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ORIGINAL RESEARCH

Loop-Mediated Isothermal Amplification (LAMP) for Rapid and Sensitive Detection of Carbapenemase Genes in CRE: A Diagnostic Validation Study

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Objective: To evaluate the clinical utility of loop-mediated isothermal amplification (LAMP) for detecting carbapenemase genes in carbapenem-resistant Enterobacteriaceae (CRE).

Methods: From January 2023 to December 2024, 112 clinical CRE isolates were collected, including 104 carbapenem-resistant Klebsiella pneumoniae (CR-KP), 7 Escherichia coli (CR-EC), and 1 Klebsiella oxytoca (CR-KO). These isolates were obtained primarily from sputum (n=65), urine (n=29), and bronchoalveolar lavage fluid (n=9). The isolates were predominantly isolated from neurosurgery (38.39%), intensive care unit (21.42%), and respiratory critical care medicine (15.18%). Carbapenemase genes were detected in parallel using both sequencing (reference standard) and LAMP methods under blinded conditions. Statistical analysis included cross-tabulation and Cohen's kappa coefficient for agreement assessment.

Results: Among the 112 CRE isolates, 96.43% (108/112) carried carbapenemase resistance genes. KPC variants predominated (89/108, 82.4%), including blaKPC-2 (n=87) and blaKPC-33 (n=2). NDM variants were detected in 29 isolates (26.9%), comprising blaNDM-1 (n=14) and blaNDM-5 (n=15). OXA-48 was identified in 3 isolates (2.8%). Compared with sequencing, LAMP demonstrated perfect sensitivity (100%) for all three gene types, with specificities of 91.30% (KPC), 96.38% (NDM), and 100% (OXA-48). However, the performance data for OXA-48 should be considered preliminary due to the low number of positive isolates (n=3).The kappa values indicated excellent agreement: 0.943 (KPC), 0.932 (NDM), and 1.000 (OXA-48).

Conclusion: LAMP technology shows high diagnostic accuracy for detecting major carbapenemase genes particularly KPC and NDM in CRE isolates, offering a reliable tool for guiding appropriate antibiotic therapy. Its operational simplicity and cost-effectiveness make it particularly suitable for implementation in primary healthcare settings.

Keywords: carbapenem-resistant Enterobacteriaceae, CRE, loop-mediated isothermal amplification, LAMP, carbapenemase genes, rapid diagnostics

Introduction

The emergence of antimicrobial resistance has become a critical global public health challenge, with infections caused by carbapenem-resistant Enterobacteriaceae (CRE) being particularly concerning.[1] According to data from the CHINET Antimicrobial Resistance Surveillance Network in China,[2,3] the resistance rate of clinical Klebsiella pneumoniae isolates to carbapenem antibiotics has risen dramatically from 3% in 2005 to over 23% in 2023, severely limiting treatment options for CRE infections. This escalating carbapenem resistance, recognized as a major public health threat worldwide,[4–7] not only complicates clinical management but also significantly increases healthcare costs.

The primary mechanism of carbapenem resistance in Enterobacteriaceae involves the production of carbapenemases,[8] including KPC, NDM, IMP, VIM, and OXA-48 variants and so on. Different carbapenemase types exhibit distinct antibiotic hydrolysis profiles, necessitating precise genotyping to guide appropriate antimicrobial therapy.[9] Current

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detection methods, such as sequencing and PCR, require sophisticated equipment and technical expertise, limiting their widespread adoption in clinical settings.

LAMP, as a novel molecular diagnostic approach, offers several advantages including operational simplicity, rapid turnaround time, and minimal equipment requirements.This technique has been successfully applied for the rapid detection of various pathogens and resistance genes, such as mecA in methicillin-resistant Staphylococcus aureus (MRSA) and Mycobacterium tuberculosis, demonstrating its reliability and versatility in molecular diagnostics.[10,11] While this technology has been routinely applied for screening other pathogens and resistance genes in clinical practice, its potential utility for detecting carbapenemase genes in CRE remains unexplored. This study aims to evaluate the application value of this promising technology in CRE carbapenemase gene detection.

