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*Original Article*

# Development and rapid identification of a self-constructed MALDI-TOF MS library for *Mycoplasma pneumoniae* clinical isolates

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## Abstract

Introduction: To assess the diagnostic efficacy of matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) for rapid and precise identification of *Mycoplasma pneumoniae* in clinical microbiology workflows.

Methodology: A reference-calibrated MALDI-TOF MS spectral database was generated through a standardized formic acid/acetonitrile extraction protocol from the *M. pneumoniae* reference strain(M129). Forty-nine prospectively collected *M. pneumoniae* clinical isolates were obtained from respiratory tract specimens at Hangzhou First People's Hospital during August-October 2023. These isolates optimized the in-house MALDI-TOF MS database for *M. pneumoniae* identification and validated the diagnostic feasibility of liquid pre-culture coupled with MALDI-TOF MS analysis.

Results: When we randomly selected 40 strains for database validation, we found that the first phase validation of the M129-derived spectral database indicated that 87.5% (35/40) of the clinical isolates were initially detected, among which 57.5% (23/40) reached the ≥ 2.0 log value (score) threshold required for species-level identification. Post-optimization through iterative spectral refinement, 100% detection rate (40/40) was attained, with 75% (30/40) meeting strict diagnostic criteria (log(score) ≥ 2.0). The integrated liquid culture-MALDI-TOF MS platform achieved a mean identification time of 9.6 ± 1.4 days, demonstrating a 19.3% reduction in turnaround time compared to traditional chromogenic detection methods (11.9 ± 1.6 days; Independent Samples t-test, $p < 0.001$).

Conclusions: This study establishes MALDI-TOF MS as a CLSI-compliant diagnostic method for *M. pneumoniae* with superior accuracy and operational efficiency. Optimized liquid culture-MALDI-TOF MS integration reduces diagnostic latency by 2.3 days, enhancing respiratory pathogen management in clinical laboratories.

**Key words:** *Mycoplasma pneumoniae*; MALDI-TOF MS; self-constructed library; rapid identification.

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## Introduction

*Mycoplasma pneumoniae* constitutes a predominant etiological agent of community-acquired pneumonia. Taxonomically classified under the *Genus Mycoplasma* (*Class Mollicutes*), this cell wall-deficient prokaryote demonstrates ultramicroscopic dimensions (0.2-0.3 µm in diameter) and streamlined genome architecture (featuring a reduced sequence length of 816 kb), reflecting evolutionary adaptation to parasitic existence [1,2]. Epidemiological data from Sun *et al.* indicate that *M. pneumoniae* accounts for approximately 20% of respiratory tract infections nationwide in China, with regional prevalence reaching up to 35%. Notably, the highest detection rate is observed among school-age children (6–9 years). While the infection incidence among adolescents (10–18 years) demonstrates a relative decline compared to the school-age group, it remains markedly elevated in comparison to adult populations [3].

Current diagnostic paradigms for *M. pneumoniae* infections predominantly utilize nucleic acid amplification tests (NAATs) as first-line detection modalities [4], reflecting the inherent constraints of conventional culture-based methodologies that necessitate prolonged incubation periods (> 21 days), specialized biosafety containment (Biosafety Level 2+), and limited phenotypic characterization capabilities. Of particular concern is the alarming epidemiological shift in antimicrobial resistance patterns, with surveillance data from Asian regions documenting 90% prevalence of macrolide-resistant genotypes (erm B and 23S rRNA mutations) among circulating *M. pneumoniae* strains [5]. This resistance crisis mandates implementation of comprehensive phenotypic drug susceptibility profiling, for which axenic culture systems remain the gold-standard prerequisite. Consequently, the development of a standardized culture-based identification platform with accelerated turnaround times represents an urgent priority to inform antimicrobial stewardship programs and optimize evidence-based therapeutic algorithms.

