleic acid analyzer were purchased from CapitalBio Corporation, Beijing. Blood agar plates and chocolate agar plates (haemophilic type) were sourced from Autobio Diagnostics Co., Ltd., Zhengzhou. The Mixing Block MB-102 shaking thermoblock was purchased from Hangzhou Bioer Technology Co., Ltd. The 5424 microcentrifuge was sourced from Eppendorf AG, Germany. The CO_2 incubator was sourced from Thermo Fisher Scientific, USA. The VITEK MS mass spectrometer was purchased from bioMérieux, France.

2.2.2. Sample requirements

Morning deep sputum samples were collected from 1723 pediatric patients (white blood cell count >25 per low-power field and epithelial cell count <10 per low-power field), with a minimum volume of 0.6 mL. The samples were divided into two portions and subjected to pathogen detection using both the LAMP and the SCM.

2.2.3. LAMP detection

Nucleic acid was extracted according to the bacterial genome DNA extraction kit manual, and the amplification and detection of target pathogen genes were completed using a one-step method on an isothermal amplification microfluidic chip nucleic acid analyzer. The software calculated the Tp value. A Tp value ≤ the positive cutoff was interpreted as positive, while a Tp value > the positive cutoff was interpreted as negative.

2.2.4. SCM detection

Sputum samples were inoculated onto blood agar plates and chocolate agar plates (haemophilic type) according to the "National Clinical Laboratory Procedures (4th Edition)" [5]. The plates were incubated at 35 °C in a 5 % CO_2 environment for 24–48 h, after which potential pathogenic bacteria were observed and selected for identification using a mass spectrometer.

2.2.5. Statistical analysis

Statistical analysis was performed using SPSS 29.0. Categorical data were expressed as frequencies or percentages. Detection rate comparisons were performed using the Chi-square test. The Kappa analysis was used to evaluate the degree of consistency between the two methods, eliminating the "false consistency" caused by accidental factors. A Kappa value ≥ 0.75 indicated high consistency, 0.4<Kappa≤0.75 indicated moderate consistency, and Kappa<0.4 indicated low consistency.In addition, basic descriptive statistical methods such as the overall positive rate, positive coincidence rate, and negative coincidence rate were employed to provide supplementary data interpretation. $P < 0.05$ is considered statistically significant.

3. Results

3.1. Comparison of the characteristics and detection rates between the LAMP and the SCM

The comparison of the characteristics of the LAMP and the SCM is shown in Table 1.The two methods have different detection

principles. The samples for both are sputum and bronchoalveolar lavage fluid. The chip method focuses on the integrity of nucleic acids, while the culture method focuses on the viability of pathogens. Regarding the turnaround time, LAMP is significantly shorter than SCM, and there is not much difference in cost.

A total of 1723 sputum samples from pediatric LRTIs patients were analyzed using both the LAMP and the SCM. As shown in Table 2, the LAMP identified 1016 positive samples, yielding a positivity rate of 58.97 % (1016/1723), while the SCM detected 752 positive samples, with a positivity rate of 43.64 % (752/1723). The LAMP demonstrated a significantly higher positive detection rate compared to the SCM, with the difference being statistically significant($P < 0.001$).

The eight most frequently detected LRTIs pathogens—Spn, Sau, Kpn, Pae, Aba, Sma, Hin, and Eco—were all detected at higher rates by the LAMP than by the SCM. Specifically, Spn (24.26 % vs. 13.52 %), Sau (13.12 % vs. 10.39 %), Aba (1.33 % vs. 0.48 %), Sma (0.58 % vs. 0.12 %), and Hin (31.05 % vs. 16.19 %) showed significant differences in detotion rates between the two methods ($P<0.05$). Furthermore, both methods ranked the top six pathogens in the same order: Hin, Spn, Sau, Kpn, Eco, and Aba.

The LAMP and the SCM both indicated that single infections were predominant in pediatric LRTIs cases. The LAMP detected a positivity rate of 65.06 % (661/1016) for single infections, while the SCM detected 87.23 % (656/752), with Hin being the most common pathogen in both methods, accounting for 49.92 % (330/661) and 33.69 % (221/656), respectively. Notably, the LAMP detected a significantly higher rate of mixed infections compared to the SCM (34.94 % vs. 12.77 %). In both methods, mixed infections of Hin and Spn were the most frequent, constituting 48.89 % (131/268) and 32.29 % (31/96) of the mixed infection cases, respectively. Comparison of the detection rates for single and mixed infections between the two methods revealed statistically significant differences in the rates for Spn, Sau, Kpn, Sma, Hin, and Eco ($P<0.05$).

