

Seasonal Assessment of Microbial Contamination and Disinfectant Efficacy in Cleanroom using MALDI-TOF MS in a NABL-Accredited Pharmaceutical Testing Laboratory

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ABSTRACT

Background: Microbial bioload control in clean rooms is critical for ensuring pharmaceutical product quality. Regular monitoring and effective disinfection practices are essential to minimize contamination risks. This study aimed to identify and analyze microbial contamination in clean rooms across different seasons using MALDI TOF MS for rapid microbial identification. **Materials and Methods:** Microbial samples were collected pre- and post-fogging from a NABL-accredited cleanroom using settle plates and active air sampling methods. The fogging was performed with a QUAT-based disinfectant. Samples were analyzed using Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI TOF MS) for rapid and reliable identification of microbial species. The study was conducted across three seasonal periods-summer, monsoon, and winter-to assess seasonal variation in microbial contamination. **Results:** MALDI TOF MS identified common microbial contaminants such as *Pseudomonas stutzeri*, *Staphylococcus* spp., *Bacillus* spp., and *Micrococci* spp. Significant seasonal variations were observed in microbial populations. Post-fogging, the microbial load was reduced by approximately 90% during the monsoon, 40% in summer, and 57% in winter. The identification process using MALDI TOF MS allowed for rapid and reliable analysis of the cleanroom microbial environment. **Conclusion:** MALDI TOF MS proved effective for quick and accurate identification of cleanroom microbial contaminants. The QUAT-based fogging process effectively reduced microbial loads, though efficacy varied seasonally. Regular monitoring, coupled with rotational disinfectant use, supports enhanced contamination control strategies in pharmaceutical cleanroom environments.

Keywords: Environmental Monitoring, Seasonal Assessment, QUAT-Based Disinfectant, Clean Rooms, MALDI TOF MS.

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INTRODUCTION

The microbiological bioload in a laboratory's clean room environment is essential for guaranteeing the safety and quality of product testing across sectors, including medicines and food processing. Effective monitoring and identification of microbial contaminants not only safeguard product integrity but also uphold public health standards (Gerba, C. and Pepper, L. 2019). At pharmaceutical the clean rooms are designed to minimize the presence of particulate and microbial contaminants (Rizwan, M., *et al.*, 2022). Its maintenance and regulation is governed by various standards and guidelines like ISO, United States Pharmacopeia (USP) Chapter <1116>. Apart from this EMA also provides guidelines for clean room classification and monitoring,

emphasizing the need for regular environmental monitoring to ensure compliance with cGMP standards (Berthod, F. *et al.*, 2020).

However, despite stringent controls, contamination can still occur from several sources. Out of which personnel are often considered the primary source, as humans shed skin cells, hair, and other particulates that can carry microorganisms. Studies have shown that even with the use of cleanroom garments, and regular cleaning and disinfection, significant levels of microbial contamination can originate (Verbeek, J. H *et al.*, 2020). Moreover the airborne microorganisms are considered as major threat due to their ability to settle on surfaces, equipment, and products, posing a significant risk of contamination (Masotti, F. *et al.*, 2019; Mohammed, S., and Balogu, T. 2023; Chai, J., *et al.*, 2018). Additionally, environmental factors such as airflow, humidity, and temperature also play crucial roles in microbial contamination.

Therefore, regular Environmental Monitoring (EM) is required to maintain the clean room. This process involves systematically



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collecting and analyzing samples from air, surfaces, and personnel to detect and quantify microbial contamination. Periodic EM, provides data that can be used to identify microbial trends, potential contamination sources, and need for potential improvement. Monitoring data can be used to identify seasonal trends and implement proactive measures to mitigate the risk of contamination (Bourdichon, F. *et al.*, 2021). Despite the advances in clean room technology and environmental monitoring, challenges remain in getting the rapid analysis of microbial load for instant actions (Das, A. K., 2023).