The MALDI-TOF MS platform represents an

established analytical platform in clinical microbiology that has undergone transformative innovation since its initial implementation [6]. This technology demonstrates superior analytical efficiency compared to conventional culture-dependent identification paradigms, featuring a streamlined analytical workflow compliant with ISO 16140 verification protocols. The standardized quadrant streak method involves sequential specimen processing steps: 1) bacterial lawn preparation via four-quadrant streaking; 2) application of saturated α-cyano-4-hydroxycinnamic acid (HCCA) matrix solution; 3) ambient temperature crystallization under controlled humidity (45-55%); followed by 4) automated spectral acquisition yielding species-level identification within ≤ 180 seconds per sample. This technological advancement achieves a 97.3% reduction in procedural duration compared to traditional biochemical identification workflows. Our research focuses on developing a strain-specific reference spectral repository for Mycoplasma pneumoniae through iterative mass spectrum optimization, coupled with synergistic integration of MALDI-TOF MS into automated liquid handling systems for enhanced mycoplasma detection. These methodological innovations establish critical infrastructure for comprehensive antimicrobial resistance surveillance (including erm B/23S rRNA mutation profiling), rapid pulsed-field gel electrophoresis (PFGE) strain typing, and quantitative virulence factor analysis (CARDS toxin/P1 adhesin), ultimately enabling phenotype-genotype correlation studies for Mycoplasma infection management.

## Methodology

*Strain collection and sampling*

*Clinical M. pneumoniae* isolates (n = 49) were prospectively collected from upper respiratory tract specimens of pediatric inpatients at the Affiliated Hangzhou First People's Hospital, Westlake University School of Medicine (Hangzhou, China) in 2023 (Supplementary Table 2). Following isolation, bacterial suspensions were prepared in 20% glycerol broth supplemented with 2% fresh sheep blood and stored at -80 °C for subsequent analysis.

*Development of an M. pneumoniae standard strain library using MALDI-TOF MS*

Cultivation and isolation of standard *M. pneumoniae* strains

The *M. pneumoniae* reference strain (ATCC® 29342™) was resuscitated from -80 ℃ cryostocks in improved SP-4 medium and homogenized through a 25G needle dispersion. After inoculating 100 μL aliquots into 3 mL improved SP-4 medium, Cultures were incubated under microaerophilic conditions (35 ℃, 5% $CO_2$) with daily monitoring of colorimetric changes. The cultures were harvested when the color shifted from red to yellow and the liquid became clear.

Master spectral profile collection of *M. pneumoniae*

Following pretreatment of *M. pneumoniae*, MALDI-TOF MS was calibrated using the BioTherapeutics Standard (BTS), and the Master Spectral Profile (MSP) was manually acquired with the Bruker Flex Control system. Individual raw spectra with a measured signal intensity greater than 1000, no blurred peaks, no flat-line spectra, and low matrix background signals were selected and added via the -add function until the cumulative signal intensity exceeded 10,000. The resulting spectrum was saved as a single fingerprint spectrum. This process was repeated three times for each well of the target plate, yielding a total of 24 spectra. The quality of the spectra was assessed using Flex Analysis, and at least 18 high-quality spectra were selected (Spectra demonstrating optimal analytical parameters (defined as ≥ $10^4$ peak intensity, complex spectral features containing ≥ 50 discernible m/z signals, and minimal background interference [signal-to-noise ratio > 20:1]) were prioritized for taxonomic classification. These spectra were then used to establish the MSP of *M. pneumoniae* strain ATCC® 29342 (M129) on the Flex Biotyper.

*Optimization and specificity validation of a self-constructed library for M. pneumoniae infection diagnosis*

The influence of *M. pneumoniae* MSP database size on identification accuracy

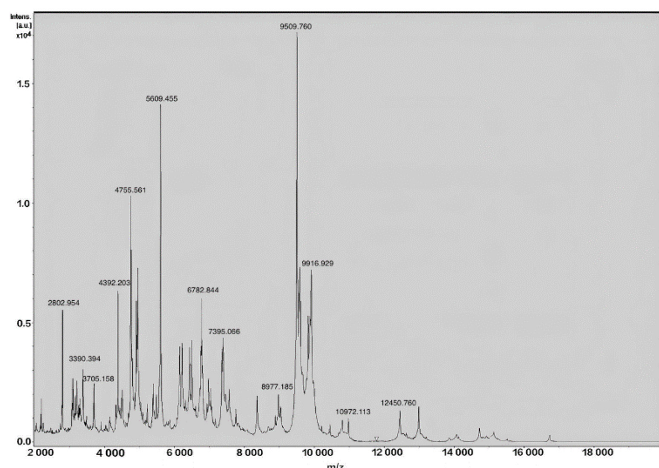
9 clinical isolates were randomly selected from a pool of 49 well-characterized *M. pneumoniae* isolates for enhancement of the in-house *M. pneumoniae* MSP database. After complete thawing, the samples were thoroughly mixed. Subsequently, 100 μL of each sample was added to 3 mL of a pre-prepared culture medium and incubated at 35 ℃ with 5% $CO_2$ in an incubator. Colour changes were monitored every 24 hours. Once the colour shifted from red to yellow and the liquid became clear, the samples were retrieved. Protein extracts were prepared using formic acid treatment as described previously. Fingerprint spectra were collected for each protein extract, ensuring that at least 24 spectra were obtained per strain. The quality of the spectra was assessed using Flex Analysis software, and at least 18 high-quality spectra were selected for each strain and incorporated into the *M. pneumoniae*

