

A Pilot Study of Quantitative Loop-mediated Isothermal Amplification-guided Target Therapies for Hospital-acquired Pneumonia

Fang Wang¹, Ran Li¹, Ying Shang¹, Can Wang², Guo-Qing Wang², De-Xun Zhou¹, Dong-Hong Yang¹, Wen Xi¹, Ke-Qiang Wang¹, Jing Bao¹, Yu Kang³, Zhan-Cheng Gao¹

¹Department of Respiratory and Critical Care Medicine, Peking University People's Hospital, Beijing 100044, China

²Department of Assay Development, CapitalBio Corporation, Beijing 102206, China

³CAS Key Laboratory of Genome Sciences and Information, Beijing Institute of Genomics, Chinese Academy of Sciences, Beijing 100029, China

Abstract

Background: It is important to achieve the definitive pathogen identification in hospital-acquired pneumonia (HAP), but the traditional culture results always delay the target antibiotic therapy. We assessed the method called quantitative loop-mediated isothermal amplification (qLAMP) as a new implement for steering of the antibiotic decision-making in HAP.

Methods: Totally, 76 respiratory tract aspiration samples were prospectively collected from 60 HAP patients. DNA was isolated from these samples. Specific DNA fragments for identifying 11 pneumonia-related bacteria were amplified by qLAMP assay. Culture results of these patients were compared with the qLAMP results. Clinical data and treatment strategies were analyzed to evaluate the effects of qLAMP results on clinical data. McNemar test and Fisher's exact test were used for statistical analysis.

Results: The detection of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Stenotrophomonas maltophilia*, *Streptococcus pneumoniae*, and *Acinetobacter baumannii* by qLAMP was consistent with sputum culture ($P > 0.05$). The qLAMP results of 4 samples for *Haemophilus influenzae*, *Legionella pneumophila*, or *Mycoplasma pneumoniae* (MP) were inconsistent with culture results; however, clinical data revealed that the qLAMP results were all reliable except 1 MP positive sample due to the lack of specific species identified in the final diagnosis. The improvement of clinical condition was more significant ($P < 0.001$) in patients with pathogen target-driven therapy based on qLAMP results than those with empirical therapy.

Conclusion: qLAMP is a more promising method for detection of pathogens in an early, rapid, sensitive, and specific manner than culture.

Key words: Hospital-acquired Pneumonia; Sputum Culture; Target-driven Therapy; Quantitative Loop-mediated Isothermal Amplification

INTRODUCTION

Hospital-acquired pneumonia (HAP) is defined as a low respiratory tract infection, which develops 48 h after hospital admission in a patient without infection at admission.^[1] HAP currently ranks second among nosocomial infections and accounts for 25% of the infections in Intensive Care Units.^[1-4] HAP has a significant impact on the financial burden of health care, and new cases drive the increasing emergence of pathogens with multi- or pan-antibiotic resistance. Therefore, identifying the infectious etiology in different settings is the key step for mitigating or obviating the severe infection in time. Early identification of specific pathogens could significantly improve the morbidity and mortality of

HAP, and lower the cost of treatment as well. So far, the most common method used to implement HAP etiology is still tied to sputum culture. Sputum culture, however, shows significant disadvantages pathogens identification. In addition to sputum culture's relatively low sensitivity and the difficulty with which it identifies atypical pathogens,^[1,5,6] the time

Address for correspondence: Prof. Zhan-Cheng Gao,
Department of Respiratory and Critical Care Medicine, Peking University
People's Hospital, Beijing 100044, China
E-Mail: zcgao@bjmu.edu.cn

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required to obtain results always leads to empirical antibiotic therapies rather than target therapies for patients with HAP. This, in turn, often increases the risk of antibiotic resistance.