Additionally, the LAMP identified 158 cases of MRS and one case of MTBC. Furthermore, it detected two cases of Lpn, three cases of Mpn, and three cases of Cpn, which are pathogens typically not isolated by conventional culture methods. Conversely, the SCM detected 46 cases of pathogens outside the detection range of the LAMP, including *Enterobacter cloacae*, *Serratia marcescens*, *Klebsiella oxytoca*, *Klebsiella aerogenes*, *Pantoea agglomerans*, *Citrobacter freundii*, *Citrobacter koseri*, *Raoultella ornithinolytica*, *Raoultella planticola*, and *Morganella morganii*.

3.2. Comparison of concordance between the LAMP and the SCM

A total of 13,784 test results were generated for the eight most frequently detected LRTIs pathogens across 1723 samples, as shown in Table 3. The overall positivity rate using the LAMP was 9.55 % (1317/13784), in contrast to 5.85 % (806/13784) for the SCM, with the LAMP demonstrating a significantly higher overall positive detection rate ($P<0.05$). Among the 806 positive results detected by the SCM 645 cases were also positive by the LAMP, resulting in a positive concordance rate of 80.02 %. The highest positive concordance rates were observed for Hin and Spn, at 91.40 % and 89.27 %, respectively. Out of the 12,978 samples that tested negative by the SCM, 12,306 samples were also negative by the LAMP, yielding a negative concordance rate of 94.82 %. The negative concordance rate exceeded 80 % for all eight pathogens. The overall concordance rate between the two methods was 93.96 %. The Kappa values for Spn (Kappa = 0.563), Sau (Kappa = 0.550), Kpn (Kappa = 0.555), Hin (Kappa = 0.526), and Eco (Kappa = 0.513) ranged between 0.4 and 0.75, indicating moderate consistency in detection results. However, lower Kappa values were observed for Pae (Kappa = 0.305), Aba (Kappa = 0.188), and Sma (Kappa = 0.165), reflecting poor consistency between the two methods for these pathogens.

4. Discussion

The World Health Organization's 2021 report highlighted that in 2019, 2.59 million people globally died from LRTIs, making it the fourth leading cause of death, with the mortality rate ranking second in low-income countries [6]. Many LRTI-related deaths are associated with AMR. A study published in The Lancet indicated that, in 2019, 1.27 million people died due to AMR, with approximately one-fifth of these deaths occurring in children under the age of five [7]. According to O'Neill J [8], by 2050, AMR could lead to 10 million deaths worldwide. AMR has undoubtedly become one of the major public health threats of the 21st century.

The diversity of LRTIs pathogens poses a significant challenge in accurate pathogen identification using conventional biochemical indicators, which are often insufficient. Serological tests are prone to false negatives, while SCM have the disadvantage of being time-consuming and lacking timeliness, leading clinicians to rely on empirical treatments that can increase the risk of AMR. With the advancement of molecular diagnostic technologies, tools such as fluorescence quantitative PCR, multiplex PCR, and high-throughput

Table 1
Comparison of the characteristics between LAMP and SCM.

Characteristics	LAMP	SCM
principles	PCR	Bacterial culture method
specific sample requirements	Sputum, bronchoalveolar lavage fluid, etc. need nucleic acid integrity, not viability	Sputum and bronchoalveolar lavage fluid samples must maintain pathogen viability
laboratory involvement	Molecular Biology Laboratory	Microbiology Laboratory
turnaround time	1–2 hours	2–7 days
cost	High for detection reagents	Low for culture media but high for identification and susceptibility tests

LAMP, loop-mediated isothermal amplification; SCM, sputum culture method; PCR,Polymerase Chain Reaction.

Table 2
Comparison of the detection rates detected by LAMP and SCM.