Materials and Methods

Bacterial Isolates

A total of 112 non-duplicate clinical CRE isolates were collected between January 2023 and December 2024. CRE was defined as Enterobacteriaceae demonstrating resistance to at least one carbapenem antibiotic (imipenem, meropenem, or ertapenem) based on antimicrobial susceptibility testing. This retrospective study utilized leftover clinical samples and was approved by the Ethics Committee of 363 hospital (Approval No. 2025065).This study was conducted in accordance with the Declaration of Helsinki.The requirement for informed consent was waived by the ethics committee due to the use of anonymized leftover samples after routine clinical testing.

Experimental Procedures

Bacterial Preservation and Revival

Pure CRE colonies were transferred using sterile swabs into cryotubes and stored at −80°C. For revival, frozen stocks were thawed at room temperature and streaked onto blood agar plates, followed by incubation at 35°C with 5% CO_2 for 48 hours. Subsequently, fresh colonies were suspended in two aliquots: one for isothermal amplification and another for whole-genome sequencing.

Nucleic Acid Extraction

Genomic DNA was extracted from bacterial colonies using the Bacteria DNA Kit (Boao Biotech, Beijing, China), according to the manufacturer's instructions. Bacterial suspensions were prepared in 1 mL sterile saline, centrifuged at 12,000 rpm for 5 min, and washed with 1 mL buffer. After repeat centrifugation, pellets were resuspended in 100 μL lysis buffer, vortexed, and transferred to extraction columns. Samples were incubated at 100°C for 10 min with agitation, followed by centrifugation at 10,000 rpm for 1 min. Purified nucleic acids were used within 1 hour.

Isothermal Amplification (LAMP)

A 26 μL aliquot of extracted DNA was mixed with 26 μL master mix (containing enzymes, fluorescent dyes, and substrates) in PCR tubes. The 52 μL reaction mixture was loaded onto microfluidic chips (CapitalBio Technologies), each pre-coated with primers (2 inner and 2 outer primers per target). The sequences of the LAMP primers used for blaKPC, blaNDM, and blaOXA-48 detection were designed by and obtained from Boao Technologies (Beijing, China). The final reaction mixture contained 1.6 μM each of inner primers (FIP and BIP), 0.2 μM each of outer primers (F3 and B3), 0.8 μM each of loop primers (LF and LB), 1.4 mM dNTPs, 0.8 M betaine, 20 mM Tris-HCl (pH 8.8), 10 mM KCl, 10 mM (NH4)2SO4, 8 mM MgSO4, and 0.1% Tween 20. Amplification was performed at 65°C for 50 min in a real-time fluorometer (CapitalBio), with results determined by fluorescence kinetics. Each run included negative and positive controls; batches with false results were excluded. All testing was conducted blinded.

Sequencing

Isolates were submitted to KingMed Diagnostics (Sichuan) for whole-genome sequencing using Illumina platforms. Sequences were analyzed via NCBI BLAST for carbapenemase gene identification.

Statistical Analysis

Data were analyzed using SPSS 22.0. Categorical variables were reported as counts/percentages. Diagnostic performance was evaluated through cross-tabulation and Cohen's kappa (κ) statistics, with $P < 0.05$ considered significant.

Results

Strain Distribution

Among the 112 CRE isolates analyzed, the majority were CR-KP (n=104, 92.9%), followed by CR-EC (n=7, 6.2%) and CR-KO (n=1, 0.9%). These isolates were primarily obtained from respiratory specimens (sputum, 58.0%; BALF, 8.0%) and urine (25.9%), consistent with their predominant association with respiratory and urinary tract infections (Figure 1). The highest isolation rates were observed in neurosurgery (38.4%), intensive care units (21.4%), and respiratory critical care departments (15.2%) (Figure 2).

Sequencing Analysis of Carbapenemase Genes

Whole-genome sequencing revealed that 96.4% (108/112) of CRE isolates carried carbapenemase resistance genes. The predominant genotype was KPC (82.4%, 89/108),with *bla*KPC-2 being most common (N=87). Two isolates harbored *bla*KPC-33. NDM variants constituted 26.9% (29/108) of positive isolates, comprising *bla*NDM-1 (n=14) and *bla*NDM-5(n=15). Notably, 10 isolates co-harbored *bla*KPC-2and *bla*NDM variants. Three isolates carried *bla*OXA-48, all in combination with *bla*NDM-5. No IMP or VIM producers were detected (Table 1).

