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Study on the clinical application of Streptococcus pneumoniae serotype detection based on MALDI-TOF MS technology

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Abstract

Streptococcus pneumoniae is related to the prognosis of infected patients. If the strain can be identified to the serotype level, it will play a key role in formulating measures to prevent Streptococcus pneumoniae infection. Therefore, this study focuses on analyzing the significance of serotype detection of Streptococcus pneumoniae under the guidance of MALDI-TOF MS technology, and provides a reference for rapid clinical typing of Streptococcus pneumoniae. A total of 500 patients were selected for treatment in our hospital from April 2019 to April 2020. The main types of diseases were patients with sinusitis, pneumonia, bacteremia, and acute otitis media. A total of 500 cases were collected, and specimens of Streptococcus pneumoniae were collected. Observe the distribution of MALDI-TOF MS mass spectrometry scores; the composition of the serotype distribution of Streptococcus pneumoniae isolates; the molecular typing of Streptococcus pneumoniae isolates; the relationship between the clinical characteristics of 500 patients and the distribution of serotypes 14, 19F; the distribution of serotypes 14, 19F and Analysis of the relationship between patient prognosis. Capsular swelling, latex agglutination test, MALDI-TOF MS detection of serotype distribution of serotype Streptococcus pneumoniae isolates, serotypes 6B, 14, 19A, 19F, 23F accounted for a large proportion, of which the highest serotype was mainly 14. 19F; The number of serotypes detected by MALDI-TOF MS was the highest, reaching 489 (97.80%), the number of serotypes detected by capsular swelling was 308 (61.60%), and the number of serotypes detected by latex agglutination test was 313 (62.40%). The distribution of serotypes detected by MALDI-TOF MS was significantly higher than the other two detection techniques ($P < 0.05$), and there was no statistical difference between the two tests of capsular swelling and latex agglutination ($P > 0.05$). The distribution rates of serotypes 14 and 19F were compared in different ages, disease types, drugs, etc., and there were statistical differences ($P < 0.05$), but there was no statistical difference in gender ($P > 0.05$). The prognosis of 500 patients, of which 487 cases improved (serotype 14: 98 strains; serotype 19F: 100 strains), 13 died (serotype 14: 12 strains; serotype 19F: 12 strains), serotypes 14, 19F There is a significant correlation between the distribution and the prognosis of patients ($P < 0.05$). MALDI-TOF MS technology can quickly, accurately and simply identify Streptococcus pneumoniae, with high sensitivity and specificity, and can save time for clinical diagnosis of Streptococcus pneumoniae infection. The laboratory can use MALDI-TOF MS to collect protein profiles of known typed strains and construct a corresponding typing mass spectrum library, and perform principal component cluster analysis on the spectrum to be tested and the library, which can quickly type Streptococcus pneumoniae.

Keywords: Streptococcus pneumoniae; serotype; MALDI-TOF MS technique; capsular swelling; latex agglutination test; clinical application; prognosis.

Practical Application: Our study shows that MALDI-TOF MS technology can quickly, accurately and simply identify Streptococcus pneumoniae, with high sensitivity and specificity, and can save time for clinical diagnosis of Streptococcus pneumoniae infection. However, this requires to be confirmed in the future studies.

1 Introduction

Streptococcus pneumoniae is a highly virulent pathogen, second only to Staphylococcus aureus in pathogenicity. As one of the main pathogens of bacterial infections, it can cause various diseases such as pneumonia, meningitis, otitis media, sinusitis and bacteremia (El-Aziz et al., 2021). People with poor resistance and more basic diseases are all susceptible (Alharbi et al., 2021). According to data reports, there are approximately 1 million deaths per year due to Streptococcus pneumoniae infection worldwide, of which 12% are from my country (Almuhayawi et al., 2021). At present, the problem of Streptococcus pneumoniae infection is a key health issue. At this stage, the only measure to control Streptococcus pneumoniae infection is vaccine (Andersen et al., 2021; Asencio-Egea et al., 2021). Therefore, it is of great significance to further explore the related issues of Streptococcus pneumoniae.

Relevant studies have shown that there is a relationship between the infection caused by Streptococcus pneumoniae and the serotype (Brodard et al., 2021; Benedittis et al., 2021; Ding et al., 2018). Clinically, the distinction is mainly based on the difference of capsular polysaccharide antigens. Streptococcus pneumoniae can be divided into more than 90 serotypes.