Pathogen	Cases of infection				Single infection		Mixed infection		χ^2	*P*
	LAMP	SCM	χ^2	*P*	LAMP	SCM	LAMP	SCM		
Spn	418(24.26)	233(13.52)	64.818	<0.001	228(54.55)	185(79.40)	190(45.45)	48(20.60)	39.844	<0.001
Sau	226(13.12)	179(10.39)	6.181	0.013	44(19.47)	131(73.18)	182(80.53)	48(26.82)	117.448	<0.001
Kpn	52(3.02)	50(2.90)	0.040	0.841	23(44.23)	36(72.00)	29(55.77)	14(28.00)	8.061	0.005
Pae	6(0.35)	7(0.41)	0.077	0.781	2(33.33)	3(42.86)	4(66.67)	4(57.14)	0.124	0.725
Aba	23(1.33)	8(0.48)	7.324	0.007	6(26.09)	5(62.50)	17(73.91)	3(37.50)	3.438	0.064
Sma	10(0.58)	2(0.12)	5.352	0.021	0(0.00)	1(50.00)	10(100.00)	1(50.00)	5.455	0.020
Hin	535(31.05)	279(16.19)	105.411	<0.001	330(61.68)	221(79.21)	205(38.32)	58(20.79)	25.763	<0.001
MRS	158(9.17)	–	–	–	2(1.27)	–	156(98.73)	–	–	–
Eco	47(2.73)	48(2.79)	0.011	0.917	21(44.68)	40(83.33)	26(55.32)	8(16.67)	15.439	<0.001
Lpn	2(0.12)	–	–	–	2(100.00)	–	0(0.00)	–	–	–
Mpn	3(0.17)	–	–	–	2(66.67)	–	1(33.33)	–	–	–
Cpn	3(0.17)	–	–	–	0(0.00)	–	3(100.00)	–	–	–
MTBC	1(0.06)	–	–	–	1(100.00)	–	0(0.00)	–	–	–
Other	–	46(2.67)	–	–	–	34(73.91)	–	12(26.09)	–	–
Total (isolates/cases)	1484/1016	852/752	80.956	<0.001	661/661	656/656	823/355	196/96	111.830	<0.001

Spn, *Streptococcus pneumoniae*; Sau, *Staphylococcus aureus*; Kpn, *Klebsiella pneumoniae*; Pae, *Pseudomonas aeruginosa*; Aba, *Acinetobacter baumannii*; Sma, *Stenotrophomonas maltophilia*; Hin, *Haemophilus influenzae*; MRS, methicillin-resistant *Staphylococcus*; Eco, *Escherichia coli*; Lpn, *Legionella pneumophila*; Mpn, *Mycoplasma pneumoniae*; Cpn, *Chlamydia pneumoniae*; MTBC, *Mycobacterium tuberculosis* complex; LAMP, loop-mediated isothermal amplification; SCM, sputum culture method.

Table 3
Comparison of consistency detected by LAMP and SCM.

Pathogen	LAMP(+) SCM (+)	LAMP(−) SCM (+)	LAMP(+) SCM (−)	LAMP(−) SCM (−)	Positive coincidence rate	Negative coincidence rate	Kappa	*P*
Spn	208	25	210	1280	89.27 %	85.91 %	0.563	<0.001
Sau	122	57	104	1440	68.16 %	93.26 %	0.550	<0.001
Kpn	29	21	23	1650	58.00 %	98.63 %	0.555	0.880
Pae	2	5	4	1712	28.57 %	99.77 %	0.305	1.000
Aba	3	5	20	1695	37.50 %	98.83 %	0.188	0.004
Sma	1	1	9	1712	50.00 %	99.48 %	0.165	0.021
Hin	255	24	280	1164	91.40 %	80.61 %	0.526	<0.001
Eco	25	23	22	1653	52.08 %	98.69 %	0.513	1.000
Total	645	161	672	12306	80.02 %	94.82 %	–	–

Spn, *Streptococcus pneumoniae*; Sau, *Staphylococcus aureus*; Kpn, *Klebsiella pneumoniae*; Pae, *Pseudomonas aeruginosa*; Aba, *Acinetobacter baumannii*. Sma, *Stenotrophomonas maltophilia*; Hin, *Haemophilus influenzae*; Eco, *Escherichia coli*; LAMP, loop-mediated isothermal amplification; SCM, sputum culture method.

sequencing are increasingly utilized in clinical pathogen detection [9]. However, these techniques have their limitations, especially when it comes to applicability in primary healthcare settings [10,11]. The LAMP, which utilizes isothermal amplification of pathogen nucleic acids at 65 °C, stands out for its high specificity, efficiency, simplicity of operation, and affordability. This study aimed to determine whether the LAMP is superior to the SCM in diagnosing pediatric LRTIs by comparing the positive detection rates and consistency of results between the two methods in 1723 pediatric sputum samples. The goal was to provide clinical guidance on selecting appropriate diagnostic methods, thereby improving diagnostic accuracy, treatment outcomes, and reducing the incidence of AMR.