LAMP Analysis of Carbapenemase Genes

KPC variants were identified in 91 isolates (76 single and 15 co-occurring with NDM), while NDM was detected in 33 isolates (14 single and 18 co-occurring). All three OXA-48-positive isolates were found in combination with NDM. No IMP or VIM producers were detected (Table 2).

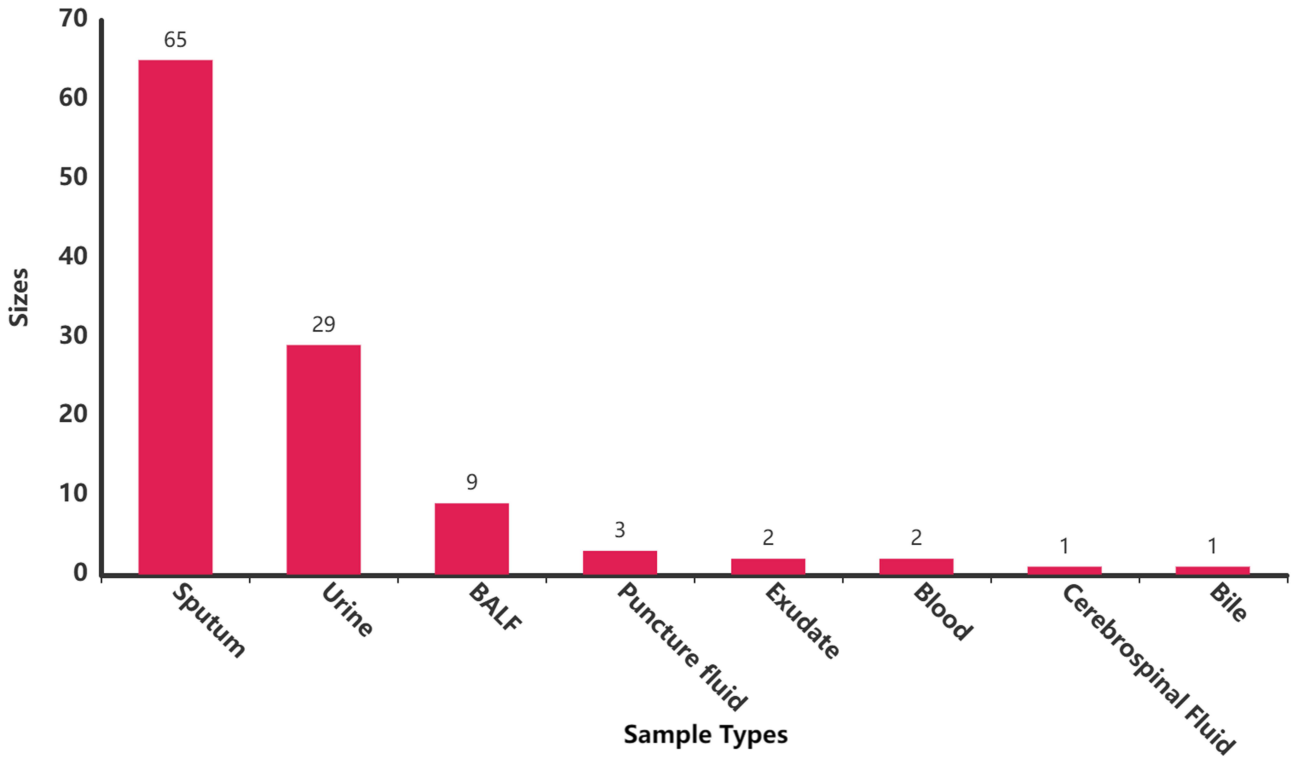

Figure 1 Specimen type distribution of CRE isolates.