MSP database. Following the addition of each MSP, the fingerprint spectra matching process for the 40 clinical strains to be identified was repeated using Flex Biotyper software.

Identification of clinical isolates of *M. pneumoniae*

Of the remaining 40 clinical isolates of *M. pneumoniae*, each sample was completely thawed and homogenized. Subsequently, 100 µL of the homogenized suspension was transferred into 3 mL of freshly prepared culture medium for further cultivation. These samples were then incubated at 35 °C in an atmosphere containing 5% $CO_2$. Color changes were monitored every 24 hours. Once the color shifted from red to yellow and the liquid became clear, the cultures were harvested. A volume of 200 µL of the bacterial suspension was aspirated and added to the extraction reagent provided with the Smart 32 DNA Extractor for DNA extraction. Subsequently, fluorescence quantitative PCR was performed to verify the resuscitated strains. Meanwhile, the pure cultures after color change were subjected to formic acid extraction to obtain protein extracts, which were used for fingerprint spectrum acquisition. For each protein extract, three spectral profiles were repetitively collected, and their quality was assessed using Flex Analysis software. Profiles with high protein signal intensity, rich spectral profiles, and low background noise were selected as optimal candidates. Spectral matching was conducted using Flex Biotyper software, and the results were classified based on the obtained identification scores. Scores greater than 2.000 were considered fully reliable; scores ranging from 1.700 to 1.999 were regarded as relatively reliable; and scores below 1.699 were deemed unreliable.

**Figure 1.** The proteomic profile of *M. pneumoniae* strain ATCC® 29342 (M129).




Validation of the specificity of *M. pneumoniae* MSP in clinical identification

The curated MSP reference database was incorporated into the primary microbial identification repository. Five representative strains per species of *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, *Mycoplasma hominis*, and *M. pneumoniae* were randomly subjected to standardized culturing protocols. Post-cultivation, microbial specimens were processed in accordance with the aforementioned sample preparation methodology, followed by systematic acquisition of MALDI-TOF mass spectral profiles. Triplicate spectral acquisitions were performed for each protein lysate, with spectral fidelity quantitatively evaluated by Flex Analysis (version 3.0) software. Spectra demonstrating optimal analytical parameters (defined as $\geq 10^4$ peak intensity, complex spectral features containing $\geq 50$ discernible m/z signals, and minimal background interference [signal-to-noise ratio > 20:1]) were prioritized for taxonomic classification.

*Investigation into the rapid and accurate identification of M. pneumoniae in liquid culture systems*

Twenty clinical isolates of *M. pneumoniae* were randomly selected. Following complete thawing and homogenization, 500 µL of bacterial suspension from each isolate was aseptically transferred into 10 mL of modified SP-4 liquid culture medium, with dual technical replicates prepared in sterile polypropylene tubes. The cultures were subsequently incubated in a humidified $CO_2$ incubator maintained at 35 °C with 5% $CO_2$. Commencing at 72 hours post-inoculation, 1 mL aliquots from the first replicate were periodically sampled using sterile pipettes every 24 hours for MALDI-TOF MS-based identification via a standardized formic acid-acetonitrile precipitation protocol. Concurrently, the second replicate was monitored for phenol red indicator transition, with chronometrically recorded time-to-colour-change events (red-to-yellow transition at pH < 6.8).

## Results

*Development and application of a self-constructed MALDI-TOF MS library for M. pneumoniae identification*

The proteomic profile of *M. pneumoniae* strain ATCC® 29342 (M129) is demonstrated in Figure 1.