MALDI-TOF MS is a reliable tool for identifying bacterial species as it analyses the molecular spectra of bacterial cells. The procedure is expedited relative to existing approaches, requiring less than 5 min to acquire a spectrum and validate it against a reference library, which is, on average, 1.45 days swifter than conventional techniques for detecting bacterial isolates. In a clinical context, the utilization of MALDI-TOF MS identification has been shown to reduce patient mortality due to the swift identification process and aids in lowering the expenses associated with standard microbiological recognition (Elbehiry, A., *et al.*, 2022). MALDI-TOF MS recognition is both quick and reportedly very accurate. More than 90% of Gram-positive strains were accurately identified using a MALDI-TOF MS system in comparison to molecular approaches, while around 90% of Gram-negative strains were correctly determined to at least a genus level in a clinical context involving humans (Torres-Sangiao, *et al.*, 2021).

The current study aims to assess the efficacy of MALDI-TOF MS in the context of microbiological monitoring within NABL-accredited pharmaceutical testing laboratories. The research assesses the utility of this instrument in detecting airborne contamination rapidly, thereby contributing to the optimization of cleanroom monitoring protocols. By enhancing our understanding of microbial dynamics within clean room environments and refining monitoring strategies, this research seeks to support pharmaceutical manufacturing processes, uphold product quality, and strengthen regulatory compliance efforts.

MATERIALS AND METHODS

Chosen areas

This study aims to isolate, characterize, and identify microorganisms through microbial monitoring at a pharmaceutical testing laboratory accredited to NABL, ISO/IEC 17025:2017 standards located in the Mumbai suburb region, Maharashtra, India. The environment monitoring sampling was conducted within the laboratory's cleanrooms. The area was thoroughly inspected, and all doors were securely closed to maintain a controlled environment before the fogging. A quaternary ammonium compound (QUAT)-based disinfectant was used by diluting it with water at a ratio of 1:15. The diluted disinfectant solution was then added to the fogger machine's

tank, which was placed in a corner of the cleanroom. According to EPA guidelines, the fogging process was conducted for 20 min to ensure comprehensive distribution of the disinfectant. Following the fogging, the area was left undisturbed for an additional 30 min to allow the disinfectant to settle and achieve effective sterilization. Microbial samples were collected across three different seasons (summer, monsoon, and winter), both before and after fogging, to assess the microbial load within the cleanroom. This approach provided a quantitative measure of airborne microbial contamination in the cleanroom across different seasons.

Microbiological methods

Settle plate

The settle plate method (Pasquarella, C. *et al.*, 2000) was used with few modifications, involving exposing sterile agar plates. Soybean-Casein Digest Agar (SCDA) and Potato Dextrose Agar with chloramphenicol (PDAC) was exposed in the cleanroom at a height of approximately one meter above the floor in an upright position. The lids were kept open, in the center of the designated sampling areas for a duration of 20 min. The locations and start times of the exposures were meticulously recorded. The procedure was carried out both before and after fogging with a QUAT-based disinfectant to evaluate the effectiveness of the disinfection process. The PCA plates were incubated at 30-35°C for 24-48 hr, while the PDAC plates were incubated at 20-25°C for 5 days. Following incubation, colonies were counted and recorded as CFU per plate.

Air sampling

The air sampling method (Pitt, J.I. and Hocking, A.D. 1999) was used with few modifications, by positioning the air sampler at approximately one meter above the floor to simulate typical human exposure levels. Sampling locations were strategically selected to represent critical areas within the cleanroom, including high-traffic zones. Sterile SCDA and PDAC were utilized as the culture media for microbial collection. This procedure was carried out both before and after fogging with a QUAT-based disinfectant to evaluate the effectiveness of the disinfection process. The air sampler was set to operate for 5-15 min, upon completion of the sampling period, the agar plates were carefully removed, sealed to prevent contamination, and labelled with pertinent information such as date, time, and sampling location. The PCA plates were incubated at 30-35°C for 24-48 hr, while the PDAC plates were incubated at 20-25°C for 5 days. After incubation, colonies were counted and recorded as CFU/m³ of air sampled.

Microbial identification

MALDI-TOF MS technique

Microbial species identification was conducted using MALDI-TOF MS (HiMedia), employing a metal reusable target slide. Before analysis, microbial samples were prepared by

direct deposit method and applied to the target slide. A solitary colony was applied to the designated slide to create a uniform smear, followed by treatment with the prepared matrix solution, featuring HCCA as the primary ingredient. Following ambient temperature drying, the target slide was placed into the hatch of the MALDI-TOF MS machine. Data acquisition was performed using the Autof Acquirer software, optimizing parameters such as laser intensity and number of shots for each sample. Subsequently, acquired spectra were analyzed using the latest version of Autof Analyzer software, where spectral patterns were compared against a comprehensive reference database to ascertain microbial species. A score >9.0 is considered highly reliable at the species level, whereas scores below 6.0 are considered indicative of unidentified isolates. Calibration of the system was routinely performed using an Auto Bio calibrating agent to ensure accuracy and consistency of results. The clear peaks were studied and based on the reliable score the identification was done.