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Different serotypes also have certain differences in virulence, pathogenicity and physiological characteristics (Fanaei et al., 2021; Farooq et al., 2021; Fernández-Esgueva et al., 2021). Clinically, serology or phage test is mainly used to detect the serotype of Streptococcus pneumoniae. However, in view of the high cost and high cost of such technology, it is impossible to conduct batch testing, which limits its use to a certain extent. Therefore, actively seek the rapid detection of Streptococcus pneumoniae serotypes is currently the main research direction (García-Salguero et al., 2021).

In recent years, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has gradually been widely used for bacterial identification, especially in clinical microbiology laboratories (Ghaith et al., 2021). MALDI-TOF MS is a high throughput technology which is based on the comparison of protein fingerprint acquired by microbial cells with a database of reference spectra by means of the utilization of various algorithms integrated in systems recently made commercially available. Any kind of microorganisms are composed of specific proteins. Therefore, it has a unique protein fingerprint automatic and rapid microbial mass spectrometry detection system. The protein fingerprint of microorganisms is obtained through MALDI-TOF MS technology, which is further compared with the protein fingerprints of known microorganisms. So as to complete the identification and typing of bacteria (Hamilton et al., 2021). MALDI-TOF MS technology has the advantages of automation, rapidity and high throughput (Jang et al., 2021). At present, the identification and classification of certain serotypes of bacteria by MALDI-TOF MS technology has been gradually promoted (Kaya et al., 2021). For example, in the study of Kmetova et al., the results of the study found that the detection of MALDI-TOF MS technology can be used for rapid detection. The drug-resistant strains of Staphylococcus aureus (Sivoňová et al., 2021); another example is the study of Lee et al. (2021), using MALDI-TOF MS technology to monitor the expression of recombinant proteins of the whole strain, and there is no need for purification, concentration and extraction, etc. Steps to shorten the detection time and greatly improve the detection efficiency. However, as the core part of the MALDI-TOF MS identification of microorganisms-the database, due to the lack of bacterial species and number, and there is no relevant data in China, the relevant data of Streptococcus pneumoniae is obtained, and the protein fingerprints of different serotypes of Streptococcus pneumoniae are constructed. Characteristic fingerprint map database, has great practical significance (Li et al., 2021). In addition, clinical practice has shown that Streptococcus pneumoniae infection is related to the prognosis of patients. If the strain can be identified to the serotype level, it will play a key role in formulating measures to prevent Streptococcus pneumoniae infection.

2 Materials and methods

2.1 Experimental reagents and instruments

Incubator (Changzhou Langyue), Optosin paper (Shanghai Jinsui), MALDI-TOF mass spectrometer (Bruker).

2.2 Research methods

Collection of test specimens for Streptococcus pneumoniae

Strain collection: The SP in this study came from the isolation of Streptococcus pneumoniae from clinical specimens submitted by our hospital. Selected from April 2019 to April 2020, the study subjects and the number of cases were 150 cases of sinusitis, 131 cases of pneumonia, 119 cases of bacteremia, and 100 cases of acute otitis media. Among them, there were 234 cases of male patients and 266 cases of female patients. The youngest patient was 5 years old and the oldest was 82 years old, with an average age of (46.29 ± 5.81) years old. Prognosis: 487 cases improved, 13 cases died. All patients signed an informed consent form; all patients' diseases were diagnosed by pathology and imaging techniques, corresponding to the diagnostic criteria for sinusitis, pneumonia, bacteremia, and acute otitis media.

Acquisition and identification of Streptococcus pneumoniae (McDaniel & Derscheid, 2021; Mehainaoui et al., 2021)

Obtain sterile body fluids such as blood and pleural effusion when the patient is admitted to the hospital, inoculate, place in an incubator for culture, incubator conditions: 35 °C, 5% CO2, use Optosin paper for identification, diameter of inhibition ring ≥ 14mm indicates that the test strain is sensitive to optoxin, and it can be determined to be Streptococcus pneumoniae.

Detection of Streptococcus pneumoniae specimens with traditional serotyping and molecular typing techniques

- Detection of serotype: capsular swelling and latex agglutination test is used to detect the serotype of Streptococcus pneumoniae.

Capsular swelling: Take the above-identified Streptococcus pneumoniae and resuscitate it. Take an appropriate amount of colonies and place them in a test tube to make a suspension. Add the suspension of the tested colony to the slide, and then add the undiluted Streptococcus pneumoniae. Evenly, add methylene blue solution, mix well, place in a wet box, and check with oil microscope. If the capsule is obviously swollen and there is a colorless and wide ring near the bacteria, it is positive. Determine the serotype.