An rapid innovative method for etiology identification of HAP, the quantitative loop-mediated isothermal amplification (qLAMP), has already been used in the diagnosis of virus, fungus, parasite, and tuberculosis infections and is now commercially available.^[7-12] It is a novel assay that focuses on the genetics of pathogens based on rapid nucleic acid amplification method. Therefore, this technique has two important advantages such as rapid diagnosis and high sensitivity.^[13-15] In addition, qLAMP is also a high specific assay, which could detect different bacteria with quantified copies.^[16] Since the excellent timeliness and accuracy of qLAMP for etiological diagnosis to the lower respiratory tract infection has been confirmed by our group,^[16] we initiated a pilot, prospective, and interventional study to investigate the value of qLAMP to guide target antibiotics therapies in a small group of patients with HAP.

METHODS

Study design

Patients with suspected HAP from August 2011 to March 2014 at Peking University People's Hospital (Beijing, China) were recruited in the study, which were approved by the Ethical Committee of Peking University People's Hospital (No. 2011-83). All participants provided written informed consents. These patients were initially diagnosed as suspected cases of HAP occurring more than 48 h after admission and were not incubated at the time of admission, having typical characteristics of pneumonia, which were firmly inferred from chest X-rays and the following criteria:^[17] [I] at least one of the following: (1) fever ($>38.5^{\circ}\text{C}$), (2) leukopenia (peripheral white blood cell count [WBC] $<4.0 \times 10^9/\text{L}$) or leukocytosis (WBC $>10.0 \times 10^9/\text{L}$), and (3) for adults 70 years old or older, mental status changes with no other recognized cause; [II] at least two of the following: (1) new-onset of purulent sputum, or change in character of sputum, or increased respiratory secretion, or increased suctioning requirements, (2) new-onset or worsening cough, or dyspnea, or tachycardia, (3) rales or bronchial breath sounds, and (4) worsening gas exchange ($\text{PaO}_2/\text{FiO}_2 \leq 240$), increased oxygen requirements, or increased ventilation demand. Patients with noninfectious diseases, viral infection, fungal infection, or tuberculosis were subsequently excluded from the study.

Once patients were enrolled, lower respiratory secretion samples were collected on the 1st day for both routine culture and qLAMP assays, of which the results were reported to the clinicians. Data of each patient were also collected from the medical records, with particular attention to clinical manifestations and treatment strategies before and after the qLAMP results reporting.

To determine the final diagnosis and assess the treatment response for each patient, 2 independent pulmonologists blinded to qLAMP results reviewed all available medical records (including patient history, physical examination,

and results of laboratory tests, including blood routine examination, biochemical indicators, plasma electrolytes, blood gas analysis, and chest radiograph) pertaining to the patient from the time of HAP presentation to discharge/death. Cases were reviewed and adjudicated by a third pulmonologist when confronting a disagreement.

Procedures

After liquefied in an equal volume of 10% NaOH, DNA specimen of each sample was isolated using the Universal Kit for Bacterial DNA Extraction (Capitalbio Corporation, China). The specimens were then prepared for qLAMP using a set of specific primers for *Streptococcus pneumoniae* (SP), *Staphylococcus aureus* (SA), *Escherichia coli* (EC), *Klebsiella pneumoniae* (KP), *Pseudomonas aeruginosa* (PA), *Acinetobacter baumannii* (AB), *Stenotrophomonas maltophilia* (SM), *Haemophilus influenzae* (HI), *Legionella pneumophila* (LP), *Mycoplasma pneumoniae* (MP), and *Chlamydomphila pneumoniae* (CP). qLAMP primer system of each species of pathogen is composed of six primers recognizing eight distinct regions on the target DNA, termed a forward outer primer (F3), a backward internal primer (BIP) (B3), a forward internal primer (FIP), a BIP, and loop primers (LF and LB). Eight-pathogen primer sequences are used same as we did before, including SP, SA, EC, KP, PA, AB, SM, and HI.^[16] Those for atypical pathogens were redesigned as shown in Table 1, and both their sensitivity and specificity were ensured by quantified DNA isolated in 27 bacterial species as we did before.^[16]