RESULTS

Microbial identification using MALDI TOF MAS revealed the presence of various microbial species in all different seasons. Forty-seven bacterial isolates were from the clean room were subjected to identification. The results showed that the MALDI-TOF MAS analysis was effective, with score more than nine that is considered reliable. Out of all the strains, about thirty-three isolates are identified upto genus level. The common isolates included *Pseudomonas stutzeri* and *Staphylococcus* spp. *Bacillus* spp. *Micrococci* spp., *Kocuria* spp. etc. The *Pseudomonas stutzeri* dominated in all seasons and *Staphylococcus* had variety of species (*Staphylococcus hominis*, *Staphylococcus haemolyticus*, *Staphylococcus warneri*, *Staphylococcus saprophyticus*, *Staphylococcus aureus*, *Staphylococcus arlettae*, and *Staphylococcus capitis*), followed by *Bacillus* spp. (*Bacillus altitudinis*, *Bacillus oceanisediminis*, *Bacillus megaterium*), *Kocuria* spp. (*Kocuria rhizophila*, *Kocuria marina*, *Kocuria varians*). The mass spectra revealed intricate assemblies of distinct ions with m/z ratios spanning from 2000 to 10,000. Table 1 presents the peak masses of 12 *Pseudomonas stutzeri* strains, with the peaks emphasized in bold representing those obtained by the majority of the strains also represented in Figure 1. The produced peaks exhibit significant intensity, enabling the identification of biomarkers, as evidenced by the peaks at 4415 and 5559 m/z .

Similarly, Figure 2 shows peak masses of 5 strains of *Bacillus* and 2 strains of *Paenibacillus*, which are closely related thus showing the similarity in protein peaks. The peak intensity of *Paenibacillus* and *Bacillus* are recorded at m/z of 7150, which are similar Table 2. Consequently, these identified peaks are considered specific biomarkers for the recognized genera present in the database. This indicates that these two protein peaks may serve as potential species-specific biomarkers for *Bacillus*.

In the summer season, a variety of bacterial species were identified pre and post-fogging. The genera and the total bacterial and fungal CFU/ m^3 were isolated from the cleanroom during the summer seasons pre- and post-fogging using the settle plate and air sampling method. Most of the sample had scores above nine.

The microbial population detected from the cleanroom in the summer season consisted mainly of bacteria from the genera *Staphylococcus* sp, *Pseudomonas* sp, *Micrococcus* sp and *Bacillus* sp. The microbes isolated from the settle plate identified four different microbial species that had reliable scores that is more than 9.0 which is credible up to species and subspecies level whereas *Paenibacillus glucanolyticus* scored between 6-9 which is credible up to species level. The air sampling identified five microbial species that had reliable scores that are more than 9.0 which is credible up to species and subspecies level. Post fogging there was a significant reduction in microbial growth Table 3.

On the other hand, during the monsoon season, the settle plate method identified six different microbial species, including *Staphylococcus* sp., *Bacillus* sp., *Pseudomonas* sp., *Kodamaea* sp. and *Moraxella* sp. with scores more than 9 whereas *Curtobacterium* sp had score between 6-9. The microbial load was reduced after fogging, with the identified spices. In comparison, air sampling identified five microbial species of *Pseudomonas* sp, *Staphylococcus* sp, and *Kodamaea* sp. before fogging and consistency of *Pseudomonas* sp., *Staphylococcus* sp., remained with lesser microbial load and a new strain was found of *Moraxella osloensis* as illustrated in (Table 4).