*Identification of clinical strains of M. pneumoniae*

Following in vitro resuscitation, forty *M. pneumoniae* clinical isolates underwent formic acid-

based proteomic identification. MALDI-TOF MS analysis revealed distinct spectral clusters: 23 isolates (57.5%) demonstrated diagnostic confidence scores > 2.300, 8 isolates (20.0%) scored 2.000-2.299, 4 isolates (10.0%) registered 1.700-1.999, while 5 isolates (12.5%) fell below the species-level identification threshold (≤ 1.699). Comprehensive mass spectral profiles are cataloged in Table 1.

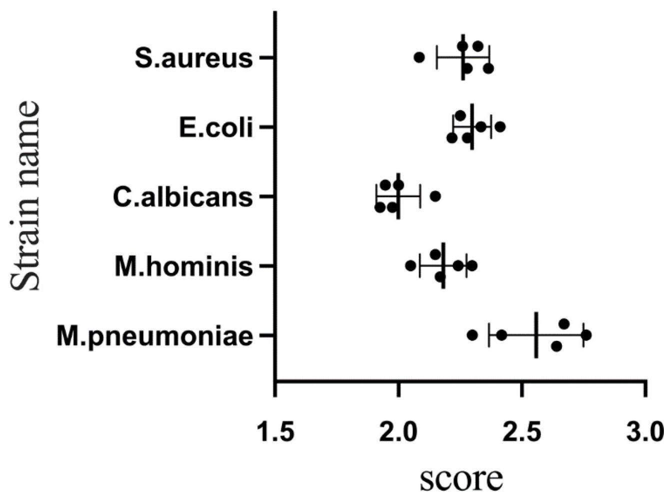
*Optimization of the MALDI-TOF MS-based spectral reference library for M. pneumoniae strains*

The establishment of a MALDI-TOF MS spectral reference library utilizing *M. pneumoniae* reference strains revealed incomplete species-level resolution for five clinical isolates (identification score range: 0-1.699), while achieving definitive identification for twenty-three isolates (score range: 2.300-3.000). Progressive augmentation of spectral entries within the database resulted in reliable identification of all clinical isolates (scores > 1.700), with thirty isolates attaining species-level confidence thresholds (score range: 2.300-3.000). Quantitative analysis demonstrated a sustained elevation of mean matching scores without plateau formation, with statistical trends detailed in Figure 2.

*Analytical validation of MALDI-TOF MS spectral profile specificity for M. pneumoniae identification*

The *M. pneumoniae* MSP reference repository was assimilated into the reference identification database.

**Figure 3.** Analytical specificity verification of *M. pneumoniae* diagnostic discrimination.




All bacterial strains were successfully identified with high confidence. The mean identification scores were as follows: 2.558 for *M. pneumoniae*, 2.182 for *M. hominis*, 1.999 for *C. albicans*, 2.299 for *E. coli*, and 2.262 for *S. aureus*.

**Table 1.** Distribution of Identification Scores for Clinical Strains of *M. pneumoniae.*

| Identification Score | Number of strains (n) |
|---|---|
| 2.300~3.000 | 23 |
| 2.000~2.300 | 8 |
| 1.700~1.999 | 4 |
| 0~1.6999 | 5 |

**Figure 2.** Correlation between MALDI-TOF MS reference spectral repository expansion and clinical isolate matching score dynamics.

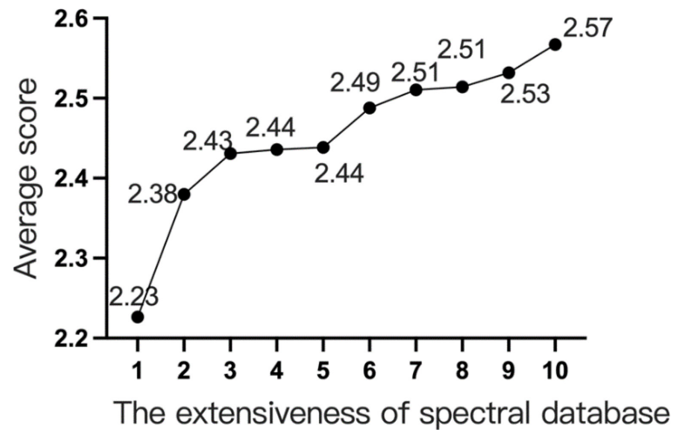
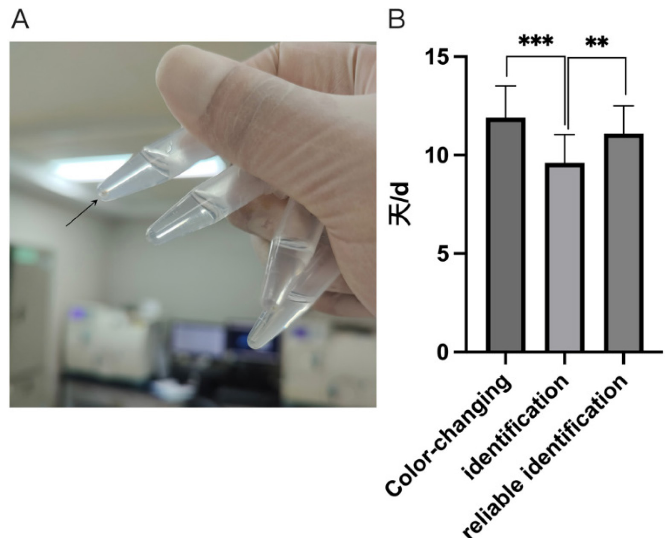