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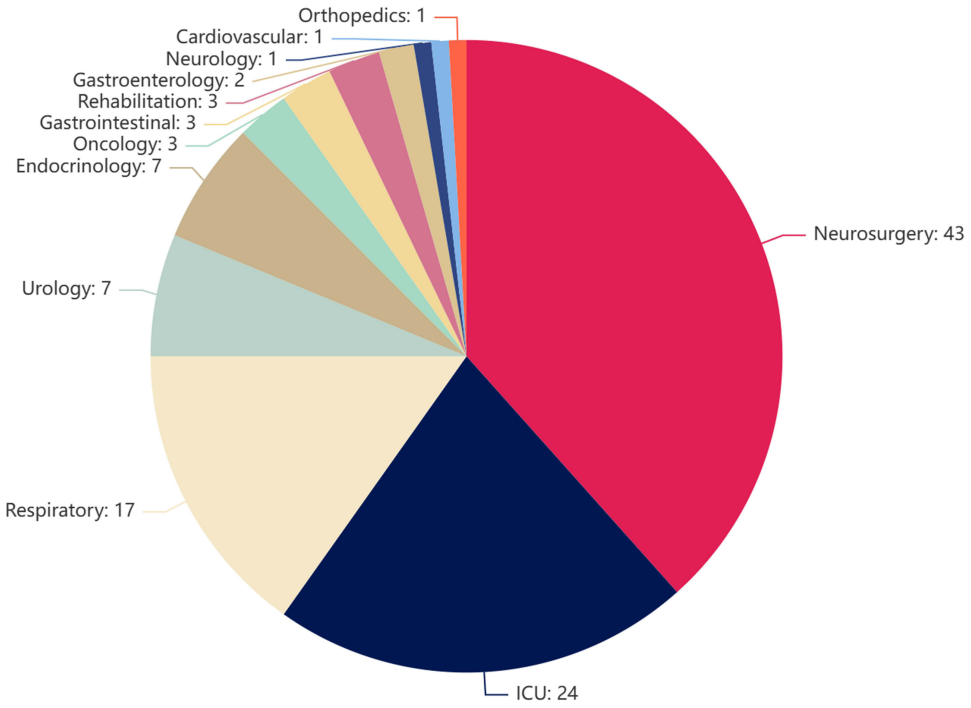

Figure 2 Clinical department distribution of CRE isolates.

Diagnostic Performance Evaluation of LAMP

Compared with sequencing, LAMP assay demonstrated excellent concordance in detecting carbapenemase genes KPC, NDM, and OXA-48, with Kappa values of 0.943, 0.932, and 1.000 respectively (Tables 3–5). The assay achieved perfect sensitivity (100%) for all three targets, while showing high specificity of 91.30% for KPC, 96.38% for NDM, and 100% for OXA-48 detection. However, the performance metrics for OXA-48, while perfect in this small subset, should be interpreted with caution due to the limited number of positive isolates (n=3). While demonstrating a broad detection range covering subtypes blaKPC-2, blaKPC-33, blaNDM-1, and blaNDM-5, the LAMP assay exhibited all its false-positives (2 for KPC and 3 for NDM) exclusively in cases of "KPC+NDM" co-detection (Figure 3A and B).

Table 1 Distribution of Carbapenemase Resistance Genes Identified by Sequencing

Genotype	n (%)
*bla*KPC-2	77 (68.8%)
*bla*KPC-2+*bla*NDM-1	8 (7.1%)
*bla*KPC-2+*bla*NDM-5	2 (1.8%)
*bla*KPC-33	2 (1.8%)
*bla*NDM-1	6 (5.4%)
*bla*NDM-5	10 (8.9%)
*bla*NDM-5+*bla*OXA-48	3 (2.7%)
IMP	0(0.00%)
VIM	0(0.00%)
Negative	4 (3.6%)
Total	112 (100.00%)

Table 2 Carbapenemase Gene Detection by LAMP

Genotype	n (%)
KPC	76 (67.9%)
KPC+NDM	15 (13.4%)
NDM	14 (12.5%)
NDM+OXA-48	3 (2.7%)
IMP	0(0.00%)
VIM	0(0.00%)
Negative	4 (3.6%)
Total	100.00%