Table 1: Primers for atypical pathogens used in this study

Target species	Primers	Nucleotide sequence
LP	F3	GCAAGACGCTATGAGTGG
	B3	TGATTACTTTGTATTGCAAACCA
	FIP	GCCATCAAATCTTTCTGAAACTTGTCTCAATTGGCTTTAACCGAAC
	BIP	GCGGATGAAAATAAAGTAAAGGGGGCTTGGCAATACAACAACGC
	LF	TAAGAACGCTTTTCATTTGCT
	LB	CTGAAAACAACAAACAAGCCAG
MP	F3	GTAAACCCGCAAACGCC
	B3	TGCTCATAGTACACCACGCT
	FIP	TGCAGCCCCACTCAAACCAAGACCAAACCGGGCAGATC
	BIP	TCAAAAACAAGGTCCCCGTCGAGGCACGAGTAAACGGCAA
	LF	CGCCAAAAGGGGTTAAAGGT
	LB	CAAGACCCCTCCAATCCCT
CP	F3	AATTATAAGACTGAAGTTGAGCA
	B3	AGAGAGATATGGCATATCCG
	FIP	TTCTCTTAGAGGCAACGTAGACTTTGGGAGATGCAGATTTAGATCA
	BIP	TCAAGTTGGAGATAAAAATGGCTGGCGGGAACGATTTTGGAAAC
	LF	ACCTTGGCGAATGACACCA
	LB	ACGACACGGAAATAAAGGTGTT

FIP: Forward internal primer; BIP: Backward internal primer; LP: *Legionella pneumophila*; MP: *Mycoplasma pneumoniae*; CP: *Chlamydomphila pneumoniae*.

The reaction was performed at 65°C for 45 min in a 25-μl reaction mixture consisting of 1.6 μmol/L each of FIP and BIP, 0.2 μmol/L each of F3 and B3, 0.4 μmol/L each of LF and LB, 8 U of the Bst DNA polymerase large fragment (New England Biolabs Inc., Beverly, Mass., USA), 0.4 mmol/L dNTP, 0.1 mmol/L dUTP, 0.8 mol/L betaine, 6 mmol/L MgSO₄, 0.5 mg/ml BSA, 0.6 × EvaGreen (Biotium, Inc., CA, USA), 0.1 U/ml Uracil-DNA Glycosylase (Fermentas Inc., MD, USA), 20 mmol/L Tris-HCl (pH 8.8 at 25°C), 10 mmol/L KCl, 10 mmol/L (NH₄)₂SO₄, 0.1% Triton X-100, and 2 μl template DNA or PCR grade H₂O as negative control, and then heated at 80°C for 5 min to terminate the reaction. All amplifications were performed with an RT-cycler Real-time Fluorescence Quantitative PCR Instrument (Capitalbio Corporation, Beijing, China). The titer was quantified according to the standard curves obtained from prequantified DNA templates as described previously.^[16] Biochip technology was introduced in January 2013 at Peking University People's Hospital, and the reaction was performed on a microfluidic device after then.

The qLAMP tests and routine cultures were conducted by two experienced technicians awareness of the sample identities in two separated laboratories of Peking University People's Hospital.

Statistical analysis

We constructed a contingency table and used McNemar test to evaluate the congruence of qLAMP and culture results. The differences between patients with or without treatment strategies adjustment based on qLAMP results were tested with Fisher's exact test or *t* test. Baseline data of these patients were expressed as mean ± standard deviation (SD) for normally distributed values. All analyses were performed with the use of SPSS statistics software, version 19.0 (IBM, USA). A *P* < 0.05 is considered statistically significant difference.

RESULTS

Quantitative loop-mediated isothermal amplification assaying outcome of specimen from hospital-acquired pneumonia patients

Totally, 76 samples were recruited from 110 eligible samples overall in our study [Figure 1]. The 76 samples were collected from 60 patients with HAP. None of the samples were collected from the same onset of HAP. As shown in Table 2, there were 70 samples with qLAMP results greater than 10⁵ copies/ml, 23 samples with qLAMP results between 10³ and 10⁵ copies/ml, and 16 samples with qLAMP results below 10³ copies/ml.

