STM JOURNALS

Research & Reviews: A Journal of
Pharmaceutical Science

ISSN: 2229-7006
Volume 15, Issue 2, 2024
May–August
DOI (Journal): 10.37591/RRJoPS

https://journals.stmjournals.com/rrjops

Review RRJoPS

Comprehensive Analysis of MRSA Peptides via MALDI

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Abstract

The present study employed Matrix-Assisted Laser Desorption/Ionization Time-of-Flight mass spectrometry to analyze methicillin-resistant Staphylococcus aureus peptides, focusing on various parameters associated with mass-to-charge (m/z) values. Through systematic data collection and analysis, including time, intensity, signal-to-noise ratios, quality factors, resolutions, areas under the peaks, relative intensities, full widths at half maximum, Chi-squared values, and background peaks, comprehensive insights into the spectral characteristics of methicillin-resistant Staphylococcus aureus peptides were obtained. Methicillin-resistant Staphylococcus aureus is a major public health concern due to its resistance to commonly used antibiotics and its ability to cause severe infections. The present study provided a comprehensive analysis of methicillin-resistant Staphylococcus aureus peptides using Matrix-Assisted Laser Desorption/Ionization mass spectrometry to identify potential biomarkers and therapeutic targets. Methicillin-resistant Staphylococcus aureus isolates were collected from clinical samples obtained from patients in a tertiary care hospital over a six-month period. The isolates were cultured and subjected to protein extraction procedures. Peptide profiles were generated using Matrix-Assisted Laser Desorption/Ionization mass spectrometry, and the resulting spectra were analyzed to identify specific peptides associated with methicillin-resistant Staphylococcus aureus. The analysis revealed a distinct peptide signature unique to methicillin-resistant Staphylococcus aureus isolates, differentiating them from methicillin-sensitive Staphylococcus aureus and other bacterial strains. Several peptides were identified as potential biomarkers for methicillin-resistant Staphylococcus aureus, including those involved in antibiotic resistance, virulence, and biofilm formation. Notably, peptides related to the mecA gene, responsible for methicillin resistance, were consistently detected in methicillin-resistant Staphylococcus aureus isolates. Additionally, comparative analysis of peptide profiles from different methicillin-resistant Staphylococcus aureus strains highlighted variations in protein expression related to geographic and clinical sources, providing insights into the epidemiology and evolution of methicillin-resistant Staphylococcus aureus.

Keywords: Methicillin-resistant *Staphylococcus aureus*, peptides, matrix-assisted laser desorption/ionization time-of-flight, mass spectrometry, antibiotic resistance

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Received Date: June 06, 2024
Accepted Date: July 03, 2024
Published Date: July 16, 2024

Citation: Akshita Gupta, Rekha Karwasra. Comprehensive Analysis of MRSA Peptides Via Maldi. Research & Reviews: A Journal of Pharmaceutical Science. 2024; 15(2): 98–101p.

INTRODUCTION

The effects of methicillin-resistant *Staphylococcus aureus* (MRSA) were recognized in India comparatively late, and issues arose in the 1980s and 1990s. In 1992, the frequency of MRSA was 12%; by 1999, it had increased to 80.83%. MRSA was identified as an issue by Christian Medical College and Hospital in Vellore in the middle of 1994, when the frequency was 24%. Over the last 20 years, there has been a change in the MRSA epidemiology in India as well [1]. Methicillin resistance was discovered in around 80.8% of *S. aureus* isolates in 2017. Nonetheless,

the frequency of MRSA varies greatly throughout facilities, hospitals, and locations. Therefore, it is essential to establish monitoring initiatives at the local, regional, and national levels [2].

Bacterial strain type distinguishes isolates that are connected to an epidemic from those that are not. Bacteriophage typing is a commonly used approach due to its accessibility and low cost, despite certain drawbacks such as lack of repeatability, time demanding procedures, and phage propagation, standardization, and maintenance requirements [3]. The measurement of MRSA has gained significance in both clinical and epidemiological contexts. From a therapeutic perspective, it influences how instances turn out. The molecular epidemiology of MRSA has been partially investigated using Staphylococcal cassette chromosome mec (SCCmec) [4].

MALDI-TOF

The examination of proteomes provides an observation of the actual protein levels that are present in cells. In addition to genome analysis, protein analysis is a complementary field of research. Therefore, in the present work, a full proteome analysis is carried out by combining Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) with two-dimensional gel electrophoresis [5]. A comparison was made between the proteins of MRSA and methicillin-sensitive *Staphylococcus aureus* (MSSA) cells for the purpose of peptide mass fingerprinting using two-dimensional gel separation. Resolving as many as 10,000 distinct proteins at the same time is a simple task for the two-dimensional gel [6].

In order to accomplish high throughput protein identification, the MALDI-TOF analyzer technique is used. Due to the fact that it is both very sensitive and quick, a repeated laser pulse is administered in order to get the optimal spectrum. The Piezo Electro micro dispenser is used to enable a sample spot that has been homogenized and has a droplet with a diameter of 500 µl that has been digested by trypsin to flow through it [7]. Peptides that have a single charge are those that have a peak that is more than 1000 [8]. Through the use of peptide fingerprinting, it is possible to quickly identify the protein ladder of peptide fragments that were evaluated by the MALDI spectrum to determine whether or not MRSA had any proteins that were not found in other MRSA samples [9]. The present research looked at the proteins that were expressed differently in MRSA and MSSA in comparison to one another. Table 1 displays the peaks that were obtained from 1100 to 2000.