Latex agglutination test: Obtain 1 drop of Streptococcus pneumoniae bacteria liquid and 1 drop of the latex reagent for identification of bacteria liquid greater than 6 McKernell units and mix well. The phenomenon of flake agglutination indicates positive.

- Using the multilocus sequence typing technique (MLST) to perform molecular level genetic typing of the isolated strains.

Randomly select 20 specimens for MLST typing, detect and sequence the aroE, gdh, gki, recP, spi, xpt and ddl housekeeping genes, enter the MLST website to find the relevant allele profile and sequence type (ST), and contact the website Compare the existing strains.

Establish MALDI-TOF MS technology to detect Streptococcus pneumoniae serotype (Moussa et al., 2021)

SP serotype: MALDI Biotyper high-throughput microorganisms (manufacturer: Bruker).

- Identification: obtain the above-mentioned recovered strains, use a disposable inoculation loop to obtain a single colony, evenly spread, set standard wells for control, namely protein standard wells, Escherichia coli DH5a, dry, add matrix solution, and dry again, Detected by MALDI-TOF mass spectrometer. < 1.7000 indicates that the result is unreliable; 2.300-3.000 indicates the level of bacterial species that may be identified; 2.000-2.299 indicates the level of very reliable identification of bacterial genus; 1.700-1.999 indicates that the level of bacterial genus may be identified.
- Protein fingerprints: use MALDI-TOF MS technology to detect strains typed according to the above standards, and repeatedly explore and select the best pretreatment and experimental conditions to obtain protein fingerprints of different serotypes of Streptococcus pneumoniae. See Figure 1.
- Characteristic fingerprint database: MALDI-TOF MS software is used to analyze the detection results of protein fingerprints and construct a database of characteristic fingerprints for daily work. See Figure 2.

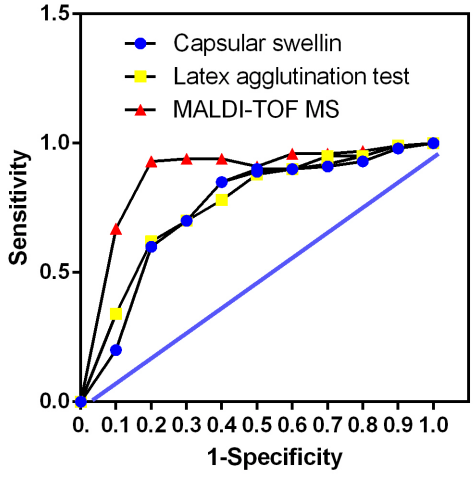

Figure 1. Protein fingerprints of different serotypes of Streptococcus pneumoniae. Note: A: 6B; B: 19F; C: 14; D: 23F.

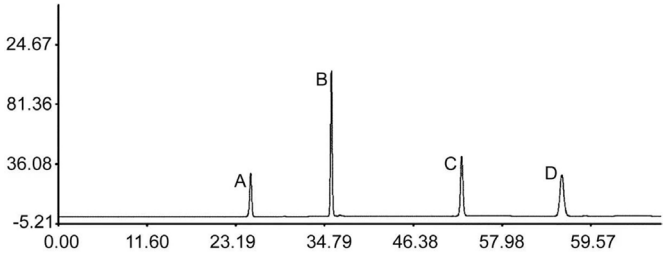

Figure 2. Database of characteristic fingerprints for standard detection of different serotypes. Note: 5: 14; 6: 23F; 9: 19F; 14: 6B.

- Sensitivity and specificity: to evaluate the sensitivity and specificity of the MALDI-TOF automatic microbial mass spectrometry detection system for serotyping of Streptococcus pneumoniae.

Follow-up

The follow-up time was 3 years. The content of the follow-up mainly included improvement and the number of deaths.

2.3 Observation indicators

Observe the distribution of MALDI-TOF MS mass spectrometry scores; the composition of the serotype distribution of Streptococcus pneumoniae isolates; the molecular typing of Streptococcus pneumoniae isolates; the relationship between the clinical characteristics of 500 patients and the distribution of serotypes 14, 19F; the distribution of serotypes 14, 19F and Analysis of the relationship between patient prognosis.