Congruence of quantitative loop-mediated isothermal amplification and culture results

The concordance rates of the two assays for detecting SA, EC, PA, KP, SM, SP, and AB are 90.79%, 98.68%, 89.47%, 93.42%, 93.42%, 100.00%, and 77.63%, respectively [Table 3]. We also evaluated the difference between qLAMP and culture results by McNemar test, in which no significant difference was found (*P* > 0.05) [Table 3 and Supplementary Tables 1-7]. The qLAMP results

of 4 samples for HI, LP, or MP were positive, while the culture results for these specimens were negative probably because of their low detectable rates in culture. We then

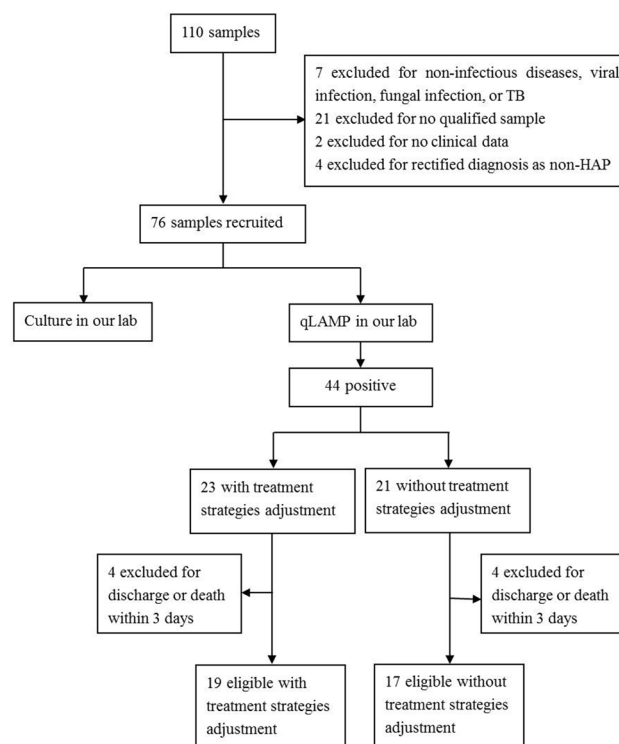


Figure 1: Study profile. For each eligible patient, we collected lower respiratory secretion samples on the 1st day for routine culture and quantitative loop-mediated isothermal amplification tests and reported the results to the clinicians. We also collected the clinical data and treatment strategies before and after reporting the quantitative loop-mediated isothermal amplification results.

Table 2: qLAMP results of specimen from patients with hospital-acquired pneumonia

Pathogens	>10 ⁵ copies/ml	10 ³ –10 ⁵ copies/ml	<10 ³ copies/ml	Negative	Total
SP	1	0	0	75	76
SA	11	5	6	54	76
EC	1	0	1	74	76
KP	8	4	3	61	76
PA	17	3	0	56	76
AB	20	5	1	50	76
SM	8	2	0	66	76
HI	1	1	3	71	76
LP	1	3	0	72	76
MP	2	0	2	72	76
CP	0	0	0	76	76
Total	70	23	16	727	836

Data are presented as number, unless otherwise indicated. qLAMP: Quantitative loop-mediated isothermal amplification; LP: *Legionella pneumophila*; MP: *Mycoplasma pneumoniae*; CP: *Chlamydomphila pneumoniae*; SP: *Streptococcus pneumoniae*; SA: *Staphylococcus aureus*; EC: *Escherichia coli*; KP: *Klebsiella pneumoniae*; PA: *Pseudomonas aeruginosa*; AB: *Acinetobacter baumannii*; SM: *Stenotrophomonas maltophilia*; HI: *Haemophilus influenzae*.

studied the clinical data of these 4 samples and found that the qLAMP results were all reliable except 1 MP positive sample due to the lack of specific species identified in the

final diagnosis. No CP positive results were reported in the 76 samples either by qLAMP or culture assay.