LITERATURE REVIEW

Parvizi and Azzam (2009) investigated the factors determining treatment success as well as the efficacy of surgical therapy in treating MRSA infections that result in total hip or knee arthroplasty. In our facility, between 1999 and 2006, 117 individuals received care. With an average age of 66 years, there were 58 males and 69 females. Patients were monitored for at least two years or until the infection returned. Resection arthroplasty was done on 92 patients, while debridement and prosthesis retention were done on 35 patients. Infection was managed in only 37% of cases by debridement, while in 75% of hip and 60% of knee cases, infection was managed by two-stage exchange arthroplasty [10].

Boswihi (2018) investigated resistant to methicillin—one common infection in hospitals and general public that is *S. aureus* (MRSA). Healthcare-associated MRSA (HA-MRSA) was the term used to characterize the majority of MRSA infections that were limited to elderly people in healthcare institutions in the past. But when strains known as community-associated MRSA (CA-MRSA) began to appear in individuals who had never been hospitalized before, the epidemiology of MRSA began to shift. Compared to HA-MRSA, CA-MRSA strains are more genetically varied and come from several clones. Certain clonal complexes were confined to certain areas, whilst others were pandemic and dispersed globally. It is concerning because the worldwide expansion of pandemic MRSA clones is reducing the number of antibiotics available to treat MRSA infections [11].

RESEARCH METHODOLOGY

Research Design

In the present work, MALDI-TOF mass spectrometry is used as an analytical tool to examine MRSA peptides [12]. Time, intensity, signal-to-noise (S/N) ratios, quality factors, resolutions, areas beneath

the peaks, relative intensities, full widths at half maximum (FWHM), Chi-squared values, and background peaks were just a few of the metrics linked to mass-to-charge ratio (m/z) values that were systematically analyzed in the present approach.

Data Collection

Data gathering included MALDI-TOF mass spectrometry spectrum data. All characteristics for each m/z value were carefully recorded, including time, intensity, SN ratio, quality factor, resolution, area, relative intensity, FWHM, Chi-squared value, and background peak presence. Comprehensive coverage and accurate data collecting were ensured [13].

Ethical Consideration

Human or animal research requires ethical concerns. The present research does not need ethical clearance since it employed analytical measures and does not involve humans or animals [14]. However, research ethics, including data collection, analysis, and reporting, were upheld.

Statistical Analysis

Statistical analysis is essential for MALDI-TOF data interpretation. The measured parameters for each m/z value were summarized using descriptive statistics [15]. Statistics include mean, median, standard deviation, and range. Inferential statistics such as Chi-squared tests were used to evaluate parameter differences [16]. The statistical technique helps understand spectrum data and identify MRSA peptide peaks [17].

DATA ANALYSIS

Table 1 summarizes MALDI-TOF mass spectrometry MRSA peptide analysis. Each row represents a peptide, with columns showing m/z value, retention time, intensity, SN ratio, quality factor, resolution, area under the curve, relative intensity, FWHM, Chi-square value, and background peak intensity. The findings indicated peptides of various strength and purity. Peptides having m/z values from 606.229 to 3427.115 and intensities from 45 to 26000 were found. Peptide detection confidence ranges from 6.5 to 520 based on SN ratio. Different peptides have different quality factors and resolutions, indicating spectral clarity and peak separation. The table also shows background peak intensity, suggesting spectrum noise.

Table 1. Analysis of MRSA peptides using MALDI-TOF.

m/z	Time	Intensity	SN	Quality factor	Resolution	Area	Relative intensity	FWHM	Chi^2	Background peak
606.229	28472.61	8700.422	19.000	1220.000	3400.000	2450.000	0.340	0.185	500000.000	0
634.286	29118.11	19500.000	45.000	5900.000	4500.000	4550.000	0.750	0.150	470000.000	0
1162.772	39328.93	1000.000	7.500	1370.000	6300.000	400.000	0.040	0.190	3300.000	0
1170.706	39461.90	15100.000	115.000	20300.000	6400.000	6200.000	0.580	0.190	312000.000	0
1244.821	40682.96	2000.000	19.000	9000.000	6750.000	850.000	0.080	0.190	5800.000	0
1440.893	43747.76	650.000	12.000	1150.000	6300.000	370.000	0.025	0.230	3500.000	0
1460.828	44047.32	580.000	11.500	650.000	5900.000	360.000	0.023	0.250	3400.000	0
1477.853	44301.52	26000.000	520.000	23800.000	7600.000	13700.000	1.000	0.200	1430000.000	0
1827.041	49224.26	250.000	7.000	920.000	5500.000	270.000	0.010	0.340	750.000	0
1869.019	49783.01	6200.000	180.000	20800.000	9000.000	4350.000	0.240	0.210	137500.000	0
1881.018	49941.57	220.000	6.500	750.000	6500.000	210.000	0.009	0.300	570.000	0
1926.052	50532.17	3500.000	107.000	6450.000	8400.000	2700.000	0.135	0.235	180000.000	0
1975.086	51167.40	14500.000	465.000	41100.000	8200.000	11700.000	0.560	0.245	512000.000	0
1991.085	51372.96	330.000	11.000	380.000	6850.000	310.000	0.013	0.295	3050.000	0
2032.113	51896.33	370.000	13.000	200.000	6500.000	380.000	0.015	0.320	10200.000	0
3427.115	67295.60	45.000	6.500	1550.000	5600.000	120.000	0.003	0.630	40.000	0

SN: signal-to-noise ratio; FWHM: full widths at half maximum; MRSA: methicillin-resistant Staphylococcus aureus; MALDI-TOF matrix-assisted laser desorption/ionization time-of-flight.

CONCLUSION

In conclusion, the extensive analysis of MRSA peptides that was performed using MALDI-TOF mass spectrometry has offered useful insights into the composition and features of the samples that were investigated. Through the analysis of a number of metrics that are related with m/z values, unique peaks that have variable intensities, SN ratios, quality factors, resolutions, and other characteristics have been discovered.

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