**Figure 4.** Rapid identification of *M. pneumoniae* in liquid culture using MALDI-TOF MS.




**A:** the enriched protein fraction isolated after washing the liquid culture medium of *M. pneumoniae*. **B:** the mean time required for reliable identification (identification score ≥ 2.000, 10.6±1.1 days), significant identification (identification score ≥ 1.700, 9.6 ± 1.4 days), and phenotypic detection via color change in the medium (11.9 ± 1.6 days). Statistically significant differences were observed among these three parameters (** $p < 0.01$, *** $p < 0.001$).

Flex Biotyper-based validation studies with five randomly selected clinical isolates from each species (*Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, *Mycoplasma hominis*, and *M. pneumoniae*) demonstrated perfect discrimination (100% specificity) across all microbial targets. Comprehensive analytical workflows are graphically detailed in Figure 3.

*Rapid identification achieved via MALDI-TOF MS analysis*

Following standardized culture protocols with 24-hour interval assay procedures, MALDI-TOF MS demonstrated a mean analytical processing time of 9.6 ± 1.4 days, versus 11.9 ± 1.6 days for color-changing in conventional culture. Following verification of normal distribution and homogeneity of variance, an independent samples t-test was performed to compare the two datasets, revealing statistically significant differences between the groups ($p < 0.001$). Time-to-reliable identification (spectral matching score ≥ 2.000) was achieved at 10.6 ± 1.1 days, with significant variance observed ($p < 0.01$) (Figure 4; see Supplementary Table 1 for full statistical breakdown).

## Discussion

MALDI-TOF MS is a validated and emerging analytical platform that has been extensively implemented in clinical microbiology laboratories. In comparison to conventional phenotypic methods, MALDI-TOF MS achieves a 1.45-day reduction in microbial identification time [7]. Owing to the reduced genomic complexity, submicron cellular dimensions, and fastidious growth requirements of *M. pneumoniae*, no validated MALDI-TOF spectral database currently exists for this pathogen. In strict adherence to the manufacturer's standardized protocol, we established a reference spectral database for the *M. pneumoniae* ATCC29342 type strain through optimized formic acid extraction methodology [7,8]. Researchers in China have previously demonstrated the efficacy of this protocol through successful construction of reference spectral databases for adult Schistosoma worms [9] and pathogenic Vibrio species [10]. Comparative analysis of clinical isolates against the ATCC29342-derived spectral database resulted in unambiguous identification of 31 strains (77.50%, 31/40). Given the established correlation between spectral database comprehensiveness and matching score reliability [10], subsequent expansion of the *M. pneumoniae* spectral database with residual isolates facilitated complete clinical isolate identification. Longitudinal analysis revealed a progressive increase in mean matching scores among clinical isolates, with no evidence of plateau formation.

The analytical specificity of the *M. pneumoniae* reference spectral database was assessed using *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, *Mycoplasma hominis*, and *M. pneumoniae* as challenge strains. Validation data revealed 100% specificity, with all 40 *M. pneumoniae* isolates correctly identified and complete exclusion of non-target organisms, including *Enterobacteriaceae*, *Staphylococcaceae*, *Candida spp*., and *Mollicutes* species.