Clinical benefit of quantitative loop-mediated isothermal amplification guided target therapy

A total of 44 qLAMP-positive samples were identified in the study. Treatment strategies were established or adjusted in 23 of them based on qLAMP results. Eight samples were subsequently excluded from analysis because of discharge or death within 3 days after admission. The final analysis group of 36 patients consisted of 19 with treatment established or adjusted to target antibiotics therapies according to qLAMP results (pathogen target-driven therapy group) and 17 without treatment strategies adjustment whose treatment strategy was inconsistent with qLAMP results (empirical therapy group).

Demographic and clinical characteristics of the two groups

Demographic and clinical characteristics of the two groups are shown in Table 4. There is no significant

Table 3: Congruence of qLAMP and culture results in patients with hospital-acquired pneumonia

Pathogens	Concordance rate (%)	P
SA	90.79	0.453
EC	98.68	1.000
PA	89.47	0.070
KP	93.42	0.375
SM	93.42	0.063
SP	100.00	1.000
AB	77.63	0.332

qLAMP: Quantitative loop-mediated isothermal amplification; SP: *Streptococcus pneumoniae*; SA: *Staphylococcus aureus*; EC: *Escherichia coli*; KP: *Klebsiella pneumoniae*; PA: *Pseudomonas aeruginosa*; AB: *Acinetobacter baumannii*; SM: *Stenotrophomonas maltophilia*.

Table 4: Demographic and clinical characteristics of patients in the two groups

Characteristics	Patients with pathogen target-driven therapy (n = 19)	Patients with empirical therapy (n = 17)	Statistics	P
Male, n (%)	15 (79)	9 (53)	2.73*	0.16
Age (years)	74.26 ± 10.99	78.00 ± 8.48	-1.13†	0.27
Complications, n (%)				
Hypoproteinemia	15 (79)	12 (71)	0.33*	0.71
Coronary heart disease	9 (47)	4 (24)	2.21*	0.18
Acute cerebrovascular disease	4 (21)	5 (29)	0.33*	0.71
Clinical manifestation				
Temperature, °C	37.95 ± 1.06	37.68 ± 0.81	0.85†	0.40
Cough, n	19	17	-	-
Sputum, n	19	17	-	-
Rales, n	19	17	-	-
Blood routine examination (normal value)				
WBC, ×10 ⁹ /L (4.0–10.0)	11.63 ± 5.26	10.45 ± 4.55	0.72†	0.48
NE, % (50–70)	81.48 ± 10.74	85.37 ± 9.80	-1.13†	0.27
NE, ×10 ⁹ /L (2.0–7.0)	9.60 ± 5.01	9.08 ± 4.50	0.33†	0.74
Hb, g/L (110–170)	103.32 ± 19.83	96.84 ± 19.51	0.99†	0.33
Platelet, ×10 ⁹ /L (100–300)	211.21 ± 104.93	197.20 ± 88.14	0.43†	0.67
Biochemical indicators (normal value)				
ALT, U/L (0–40)	41.11 ± 68.58	28.35 ± 17.39	0.74†	0.46
AST, U/L (0–40)	36.95 ± 29.20	35.06 ± 20.13	0.22†	0.83
ALB, g/L (35–55)	31.59 ± 3.57	30.38 ± 5.46	0.80†	0.43
CRE, μmol/L (20–106)	76.21 ± 73.42	70.00 ± 39.52	0.31†	0.76
BUN, mmol/L (2.9–8.3)	10.28 ± 7.80	10.28 ± 5.64	0.0002†	1.00
Blood gas analysis (normal value)				
pH (7.35–7.45)	7.52 ± 0.05	7.51 ± 0.06	0.91†	0.37
PaO ₂ , mmHg (80.0–100.0)	115.17 ± 43.97	97.47 ± 32.29	1.36†	0.18
PaCO ₂ , mmHg (35–45)	38.79 ± 8.03	40.12 ± 9.56	-0.45†	0.65
HCO ₃ ⁻ , mmol/L (21.4–27.3)	31.97 ± 6.47	31.57 ± 6.37	0.19†	0.85
Oxygenation index, mmHg (400–500)	230.02 ± 113.91	229.10 ± 96.36	0.03†	0.98
Blood coagulation index (normal value)				
PT, s (9.8–13.1)	12.81 ± 2.13	14.05 ± 5.90	-0.82†	0.42
APTT, s (25.4–38.4)	33.22 ± 6.85	32.88 ± 6.32	0.15†	0.88
Chest radiograph infiltration, n (%)	19 (100)	17 (100)	-	-