2.4 Statistical methods

SPSS26.0 analysis data, the serotype distribution of Streptococcus pneumoniae isolates is expressed in %, the comparison between groups is performed by χ^2 test, and $P < 0.05$ indicates statistical significance.

3 Results and discussion

3.1 Identification of Streptococcus pneumoniae bacteria

The identification results were all Streptococcus pneumoniae (Table 1).

3.2 The composition of the serotype distribution of Streptococcus pneumoniae isolates

Capsule swelling, latex agglutination test, MALDI-TOF MS detection of serotypes of Streptococcus pneumoniae isolates, serotypes 6B, 14, 19A, 19F, 23F accounted for a large proportion, of which the highest serotypes were mainly 14. 19F; The number of serotypes detected by MALDI-TOF MS was the highest, reaching 489 (97.80%), the number of serotypes detected by capsular swelling was 308 (61.60%), and the number of serotypes detected by latex agglutination test was 313 (62.40%). The distribution of serotypes detected by MALDI-TOF MS was significantly higher than the other two detection techniques ($P < 0.05$), and there was no statistical difference between the two tests of capsular swelling and latex agglutination ($P > 0.05$) (Table 2).

Table 1. Mass spectrum distribution of 500 strains of Streptococcus pneumoniae detected by MALDI-TOF MS.

Score range	Sign	Number of specimens	Composition ratio (%)
1.700-1.999	+	71	14.20%
2.000-2.299	++	315	63.00%
2.300-3.000	+++	114	22.80%

The standard Streptococcus pneumoniae is 1.897, and the score > 1.700 indicates that the identification result is highly reliable.

The sensitivity and specificity of the MALDI-TOF MS detection technique are significantly higher than those of the capsular swelling and latex agglutination experiments (Table 3 and Figure 3).

3.3 Molecular typing of Streptococcus pneumoniae isolates

Twenty strains of Streptococcus pneumoniae with the lowest inhibitory concentration of penicillin were randomly selected and typed using MLST technology. The molecular typing is shown in Table 4.

3.4 Analysis of the relationship between the clinical characteristics of 500 patients and the distribution of serotypes 14 and 19F

The distribution rates of serotypes 14 and 19F were compared in different ages, disease types, drugs, etc., and there were statistical differences ($P < 0.05$). The distribution rates of serotypes 14 and 19F were not significantly different in different genders ($P > 0.05$)(Table 5).

3.5 Analysis of the relationship between the distribution of serotypes 14 and 19F and the prognosis of patients

The prognosis of 500 patients, of which 487 cases improved (serotype 14: 98 strains; serotype 19F: 100 strains), 13 died (serotype 14: 12 strains; serotype 19F: 12 strains), There is a significant correlation between the serotypes 14, 19F distribution and the prognosis of patients ($P < 0.05$).

3.6 Discussion

The main purpose of this study is to establish a method for rapid detection of SP serotype using MALDI-TOF MS technology; to establish and improve the regional SP characteristic fingerprint

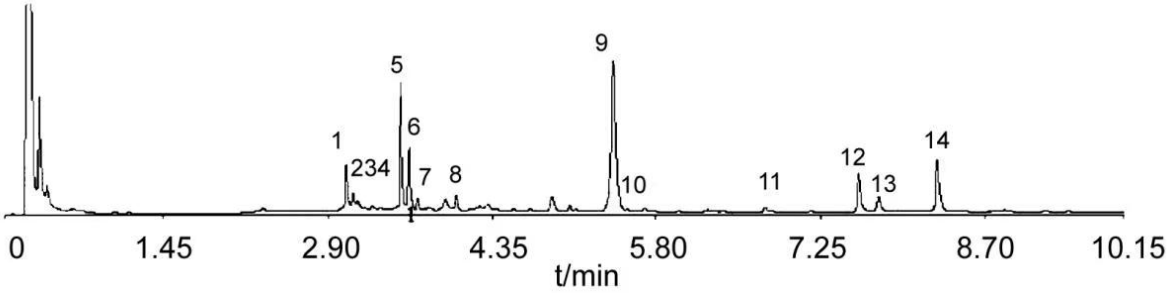

Figure 3. ROC curve analysis of the application value of three detection techniques for serotyping.

Table 2. Serotype distribution composition of Streptococcus pneumoniae isolates.