In the winter season, there was a reduction in the number of species identified in both settle plates and air samples post-fumigation. The settle plate method identified three microbial species of *Pseudomonas* sp, *Staphylococcus* sp., and *Micrococcus* spp., with multiple colonies noted for some species. In comparison, air sampling identified six microbial species of *Pseudomonas* sp, *Staphylococcus* sp, *Micrococcus* spp., *Bacillus* sp, and *Janibacter* sp, also with multiple colonies noted for some species. After the fogging process settle plate reported three species of *Micrococcus* spp., *Kocuria* sp. and *Staphylococcus* sp., whereas the air sampling reported the presence of *Staphylococcus* sp. and *Janibacter indicus* (Table 5).

In air sampling, the fogging process resulted in a 6% and 17% reduction in microbial load reduction in summer, and winter season respectively. This minimal reduction suggests that the QUAT-based disinfectant used may be less effective under the environmental conditions typical of summer, such as higher temperatures and potentially lower humidity. The mean microbial count was 15.5, indicating a relatively stable microbial load before and after fogging (Table 6). The low SD reflects low variability in the microbial counts, suggesting that the microbial population was fairly consistent across samples and the SE indicates a small margin of error in the mean estimate, reinforcing the stability of

Table 1: Characteristic peak masses of *Pseudomonas stutzeri*; m/z values.

Strain code	SPB01PR	SPB02PR	SPB06PR	ASB01PR	ASB05PR	SPB01PO	SPB02PO	SPB06PO	ASB06PO	SPB05PO	ASB01PO	ASB02PO
m/z	3633	2208	2790	2754	2983	3635	2069	3077	2603	3478	2750	2750
	4124	3649	3311	3076	3227	3772	3775	3555	3612	3639	3477	3079
	4134	3772	3667	4132	4221	4125	4135	4133	4143	4112	4158	4126
	4144	4133	4132	4151	4387	4136	4394	4153	4400	4128	4392	4391
	4415	4415	4415	4415	4415	4415	4415	4415	4415	4415	4415	4415
	4434	4436	4443	4435	4432	4437	4439	4437	4436	4601	4439	4438
	4938	4941	4940	4532	4468	4446	4601	4939	4456	5082	4600	4456
	4996	4998	5078	4597	4486	5080	4943	4998	4939	5094	4633	4600
	5077	5079	5135	4938	4530	5090	4961	5079	4997	5644	4943	4633
	5659	5659	5659	5659	5659	5659	5665	5659	5659	5659	5659	5663
	5673	5704	5677	6155	5700	7113	5976	5973	5675	6091	5976	6091
	6155	5960	5958	7111	5966	7273	6446	6090	5693	6435	6161	6160
	7110	5973	5972	7269	6230	7295	6466	6141	5957	6957	6240	6239
	7269	6462	6010	7298	6455	7311	7274	6158	5972	6996	6436	6588

Table 2: Characteristic peak masses of 5 strains of *Bacillus* and 2 strains of *Paenibacillus*; m/z values.

			<i>Bacillus</i>			<i>Paenibacillus</i>	
Strain code	ASB06PR	SPB04PO	ASB06PO	SPB03PR	SPB07PRR	ASB05PO	ASB06PO
	2210	2164	2540	3071	2580	3064	3448
	2231	2567	2562	3706	2589	3091	3502
	4343	3192	4694	5228	3404	5094	4845
	5167	5167	5167	5247	3478	5156	5151
	5190	5212	5207	5362	3545	5176	5176
m/z	5206	5253	5226	6143	3576	5196	5395
	5225	5608	5245	7087	10054	6682	6522
	5531	5622	5813	7115	10411	6778	6633
	5796	5660	5833	7150	10455	7150	6649
	5817	5791	5871	7174	10492	7191	6741
	5832	6312	6398	7193		7580	6761
	5869	6326	6516	7212		7657	6903
	6262	6348	6534	7234		7708	6955
	6396	6385	6562	7508		7748	6992

the microbial counts during this season. On the other hand, a significant 50% reduction in microbial load was observed during the monsoon season. This substantial decrease suggests that the fogging process is particularly effective. The mean microbial count was 21.0, the highest among the seasons studied, indicating a higher initial microbial load before fogging and the SD shows high variability in microbial counts, suggesting fluctuating environmental conditions and microbial populations (Table 7).