Data are presented as mean ± SD unless otherwise indicated. * χ^2 value; †t value. “-”: Data not applicable; SD: Standard deviation; WBC: White blood cell; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALB: Albumin; CRE: Creatinine; BUN: Blood urea nitrogen; PT: Prothrombin time; APTT: Activated partial thromboplastin time; NE: Neutrophil count.

difference ($P > 0.05$) in the characteristics of the patients between the two groups, including gender, age, complications, clinical manifestation (temperature, cough, sputum, and rales), blood routine examination, biochemical indicators, blood gas analysis, blood coagulation index, and chest radiograph infiltration.

Patients taking an advantage for clinical condition with pathogen target-driven therapy

There are 16 patients from the pathogen target-driven therapy group with clinical condition improvement 3 days after qLAMP results reported, while there is only 1 from the empirical therapy group. The remission rate is 84.2% in the group with pathogen target-driven therapy, and 5.9% in the group with empirical therapy. The differences in the remission rates between these two groups evaluated by Fisher's exact tests are statistically significant ($P = 2 \times 10^{-6}$).

Daily mean temperature of the group with pathogen target-driven therapy shows a more obvious tendency of improvement than the group with empirical therapy [Figure 2]. On the 1st day when samples were collected, the mean temperature of target-driven therapy group and empirical therapy group were 37.95°C and 37.68°C, respectively, while the mean temperature of those two groups changed to 37.42°C and 38.02°C 3 days later. The decrease of daily mean temperature was 0.53°C in the group with pathogen target-driven therapy while the decrease in the group with empirical therapy was -0.34°C. Similarly, decrease of total WBC number in group with pathogen target-driven therapy is more significant than the group with empirical therapy ($2.15 \times 10^9/L$ with pathogen target-driven therapy vs. $0.70 \times 10^9/L$ with empirical therapy) [Figure 3].

DISCUSSION

This is a pilot study to assess the value of qLAMP in guiding early target antibiotic therapies of HAP, which may have significant effects on the mortality of HAP and reduce the cost. Although bacterial pneumonia is a kind of curable

diseases due to the advent of the antibiotics, the mortality of bacterial HAP is still high, which may contribute to the delay of target antibiotic therapies according to the results of sputum culture.

As a new manner of detecting the etiology of different kinds of infections, qLAMP is now commercially available. With the availability of this rapid (results are available within 1–2 h), sensitive, and specific test, early target antibiotic therapy of infection is now possibly feasible. Therefore, we apply qLAMP for the decision-making regarding whether we selected empirical antibiotic therapies or the target antibiotic therapies for HAP patients.

Since we would investigate the value of qLAMP steering therapies, the first important issue was whether qLAMP can etiologically diagnose HAP in time. As qLAMP assay was much more rapid than sputum culture, the most common assay in recent clinical practice, we first focus on the congruency of the results of qLAMP and sputum culture. Fortunately, there was no significance between qLAMP and culture results of HAP patients with infections of SA, EC, PA, KP, SM, SP, and AB. In addition, qLAMP can detect HI, LP, and MP, which were not detectable by culture. Besides, qLAMP was a candidate method which could differentiate the pathogens between colonized and infectious status. After that, we prospectively enrolled 36 patients with HAP with the same baseline data to evaluate the value of qLAMP steering early target therapies. Among these patients, the qLAMP results were all positive based on the cut-off value ($>1.0 \times 10^5$ copies/ml) which was established in our former work (data not shown). We randomly adjusted the regimen of these patients with empirical therapies according to the 2005 American Thoracic Society/Infectious Diseases Society of America HAP guideline^[1] or target therapies based on the results of qLAMP. Interestingly, we found that the clinical condition was significantly improved in