Serotype	Capsular swelling		Latex agglutination test		MALDI-TOF MS	
	Number of plants	Percentage	Number of plants	Percentage	Number of plants	Percentage
4	0	0.00%	2	0.40%	4	0.80%
3	10	2.00%	8	1.60%	12	2.40%
6B	44	8.80%	46	9.20%	70	14.00%
9V	7	1.40%	5	1.00%	11	2.20%
14	71	14.20%	72	14.40%	110	22.00%
15A	0	0.00%	0	0.00%	2	0.40%
19A	51	10.20%	50	10.00%	81	16.20%
19F	71	14.20%	74	14.80%	112	22.40%
23F	44	8.80%	42	8.40%	70	14.00%
19C	7	1.40%	10	2.00%	11	2.20%
15C	1	0.20%	2	0.40%	1	0.20%
18C	0	0.00%	0	0.00%	1	0.20%
23A	1	0.20%	0	0.00%	1	0.20%
29	0	0.00%	0	0.00%	1	0.20%
42	1	0.20%	1	0.20%	2	0.40%
Total	308	61.60%	313	62.40%	489	97.80%

Table 3. ROC curve analysis of the application value of three detection techniques for serotyping.

Technology	AUC	Sensitivity	Specificity	95% CI	Yorden Index
Capsular swelling	0.709	0.623	0.632	0.588-0.815	0.255
Latex agglutination test	0.738	0.601	0.670	0.617-0.856	0.271
MALDI-TOF MS	0.769	0.756	0.881	0.723-0.894	0.637

Roc: receiver operating characteristic.

Table 4. Molecular typing of Streptococcus pneumoniae isolates.

Sample number	serotype	aroE	gdh	gki	recP	spi	xpt	ddl	ST
6	4	7	13	1	6	6	6	14	4542
8	3	15	16	19	15	6	20	26	236
9	6B	15	17	4	16	6	1	17	280
10	9V	93	5	7	113	17	28	70	6990
11	14	46	8	2	10	6	1	22	505
12	15A	4	16	19	15	6	20	1	320
18	19A	46	8	2	10	6	1	22	505
20	19F	4	4	2	4	4	1	1	81
21	23F	8	13	14	4	6	4	14	876
35	19C	4	16	19	15	6	20	1	320
40	15C	8	5	6	1	9	10	14	1931
61	18C	10	13	34	16	15	28	31	2752
64	23A	4	16	19	15	6	20	1	320
76	29	10	12	2	1	6	1	14	4450
79	42	5	5	6	16	9	1	14	4560

ST: sequence type.

Table 5. Analysis of clinical characteristics and distribution of serotypes 14 and 19F in 500 patients.

Index		14 (n = 110)			19F (n = 112)		
		Distribution	Distribution rate	χ^2/P	Distribution	Distribution rate	χ^2/P
gender	Male (n = 234)	57	51.82%	1.426/0.232	60	54.55%	2.658/0.103
	Female (n = 266)	53	48.18%		52	47.27%	
age	5-16 (n = 166)	33	30.00%	12.655/0.000	37	33.64%	13.825/0.000
	17-59 (n = 211)	13	11.82%		15	13.64%	
	60-82 (n = 123)	64	58.18%		60	54.55%	
Type of disease	Sinusitis (n = 108)	7	6.36%	32.792/0.000	9	8.18%	26.737/0.000
	Pneumonia (n = 145)	52	47.27%		50	45.45%	
	Bacteremia (n = 150)	43	39.09%		45	40.91%	
	Acute otitis media (n = 97)	8	7.27%		8	7.27%	
drug	Erythromycin (n = 158)	50	45.45%	27.999/0.000	48	43.64%	27.740/0.000
	Clindamycin (n = 165)	45	40.91%		49	44.55%	
	Penicillin (n = 100)	6	5.45%		8	7.27%	
	Other (n = 77)	9	8.18%		7	6.36%	

library; to clarify the correlation between SP serotype and infection and prognosis, and to further evaluate MALDI-TOF MS Application value of technology. In this study, 500 strains of Streptococcus pneumoniae were first obtained. First, 500 strains were identified using MALDI-TOF MS technology. The standard Streptococcus pneumoniae was 1.897. Observation of the identification results showed that all strains had scores above 1.700, and the scores were Strains from 2.000-2.299 accounted for 63.00%, among which strains with a score of 2.300-3.000 accounted for 22.80%, all of which were successfully identified as Streptococcus pneumoniae, and subsequent experiments can be carried out.