The findings from this study revealed that the positive detection rate of the LAMP was significantly higher than that of the SCM, with the most commonly detected pathogens being Hin, Spn, and Sau. These results are consistent with the studies of Zhao Liu et al. [12] and Yonghong Wang et al. [13], which also identified these pathogens as the predominant causes of LRTIs in children. Upon comparison, the top six pathogens ranked by positive detection rates in both methods were identical: Hin, Spn, Sau, Kpn, Eco, and Aba. Among these common pediatric LRTI pathogens, the positive concordance rates were all greater than 50.00 %, demonstrating a high degree of consistency. The ranking of isolation rates aligns with the 2022 data released by the China Pediatric Bacterial Resistance Surveillance Group [14] and is consistent with findings from studies by Hua Feng Li et al. [15] and Hou JY et al. [16]. Conversely, studies by CZ Wang et al. [17] and YY Si et al. [18] found that the major pathogens of adult LRTIs were Kpn, Pae, and Aba, highlighting the significant differences in the primary pathogens between children and adults. The LAMP detected higher positivity rates for Spn, Sau, Aba, Sma, and Hin compared to the SCM, whereas the detection rates for typical adult LRTIs pathogens, such as Kpn, Pae, and Eco, demonstrated minimal differences between the two methods. These findings are consistent with the study by Xiaona Xu et al. [19], which also demonstrated that the overall sensitivity of the LAMP exceeds that of the SCM.

In this study, inconsistent results were obtained between the "gold standard" SCM and the LAMP, mainly for common pediatric pathogens like Hin and Spn. This is likely due to the special requirements for culturing conditions and specimen collection time,

making the culturing and identification processes more complex and error-prone. For instance, Hin can be affected by pre-hospital medications [20], leading to false negatives. When facing inconsistent results, retesting is indispensable to reduce potential random errors in a single test. Meanwhile, comprehensive judgment should be made in close combination with the patient's clinical manifestations. If possible, fluorescence quantitative PCR or high-throughput sequencing can be introduced for auxiliary diagnosis. Studies have shown that the LAMP is advantageous in detecting fastidious and slow-growing organisms like Hin and Spn. This finding aligns with the "Expert Consensus on Nucleic Acid Detection of Respiratory Pathogens in Children" [21], which recommends the LAMP as the first choice for detecting respiratory pathogens in outpatient and emergency pediatric cases. Furthermore, our study demonstrated a significant difference in the detection rates of mixed infections between the two methods. While the SCM detected only four cases of mixed infections involving three pathogens, the LAMP identified 87 cases with $\geq$3 mixed infections. The LAMP also revealed that Pae, Aba, and Sma predominantly occurred in mixed infections, whereas the SCM had a lower detection rate for these pathogens. This suggests that the LAMP can overcome the limitations of the SCM, which may miss certain pathogens due to nutrient competition. The study by Xiao Li et al. [22] also demonstrated that the LAMP is capable of detecting a greater number of mixed infections, making it particularly suitable for pathogen detection in critically ill pediatric LRTIs patients. Early identification of mixed infections can guide the early administration of combination antibiotic therapy, improving clinical outcomes.

The LAMP reagent kit used in this study also detected Mpn, Cpn, and Lpn. Although the study by Zijing Wang et al. [23] suggests that the LAMP has strong specificity for detecting Mpn, the small number of positive samples for these pathogens in this study limits the ability to draw definitive conclusions. Future studies are needed to further validate the value of the LAMP in detecting non-bacterial pathogens.

To sum up, the LAMP has obvious advantages over the SCM. It can produce results quickly, within 1–2 h, is highly sensitive to specific pathogens, and requires less laboratory infrastructure. However, it is prone to false positives due to contamination and cannot distinguish whether the microorganisms are viable or not. The SCM is the "gold standard" for diagnosing LRTIs. It can accurately identify and characterize pathogens and conduct antibiotic sensitivity tests. Nevertheless, it takes several days for turnaround, demands strict professional facilities and personnel, and may miss microorganisms that are difficult to culture or affected by antibiotics. Therefore, the LAMP has been widely adopted for pathogen detection in respiratory viruses [24,25], fungi [26], and parasites [27,28], offering a rapid, accurate, and cost-effective diagnostic tool that is particularly valuable for primary healthcare institutions in diagnosing LRTIs. Although the LAMP has some limitations in the breadth of pathogen coverage, our study confirms its advantages over the SCM in detecting common pediatric LRTIs pathogens, particularly for fastidious and slow-growing organisms. The LAMP is simpler, faster, more sensitive, and more specific, providing higher diagnostic efficacy. Combining the LAMP with the SCM could complement each other, expand pathogen coverage, and improve the positive detection rate and efficiency, providing clinicians with accurate, reliable, and timely diagnostic information, thereby reducing the threat of AMR to public health.

CRediT authorship contribution statement

Feng Yan: Writing – original draft, Validation, Investigation, Data curation. **Shikun Xu:** Software, Data curation. **Meijing Shen:** Data curation. **Yu Zhao:** Validation. **Huabo Tong:** Validation. **Kaifeng Wu:** Resources, Funding acquisition, Data curation. **He Zha:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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