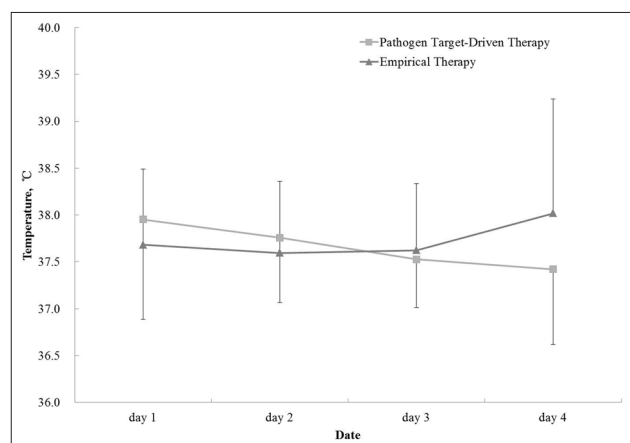


Figure 2: Temperature alteration between two groups. The body temperature of the group with pathogen target-driven therapy decreased while the group with empirical therapy had no significant improvement in body temperature.

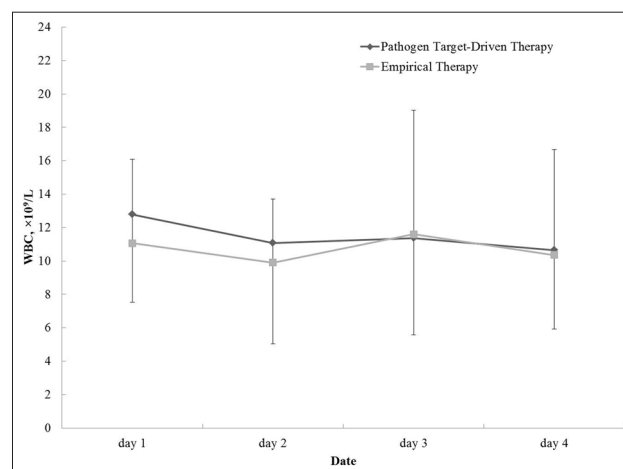


Figure 3: White blood cell (WBC) count alteration between two groups. The WBC count of the group with pathogen target-driven therapy decreased while the group with empirical therapy fluctuated in WBC count.

the group with pathogen target-driven therapy compared to the group with empirical therapies.

There are a few limitations in our studies. Firstly, it was performed with a small sample size, and the stochastic effects were too big to drive a definite conclusion. Secondly, we did not test the infection of fungus and virus of HAP, which may contribute a small number of HAP infections. Thirdly, we did not assess the immunological conditions and nutritional statuses of the patients in these two groups, which may influence the effects of antibiotic therapies. A fourth limitation was that the drug sensitivity cannot be tested by qLAMP. Perhaps, we could combine qLAMP and sputum drug sensitivity test to individualize the HAP regimens. However, the definition of conclusion can only be driven after multi-centered, randomized, and large sample sized research. Since qLAMP cannot test the drug sensitivity, the combination of qLAMP and sputum culture is the good choice for guiding early target therapies in HAP patients.

In conclusion, the qLAMP assay is a reliable alternative for steering early target therapies of HAP.