A head-to-head comparison was conducted between conventional culture and MALDI-TOF MS with traditional culture as the reference method for 20 consecutive clinical isolates. Quantitative temporal analysis demonstrated that MALDI-TOF MS achieved both presumptive identification (mean 5.2 ± 0.8 days) and confirmatory identification (mean 7.1 ± 1.2 days) significantly earlier than phenotypic detection via medium acidification (mean 9.3 ± 1.5 days; $p < 0.001$ by paired t-test). This discrepancy arises from the fundamental detection thresholds: MALDI-TOF MS requires merely $10^3$ CFU/mL for spectral acquisition, whereas visible pH-dependent colorimetric changes in SP-4 broth necessitate ≥ $10^5$ CFU/mL mycoplasmal biomass accumulation through active glucose metabolism.

Although nucleic acid amplification tests (NAATs), including loop-mediated isothermal amplification (LAMP), have rapidly advanced and become widely adopted due to their high sensitivity, strong specificity, rapid detection capability, and ability to directly analyze clinical samples [11-12], these molecular methods still exhibit certain limitations when compared with conventional culture techniques. First, NAATs depend on specialized amplification platforms and require relatively sophisticated technical infrastructure. Second, in clinical and research contexts that necessitate viable microbial strains—such as antimicrobial susceptibility testing for treatment guidance or vaccine development—culture-based approaches remain irreplaceable [13-15]. These methods enable direct assessment of pathogen viability through metabolic activity and provide live organisms essential for functional studies. Consequently, the integration of MALDI-TOF MS with rapid culture methodologies represents a promising strategy to reduce turnaround time while preserving the critical advantages of culture, thereby offering significant value in both clinical diagnostics and microbiological research.

In conclusion, MALDI-TOF MS emerges as a validated analytical approach for the precise identification of *M. pneumoniae* isolates. Notably, its identification threshold in liquid culture systems precedes chromogenic detection by a significant margin ($p < 0.01$), enabling real-time monitoring of *M. pneumoniae* viability during active proliferation phases. This technological advantage provides a critical pathway for expediting antimicrobial susceptibility profiling and optimizing evidence-based therapeutic interventions in *M. pneumoniae*-associated respiratory infections.

While extensive validation studies in clinical microbiology have established MALDI-TOF MS as capable of achieving reliable discrimination between 10 human-associated and 13 zoonotic mycoplasma strains [16,17], the restricted mycoplasma strain repository within our dataset prevented systematic spectral alignment of *M. pneumoniae* with other human or animal mycoplasma species beyond *M. hominis* as the sole human mycoplasma reference. Future methodological refinements necessitate the establishment of expanded strain collections through multicenter collaborations to enable robust cross-species mycoplasma identification under standardized protocols.

Secondarily, this study constitutes a methodological proof-of-concept for MALDI-TOF MS-based rapid identification of *M. pneumoniae*, though currently lacking clinical validation. The custom MALDI-TOF MS spectral database requires prioritized clinical verification in subsequent phases to confirm its diagnostic reliability for *M. pneumoniae* detection. Furthermore, MALDI-TOF MS enables phenotypic characterization of antimicrobial resistance profiles [18-21], with existing literature documenting its integration with single nucleotide polymorphism (SNP) analysis for molecular subtyping of *M. pneumoniae* [22]. It is noteworthy that no prior studies have utilized MALDI-TOF MS for the concurrent assessment of antimicrobial susceptibility test and genetic diversity in *M. pneumoniae*. Our research group intends to collect expanded clinical isolates through long-term surveillance initiatives, in conjunction with comprehensive antimicrobial susceptibility testing and molecular profiling of resistance determinants. These integrative strategies are anticipated to enhance comparative proteomic analyses aimed at elucidating genotype-phenotype relationships. The resulting insights may substantially improve the precision and practical relevance of epidemiological surveillance for *M. pneumoniae* infections.

## Conclusions

This study showed that MALDI-TOF MS demonstrates high applicability for the precise identification of Mycoplasma pneumoniae isolates. By integrating liquid culture with MALDI-TOF MS, the rapid identification of *M. pneumoniae* achieves a significant reduction in time compared to conventional culture-based methods.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## Ethcal approval

The research protocol was approved by the Hospital Ethics Committee and relevant departments (Ethics Approval Number: KY-20231017-0237-01).


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## Conflict of interest

No conflict of interest is declared.