In the Settle plate, the fogging process resulted in a reduction of 40% in summer, and 57% in microbial load during the winter season. This moderate reduction suggests that the QUAT-based

disinfectant used has a reasonable level of effectiveness under environmental conditions. The mean microbial count indicates a relatively high baseline microbial load before fogging. The SD reflects moderate variability in the microbial counts, suggesting some fluctuation in the microbial population across samples. The SE indicates a moderate margin of error in the mean estimate, reinforcing the variability observed during this season.

During monsoon season a substantial 90% reduction in microbial load was observed during the monsoon season. This significant decrease suggests that the fogging process is highly effective under the high humidity conditions of the monsoon season, which

may enhance the efficacy of the QUAT-based disinfectant. The mean microbial count was 17.5, lower than the summer season but still indicating a relatively high initial microbial load before fogging. The SD of 20.51 shows very high variability in microbial counts, suggesting considerable fluctuations in environmental conditions and microbial populations. The SE of 14.50 indicates a large margin of error in the mean estimate, which could be due to the inherent variability in environmental conditions during the monsoon.

DISCUSSION

This study highlights the application of MALDI-TOF MS as a rapid and reliable technique for identifying airborne microbial contaminants in pharmaceutical cleanrooms and underscores the significant impact of seasonal variation on microbial presence. MALDI-TOF MS is based on the measurement of microbial proteins that are generally well-conserved within a species. Consequently, it offers a more dependable method for identifying species at the level of specificity. The FDA has approved the MALDI Biotyper for identifying species groups, allowing for the analysis of 2,263 clinically significant species of Gram-negative bacteria. According to studies, The accuracy of MALDI-TOF MS in identifying isolates is remarkable, achieving 99.8% at the genus level and approximately 98.2% at the species level (Gato, E., *et al.*, 2022). On the other hand, for Gram-positive bacteria evaluations, 92.8% correct identification to the species level and 95.5% to the genus level (Teke, L., Barış, A., and Bayraktar, B.,

2021). Consequently, this facilitates the efficient and precise identification of a diverse array of microbial isolates under various culture conditions using MALDI-TOF MS technology.

Studies indicate that microbial load in clean rooms can vary with seasons where higher humidity and temperature, can contribute to increased microbial growth and dispersion. Seasonal variations necessitate adjustments in cleaning and disinfection protocols to maintain the required sterility levels in clean rooms (De Vecchi, F., and DeSantis, P. 2021). The results demonstrate a clear pattern: the monsoon season exhibited the highest pre-fogging microbial loads, likely due to elevated humidity and increased microbial dispersal from the surrounding environment. Fogging with QUAT-based disinfectants during this season resulted in the highest efficacy, with a 90% reduction in microbial load on settle plates. This enhanced effectiveness is possibly attributed to the stability and prolonged suspension of disinfectant droplets in humid air, leading to better surface coverage and contact with microbes. Moreover, the presence of diverse microbial populations during monsoon can be attributed to higher humidity levels, which are known to support microbial growth and dispersion (Egberongbe, *et al.*, 2021).

In contrast, the reduction in species diversity post-fogging in winter, where species like *Staphylococcus cohnii* were not detected after fogging, could be due to lower ambient temperatures and humidity levels that are less conducive to microbial proliferation (Siebielec, S., *et al.*, 2020). The appearance of fungi in air