This study further analyzed the composition of the serotype distribution of Streptococcus pneumoniae. Capsular swelling, latex agglutination test, and MALDI-TOF MS were used to detect the serotype distribution of serotype Streptococcus pneumoniae isolates. The results of the study found that serotypes 6B, 14, 19A The proportions of MALDI, 19F, and 23F are relatively large, and the highest proportion of serotypes are mainly 14 and 19F. Comparing the three detection techniques, the number of strains detected by the MALDI-TOF MS detection technique reached 489, accounting for 97.80%, while the number of strains detected by the capsular swelling detection technique was only 308, accounting for 61.60%, the number of strains detected by the latex agglutination test technique was only 313, accounting for 62.40%, and the serotype detected by MALDI-TOF MS The distribution is significantly higher than that of the other two detection technologies. The results of this study suggest that compared with conventional capsular swelling and latex agglutination experiments, the MALDI-TOF MS detection technology can quickly and accurately detect the serotype of Streptococcus pneumoniae in real time. Further evaluation of the detection value of the MALDI-TOF method, the results found that the sensitivity and specificity of the MALDI-TOF MS detection technology were significantly higher than the capsular swelling and latex agglutination experiments. The capsular swelling experiment mainly uses Streptococcus pneumoniae antiserum to swell the capsule of the same type of bacteria, which in turn makes the polysaccharides present at the surface of the streptococcus bind to the serum to form a complex, but

there are also some cases in the capsular swelling experiment. Insufficient, the formation of the complex is easily disturbed by the capsule, and after passage of Streptococcus pneumoniae, the autolyzed enzyme produced by itself is gradually dissolved, and the capsule is easily lost, which affects the application value of the capsule swelling experiment (Mulet et al., 2021). The experimental procedures of latex agglutination experiments are cumbersome and the detection cycle is relatively long. However, such experimental techniques can identify species or subspecies level. MALDI-TOF MS detection technology protein fingerprinting can effectively identify different serotypes, and give fast and accurate, but theoretically only the serotypes of Streptococcus pneumoniae can be identified. This is not the case for other strains. The analysis may be due to serological typing. There is a difference between the cell membrane protein of MALDI-TOF MS and the protein typed by MALDI-TOF MS (Nabet et al., 2021).

Further analyze the application value of MALDI-TOF MS technology, especially in daily work, combined with clinical efficacy, evaluate the application value of MALDI-TOF MS technology for serotyping of Streptococcus pneumoniae. Analyze the relationship between the serotype of Streptococcus pneumoniae and the clinical characteristics of the patient, and evaluate the relationship between the serotype, infection and prognosis at the same time. The results of the study found that the distribution rates of serotypes 14 and 19F were compared in different ages, disease types, drugs, etc., and there were statistical differences ($P < 0.05$). The distribution rates of serotypes 14 and 19F were not significantly different in different genders ($P > 0.05$). Serotypes 14 and 19F have a higher detection rate in younger or older people, especially in pneumonia or bacteremia, and in patients with erythromycin and clindamycin resistance The detection rates of serotypes 14 and 19F were higher. The prognosis of 500 patients was further found, of which 487 cases improved (serotype 14: 98 strains; serotype 19F: 100 strains), 13 died (serotype 14: 12 strains; serotype 19F: 12 strains), and serotype 14 There is a significant correlation between the 19F distribution and the prognosis of patients ($P < 0.05$). However, some studies have shown that there is no significant correlation between the serotype and the patient's prognosis, which is different from this study. The analysis of the reason may be due to the difference in detection technology and the number of selected strains. It is also necessary to select a large sample of strains for experimentation to improve the accuracy of research results (Oho et al., 2021; Oliva et al., 2021).

4 Conclusion

In summary, MALDI-TOF MS can quickly, accurately and simply identify Streptococcus pneumoniae, with high sensitivity and specificity, and can save time for clinical diagnosis of Streptococcus pneumoniae infection. The laboratory can use MALDI-TOF MS to collect protein profiles of known typed strains and construct a corresponding typing mass spectrum library, and perform principal component cluster analysis on the spectrum to be tested and the library, which can quickly type Streptococcus pneumoniae. It is of great significance to provide clinical infection information in a timely manner and formulate treatment plans for monitoring the prevalence of strains and vaccination.

Ethical approval

Research experiments conducted in this article with animals or humans were approved by the Second People's Hospital of Lishui following all guidelines, regulations, legal, and ethical standards as required for humans or animals.

Conflict of interest

There are no conflicts to declare.

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