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## Annex – Supplementary Items

**Supplementary Table 1.** Group *t*-test verification: Shapiro-Wilk test and Levene's test.

| Sample name | Sample size | Average days | Standard deviation | Shapiro-Wilk test | | Levene's test |
|---|---|---|---|---|---|---|
| | | | | W | P [a] | P [b] |
| identification time | 20 | 9.6 | 1.4 | 0.922 | 0.108 | 0.59 |
| Color-changing time | 20 | 11.9 | 1.6 | 0.944 | 0.289 | 0.55 |
| reliable identification time | 20 | 10.6 | 1.1 | 0.913 | 0.072 | |

a: All three datasets were confirmed to follow a normal distribution, as verified through the Shapiro-Wilk normality test ($p > 0.05$); b: The results of Levene's test for homogeneity of variances demonstrate that the p-values comparing Color-changing time with identification time and reliable identification time were 0.59 and 0.55. respectively, both exceeding the 0.05 significance threshold. These findings indicate that the variance of Color-changing time is statistically consistent with that of the other two measurement parameters.

**Supplementary Table 2.** Details of *M. Pneumoniae* isolates. Information Collection Form for Clinical Isolates of *M. Pneumoniae.*

| No. | Sample type | Sex | Age | Diagnosis |
|---|---|---|---|---|
| 1 | Sputum | male | 7 years | Acute bronchitis |
| 2 | Sputum | female | 5 years | Pneumonia |
| 3 | Sputum | female | 21 months | Pneumonia |
| 4 | Sputum | male | 13 months | Pneumonia |
| 5 | Sputum | female | 21 months | Bronchopneumonia |
| 6 | Sputum | female | 5 years | Bronchopneumonia |
| **7** [a] | **Sputum** | **female** | **12 months** | **Pneumonia** |
| 8 | Swab | female | 8 months | Bronchopneumonia |
| 9 | Sputum | female | 13 years | Pneumonia |
| 10 | BALF | female | 7 years | Pneumonia |
| 11 | Sputum | female | 3 years | Pneumonia |
| 12 | Sputum | male | 8 years | Pneumonia |
| 13 | Sputum | female | 5 years | Infectious mononucleosis |
| 14 | Sputum | male | 14 years | Pneumonia |
| 15 | Sputum | male | 22 months | Acute bronchitis |
| 16 | Sputum | male | 3 years | Pneumonia |
| 17 | Sputum | male | 6 years | Bronchopneumonia |
| 18 | Sputum | male | 5 years | Mycoplasma Pneumonia |
| 19 | Sputum | male | 8 years | Pneumonia |
| 20 | Sputum | male | 5 years | Pneumonia |
| **21** [b] | **Sputum** | **female** | **12 months** | **Pneumonia** |
| 22 | Sputum | male | 3 years | Pneumonia |
| 23 | Sputum | female | 8 months | Pneumonia |
| 24 | Sputum | male | 4 years | Pneumonia |
| 25 | Sputum | female | 6 years | Pneumonia |
| 26 | swab | male | 6 years | Pneumonia |
| 27 | Sputum | female | 6 years | Pneumonia |
| 28 | Sputum | female | 3 years | Bronchopneumonia |
| **29** [c] | **Sputum** | **female** | **12 months** | **Pneumonia** |
| 30 | Sputum | female | 8 years | Infectious fever |
| 31 | Sputum | female | 3 years | Pneumonia |
| 32 | Sputum | male | 11 months | Bronchopneumonia |
| 33 | BALF | male | 4 years | Pneumonia |
| 34 | Sputum | male | 3 years | Pneumonia |
| 35 | Sputum | male | 8 years | Bronchopneumonia |
| 36 | BALF | female | 6 years | Pneumonia |
| 37 | Sputum | female | 6 years | Pneumonia |
| 38 | Sputum | male | 6 years | Pneumonia |
| 39 | Sputum | male | 7 years | Pneumonia |
| 40 | Sputum | male | 6 years | Pneumonia |
| 41 | Sputum | male | 7 years | Pneumonia |
| 42 | Sputum | male | 4 years | Pneumonia |
| 43 | BALF | female | 17 years | Pneumonia |
| 44 | Sputum | male | 5 months | Bronchopneumonia |
| 45 | Sputum | female | 3 years | Pneumonia |
| 46 | BALF | female | 3 years | Pneumonia |
| 47 | BALF | female | 8 years | Pneumonia |
| 48 | Sputum | female | 6 years | Bronchopneumonia |
| 49 | BALF | male | 5 months | Pneumonia |
| 50 | BALF | female | 3 years | Pneumonia |
| 51 | BALF | female | 3 years | Pneumonia |

A, b, c: These three samples were derived from the same patient.