Table 3 Diagnostic Performance of LAMP for KPC Detection

LAMP	Sequencing			Sensitivity	Specificity	Kappa	P-value
	KPC (+)	KPC (-)	Total				
KPC (+)	89	2	91	100%	91.30%		
KPC (-)	0	21	21			0.943	0.000
Total	89	23	112				

Table 4 Diagnostic Performance of LAMP for NDM Detection

LAMP	Sequencing			Sensitivity	Specificity	Kappa	P-value
	NDM (+)	NDM (-)	Total				
NDM (+)	29	3	32	100%	96.38%		
NDM (-)	0	80	80			0.932	0.000
Total	29	83	112				

Table 5 Diagnostic Performance of LAMP for OXA-48 Detection

LAMP	Sequencing			Sensitivity	Specificity	Kappa	P-value
	OXA-48 (+)	OXA-48 (-)	Total				
OXA-48 (+)	3	0	3	100%	100%		
OXA-48 (-)	0	109	109			1.000	0.000
Total	3	109	112				

Discussion

LAMP first established by Notomi et al in 2000,[12] represents a novel nucleic acid amplification technique that employs two pairs of primers targeting six distinct regions of the target DNA sequence. Utilizing strand-displacing DNA polymerase, LAMP enables efficient amplification under isothermal conditions (65°C), with results detectable via turbidity or fluorescence. Compared to sequencing or conventional PCR, LAMP offers rapid, equipment-independent detection, making it particularly suitable for point-of-care testing in primary healthcare settings. While LAMP has been widely adopted for detecting viruses, fungi, and parasites, its application in carbapenemase gene detection remains underexplored.