Supplementary information is linked to the online version of the paper on the Chinese Medical Journal website.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. American Thoracic Society; Infectious Diseases Society of America. Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. *Am J Respir Crit Care Med* 2005;171:388-416. doi: 10.1164/rccm.200405-644ST.
2. Vincent JL. Nosocomial infections in adult intensive-care units. *Lancet* 2003;361:2068-77. doi: 10.1016/S0140-6736(03)13644-6.
3. Mehta RM, Niederman MS. Nosocomial pneumonia in the intensive care unit: Controversies and dilemmas. *J Intensive Care Med* 2003;18:175-88. doi: 10.1177/0885066603254249.
4. Diaz E, Rodríguez AH, Rello J. Ventilator-associated pneumonia: Issues related to the artificial airway. *Respir Care* 2005;50:900-6.
5. Niederman MS. The argument against using quantitative cultures in clinical trials and for the management of ventilator-associated pneumonia. *Clin Infect Dis* 2010;51 Suppl 1:S93-9. doi: 10.1086/653055.
6. Chastre J, Trouillet JL, Combes A, Luyt CE. Diagnostic techniques and procedures for establishing the microbial etiology of ventilator-associated pneumonia for clinical trials: The pros for quantitative cultures. *Clin Infect Dis* 2010;51 Suppl 1:S88-92. doi: 10.1086/653054.
7. Dinh DT, Le MT, Vuong CD, Hasebe F, Morita K. An updated loop-mediated isothermal amplification method for rapid diagnosis of H5N1 Avian influenza viruses. *Trop Med Health* 2011;39:3-7. doi: 10.2149/tmh.2010-21.
8. Curtis KA, Rudolph DL, Nejad I, Singleton J, Beddoe A, Weigl B, et al. Isothermal amplification using a chemical heating device for point-of-care detection of HIV-1. *PLoS One* 2012;7:e31432. doi: 10.1371/journal.pone.0031432.
9. Kong QM, Lu SH, Tong QB, Lou D, Chen R, Zheng B, et al. Loop-mediated isothermal amplification (LAMP): Early detection of *Toxoplasma gondii* infection in mice. *Parasit Vectors* 2012;5:2. doi: 10.1186/1756-3305-5-2.
10. Tao ZY, Zhou HY, Xia H, Xu S, Zhu HW, Culleton RL, et al. Adaptation of a visualized loop-mediated isothermal amplification technique for field detection of *Plasmodium vivax* infection. *Parasit Vectors* 2011;4:115. doi: 10.1186/1756-3305-4-115.
11. Uemura N, Makimura K, Onozaki M, Otsuka Y, Shibuya Y, Yazaki H, et al. Development of a loop-mediated isothermal amplification method for diagnosing *Pneumocystis pneumonia*. *J Med Microbiol* 2008;57(Pt 1):50-7. doi: 10.1099/jmm.0.47216-0.
12. George G, Mony P, Kenneth J. Comparison of the efficacies of loop-mediated isothermal amplification, fluorescence smear microscopy and culture for the diagnosis of tuberculosis. *PLoS One* 2011;6:e21007. doi: 10.1371/journal.pone.0021007.
13. Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N, et al. Loop-mediated isothermal amplification of DNA. *Nucleic Acids Res* 2000;28:E63. doi: 10.1093/nar/28.12.e63.
14. Parida M, Sannarangaiah S, Dash PK, Rao PV, Morita K. Loop mediated isothermal amplification (LAMP): A new generation of innovative gene amplification technique; perspectives in clinical diagnosis of infectious diseases. *Rev Med Virol* 2008;18:407-21. doi: 10.1002/rmv.593.
15. Kaneko H, Kawana T, Fukushima E, Suzutani T. Tolerance of loop-mediated isothermal amplification to a culture medium and biological substances. *J Biochem Biophys Methods* 2007;70:499-501. doi: 10.1016/j.jbbm.2006.08.008.
16. Kang Y, Deng R, Wang C, Deng T, Peng P, Cheng X, et al. Etiologic diagnosis of lower respiratory tract bacterial infections using sputum samples and quantitative loop-mediated isothermal amplification. *PLoS One* 2012;7:e38743. doi: 10.1371/journal.pone.0038743.
17. Andrews CP, Coalson JJ, Smith JD, Johanson WG Jr. Diagnosis of nosocomial bacterial pneumonia in acute, diffuse lung injury. *Chest* 1981;80:254-8. doi: 10.1378/chest.80.3.254.