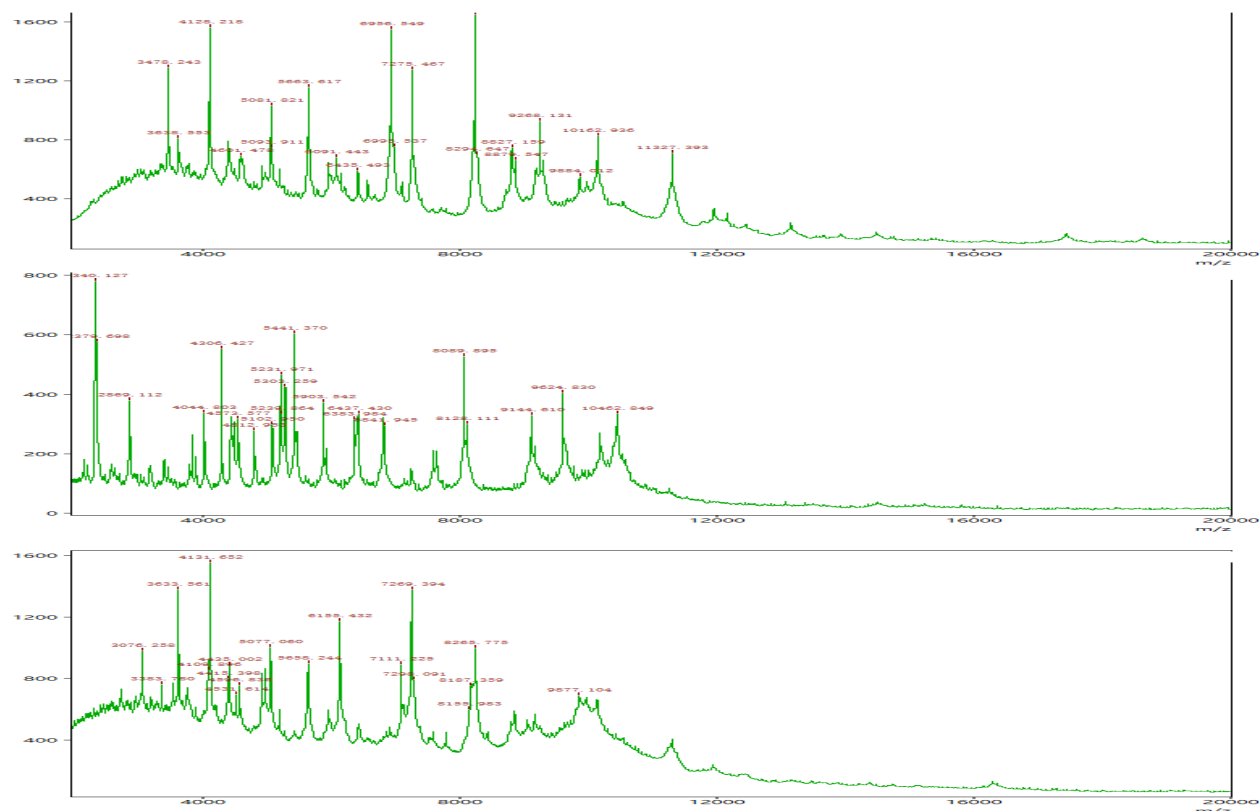


Figure 1: Spectral fingerprints obtained from various strains of *Pseudomonas stutzeri* sing HCCA matrix.

Table 3: Microbial analysis pre and post fogging in summer season.

Identified organism	Settle plate		Air sampling	
	Pre- fogging	Post-fogging	Pre- fogging	Post-fogging
<i>Pseudomonas stutzeri</i>	9.325	9.101	9.088	9.191
<i>Staphylococcus hominis</i>	9.588	-	-	9.208
<i>Staphylococcus haemolyticus</i>	-	-	9.076	-
<i>Staphylococcus warneri</i>	-	9.067	-	-
<i>Brachybacterium paraconglomeratum</i>	9.076	-	-	-
<i>Kocuria rhizophila</i>	-	-	9.262	-
<i>Kocuria marina</i>	-	9.502	-	-
<i>Micrococcus luteus</i>	-	-	-	9.243
<i>Paenibacillus glucanolyticus</i>	6.796	-	6.809	-
<i>Bacillus oceanisediminis</i>	-	9.13	-	-
<i>Bacillus megaterium</i>	-	-	9.503	9.5

Table 4: Microbial analysis pre and post fogging in Monsoon season.