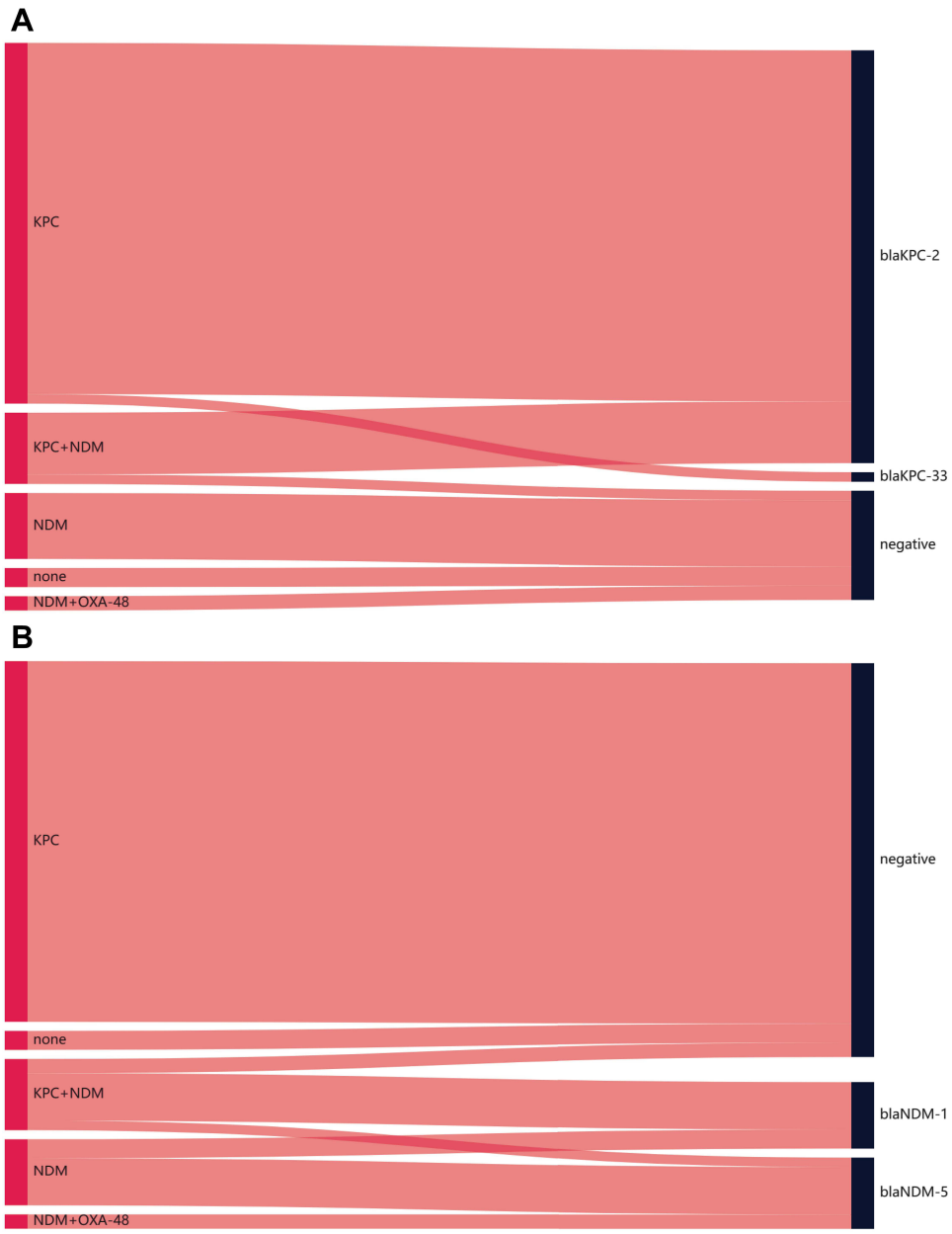

Figure 3 (**A**) Sankey diagram comparing LAMP and sequencing results for KPC detection. (**B**) Sankey diagram comparing LAMP and sequencing results for NDM detection.

The CRE isolates in this study reflected prevalent epidemiological patterns. CR-KP predominated (104/112, 92.9%), consistent with global reports.[13,14] Specimens were primarily respiratory (sputum: 58.0%; BALF: 8.0%) and urinary (25.9%) in origin, with highest isolation rates from neurosurgery (38.4%), ICUs (21.4%), and respiratory department (15.2%)—aligning with regional surveillance data.[15,16]

Carbapenemase gene distribution exhibited geographic variability.[17–19] In our cohort, 96.4% (108/112) of CRE isolates harbored carbapenemase genes, predominantly *bla*KPC-2 (87/108), followed by *bla*NDM variants (29/108) and rare *bla*OXA-48 (3/108). This profile mirrors trends in Southwest Shandong[20] but diverges from pediatric data in Soochow,[21] underscoring the need for region-specific resistance surveillance. Notably, four carbapenemase-negative isolates demonstrated alternative resistance mechanisms (*bla*OXY, *bla*CTX+*bla*SHV+*bla*TEM, or *bla*SHV), highlighting the importance of monitoring non-carbapenemase resistance (eg, AmpC, ESBLs, porin loss, efflux pumps).

Compared with sequencing, LAMP assay demonstrated excellent concordance in detecting carbapenemase genes KPC, NDM, and OXA-48, with Kappa values of 0.943, 0.932, and 1.000 respectively. The assay achieved perfect sensitivity (100%) for all three targets, while showing high specificity of 91.30% for KPC, 96.38% for NDM, and 100% for OXA-48 detection. It is important to note that the high accuracy for OXA-48 is based on a very small sample size (n=3), and thus requires validation in larger cohorts.These results parallel LAMP's utility in other pathogens (eg, 98.32% sensitivity for visceral leishmaniasis[22]) and resistance gene detection (eg, Salmonella[23]). A critical finding of this study was that the few false-positive results generated by the LAMP assay were exclusively associated with a "KPC+NDM" co-detection profile. This observation points towards a potential limitation in the current assay configuration when dealing with mixed genetic signals. The false-positives may be attributed to low-level cross-reactivity between primer sets, non-specific amplification artifacts exacerbated in dual-positive reactions, or even the presence of previously uncharacterized genetic variants that are efficiently amplified by LAMP but not confirmed by the sequencing method used as the reference. Further investigation is warranted to elucidate the exact mechanism.

Conclusion

In conclusion, LAMP demonstrates high accuracy for carbapenemase gene screening, offering particular value for resource-limited settings and point-of-care testing.

Ethics Statement

Ethical approval was granted by the Ethics Committee of 363 Hospital.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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