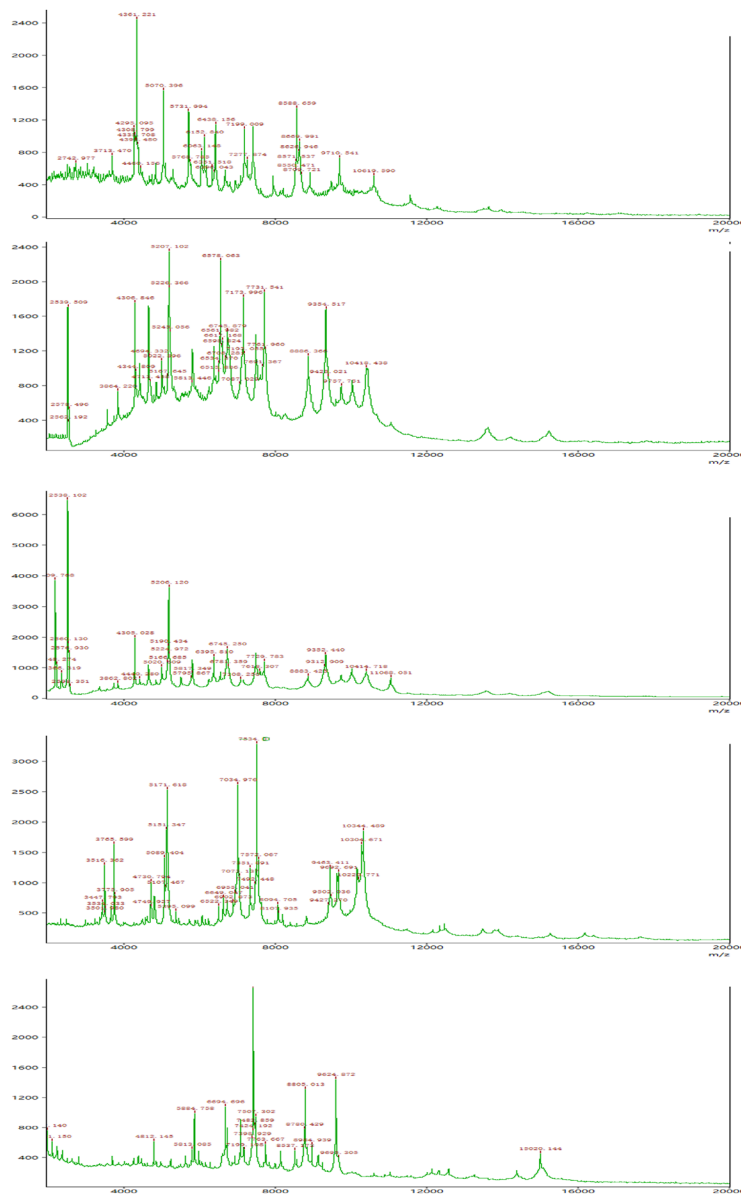
Identified organism	Settle plate		Air sampling	
	Pre- fogging	Post-fogging	Pre- fogging	Post-fogging
<i>Pseudomonas stutzeri</i>	9.069	9.042	9.226	9.165
<i>Staphylococcus hominis</i>	9.588	-	9.047	-
<i>Staphylococcus haemolyticus</i>	9.077	-	-	-
<i>Staphylococcus warneri</i>	-	9.108	-	-
<i>Staphylococcus saprophyticus</i>	9.031	-	-	6.034
<i>Bacillus altitudinis</i>	9.17	-	-	-
<i>Staphylococcus aureus</i>	-	-	9.093	-
<i>Staphylococcus arlettae</i>	-	6.652	9.33	-
<i>Curtobacterium sp.</i>	7.756	-	-	-
<i>Kodamaea ohmeri</i>	-	-	9.108	-
<i>Staphylococcus capitis</i>	-	-	-	9.00
<i>Moraxella osloensis</i>	-	-	-	9.064

Table 5: Microbial analysis pre and post fogging in winter season.

Identified organism	Settle plate		Air sampling	
	Pre- fogging	Post-fogging	Pre- fogging	Post-fogging
<i>Pseudomonas stutzeri</i>	9.066	-	9.231	-
<i>Staphylococcus hominis</i>	-	9.095	-	-
<i>Staphylococcus cohnii</i>	9.101	-	-	-
<i>Staphylococcus warneri</i>	9.002	-	-	9.325
<i>Staphylococcus sciuri</i>	-	-	9.026	-
<i>Staphylococcus epidermidis</i>	-	-	-	9.095
<i>Kocuria varians</i>	-	9.502	-	-
<i>Janibacter indicus</i>	-	-	8.281	8.452
<i>Bacillus megaterium</i>	-	-	9.244	-
<i>Staphylococcus capitis</i>	-	-	9.058	-

Table 6: Air sampling analysis pre and post-fogging.

Seasons	Percentage Reduction	Mean	Standard deviation	(CFU/m ³)
Summer	6	15.5	0.707	<50
Monsoon	50	21.0	9.899	<50
Winter	17	15.5	2.121	<50

**Figure 2:** Spectral fingerprints obtained from various strains of *Bacillus* and *Paenibacillus* using HCCA matrix.

samples post-fumigation during the winter season is particularly noteworthy. The detected fungi, can pose significant challenges in clean room environments due to their spores being highly resistant to standard disinfection methods (Andersen *et al.*, 2019). The analysis reveals that the fogging process is most effective during the monsoon season, achieving a 50% reduction in microbial load. This increased effectiveness may be due to the higher humidity levels prevalent during the monsoon, which

could enhance the activity of the QUAT-based disinfectant. Conversely, the summer season exhibited the least effectiveness, with only 6% reduction in microbial load. This suggests that higher temperatures and potentially lower humidity may reduce the efficacy of the disinfectant.

During winter season there is moderate reduction of 17%, indicating some effectiveness of the disinfectant in cooler conditions. In contrast, the fogging process was less effective

Table 7: Settle plate analysis pre- and post-fogging.

Season	Percentage Reduction	Mean	Standard deviation	(CFU/m ³)
Summer	40	21.5	7.78	<50
Monsoon	90	17.5	20.51	<50
Winter	57	10.0	5.66	<50

during the summer season, achieving only a 40% reduction in microbial load. The high ambient temperature and lower humidity may contribute to the rapid evaporation of disinfectant droplets, thereby reducing contact time with microbial cells and diminishing biocidal activity (Oliveira *et al.*, 2020). Furthermore, microbial cells exposed to heat stress can enter a physiological state where they form biofilms or upregulate stress-related genes, increasing resistance to chemical disinfectants (Bridier *et al.*, 2015). The winter season showed moderate fogging efficacy (57%), which may be attributed to more stable ambient conditions, though the reduced humidity likely limits droplet persistence and surface adherence, thus impacting the uniformity of disinfection (Masotti *et al.*, 2019).

The use of MALDI-TOF MS enabled precise identification of microbial species, including both recurring and seasonal isolates. Notably, *Pseudomonas stutzeri* was found across all seasons, while species such as *Moraxella osloensis* and *Brachybacterium paraconglomeratum* appeared post-fogging, suggesting that disinfection may suppress dominant flora and create ecological niches for less common microbes. These observations highlight the need to evaluate not just microbial count reduction, but also species-level shifts post-disinfection. The seasonal variability in microbial trends emphasizes the necessity for dynamic and data-driven contamination control strategies. Disinfection protocols may need seasonal adjustment, such as increasing fogging frequency or alternating biocides during summer months. Moreover, the emergence of new species post-fogging supports the need for regular microbial profiling to monitor potential resistance or recolonization patterns.

While the study offers valuable insights, it is limited by the exclusion of fungal profiling through MALDI-TOF MS and a focus on a single disinfection method. Future studies should include comparative efficacy of multiple disinfectants and consider real-time environmental condition logging to correlate with microbial trends.

CONCLUSION

The study aimed at rapid identification of microbial strains by MALDI-TOF-MS which is effective and well suited for routine microbial identification in any laboratory. It is one of the fastest techniques to accurately identify microorganisms grown in cleanroom. The study reveals significant seasonal variability in microbial contamination and disinfection outcomes, with monsoon showing the highest microbial load and best disinfection

response, while summer posed challenges to QUAT-based fogging efficacy. The study advocates for seasonally adaptive disinfection protocols, continuous monitoring, and broader microbial spectrum coverage to ensure consistent cleanroom hygiene. These findings are crucial for optimizing clean room maintenance and ensuring microbial control in pharmaceutical testing labs. These strategies are essential to support robust contamination control in pharmaceutical testing environments and maintain compliance with regulatory standards.

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ABBREVIATIONS

HCCA: α -Cyano-4-Hydroxycinnamic Acid; **QUAT:** Quaternary Ammonium Compound; **SCDA:** Soybean-Casein Digest Agar; **PDAC:** Potato Dextrose Agar with Chloramphenicol; **CFU:** Colony Forming Units; **USP:** United States Pharmacopeia; **ISO:** International Organization for Standardization; **EMA:** European Medicines Agency; **GMP:** Good Manufacturing Practice; **MALDI-TOF MS:** Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry; **NABL:** National Accreditation Board for Testing and Calibration Laboratories; **m/z ratio:** Mass-to-Charge Ratio.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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