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Evaluating the Efficacy of QAT-Based Disinfectants on Microbial Environment in Pharmaceutical Industries, India

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Abstract
Pharmaceuticals industries strictly follow the regulatory guidelines by various bodies like USFDA, WHO, GMP etc. QAT-based disinfectants are widely used in pharmaceutical industries and are generally effective at reducing microbial loads. This study investigates the efficacy of Quaternary Ammonium Compound (QAT)-based disinfectants in controlling microbial contamination in pharmaceutical cleanrooms across three facilities in India-Himachal Pradesh, Maharashtra, and Sikkim over 2022 and 2023. The research focuses on seasonal variations in microbial load and the challenges posed by specific bacterial and fungal contaminants. The results highlight increase in microbial counts in the year 2023 in all facilities, with Staphylococcus sp, Kocuria sp., and Micrococcus being the dominant bacteria, particularly in areas of higher human traffic and equipment use. The Maharashtra facility presented a more complex contamination profile, with the presence of both bacterial and fungal species, including Aspergillus and Stachybotrys, which are known to thrive in humid conditions and form resilient biofilms. The Sikkim facility revealed a diverse microbial spectrum, including Gram-negative bacteria such as Pseudomonas stutzeri and Enterobacter aerogenes, which pose heightened risks due to their ability to persist in damp environments and resist disinfection. The study underscores the importance of regular personnel training to ensure correct handling of disinfectants. It recommends a rotational disinfection strategy combining QATs with other biocidal agents to address a wide variety of microbial bioload and maintain pharmaceutical cleanrooms. The findings provide crucial insights for optimizing disinfection practices, ensuring compliance with cleanroom standards, and safeguarding product quality and safety.
Key words: Quaternary Ammonium Compounds, pharmaceuticals, microbial environment, USFDA, microbial contamination.

INTRODUCTION

Clean room conditions are paramount, particularly in aseptic production and non sterile production environments, where the presence of microorganisms can lead to the spoilage of products, increased risk of infections in patients, and costly product recalls. Maintaining a high level of microbial control is a critical requirement for pharmaceutical manufacturing facilities, to avoid any contamination that can have serious consequences for product quality and product safety (Willison-Parry *et al*., 2019). There are regulatory bodies like the U.S. Food and Drug Administration (USFDA), European Union (EU), World Health Organization (WHO) etc. that emphasize on the need for controlled environments, especially in cleanrooms, to limit microbial contamination. The cleanrooms are classified into different grades based on the level of permissible airborne microbial contamination, with grade A being the strictest going down to grade D and ISO 8 classifications. Failures in maintaining microbial control can result in serious consequences. In 2012, FDA and CDC reported fungal contamination of *Aspergillus* sp., *Cladosporium, Alternaria, Exophiala,* and *Penicillium* species from unopened vials of betamethasone and triamcinolone solutions and was recalled from the NECC. This lead to death of 60 patients and more than 100 people were in critical conditions. Similarly, FDA recalled several drugs during 2020-2021 due to fungal contamination (Ahmed, M. A. E. G. E. S., Abbas, H. S., & Kotakonda, M. 2024). Hence it becomes really important to maintain the required levels of cleanroom in pharmaceutical facilities, and to achieve it, one of the most important work is implementation of rigorous cleaning and disinfection protocols as per USP <1072>.

Disinfectants play a critical role in mitigating microbial contamination in pharmaceutical environments. Various classes of disinfectants are used, each with specific properties and spectrum of activity. These include alcohols, phenolics, chlorine-based compounds, peracetic acid, and Quaternary Ammonium

Compounds (QATs). Among them, QATs are extensively used in pharmaceutical facilities because they are effective against a broad range of microorganisms, including gram-positive and gram-negative bacteria, fungi, and some viruses. Their mechanism of action involves disrupting the cell membrane of microorganisms, leading to cell lysis and death (Ciumac, D., et al., 2019). Quaternary ammonium compounds are a class of synthetic antimicrobial agents that have been widely adopted as active ingredients in disinfectants used across various industries, including pharmaceuticals (Mohapatra et al., 2022) and have been used for over a century. Their chemical structure, which includes a nitrogen atom surrounded by alkyl or aryl groups, gives them their antimicrobial properties. When applied to a surface, QATs can adsorb onto microbial cell membranes and disrupt the phospholipid bilayer, leading to cell death. Their efficacy is enhanced when combined with detergents, making them ideal for surface cleaning in pharmaceutical cleanrooms. Additionally, QATs are stable, non-corrosive, and generally non-toxic, making them suitable for frequent use on various surfaces, including metals, plastics, and glass (DeLeo et al., 2020). The widespread use of QAT-based disinfectants has been further exacerbated during the COVID-19 pandemic, as these compounds have demonstrated efficacy against a broad spectrum of pathogens, including SARS-CoV-2 (Dewey et al., 2021; Mohapatra et al., 2022; Frantz, 2023). However, Kampf, G. (2018) have highlighted concerns about microbial resistance to QATs when used repeatedly over long periods. The QATs remain effective as part of a rotational disinfection strategy, exclusive reliance on QATs could lead to the development of resistant strains of microorganisms. Therefore, microbes have developed resistance mechanisms to QAC-based disinfectants, presenting a potential challenge to their long-term efficacy (Kim et al., 2018). Studies have shown that the formulation and application of QAC-based disinfectants can have a significant impact on their efficacy against target organisms (Martino et al., 2021; Lu, Z., et al., 2024). Factors such as the concentration of the active ingredient, contact time, and the presence of organic matter can all affect the disinfectant's ability to effectively eliminate microbial bioload (Factors Affecting the Efficacy of Disinfection and Sterilization, 2019). Additionally, the emergence of QAC-resistant strains of bacteria highlights the need for continuous monitoring and evaluation of the efficacy of these disinfectants. Proper training of personnel in the correct application and handling of QAC-based disinfectants is also crucial to ensure their optimal performance and prevent the development of further resistance (USP 1116). The efficacy of QAT-based disinfectants in microbial monitoring within pharmaceutical facilities has been a subject of ongoing research. Studies have shown that these disinfectants are generally effective against a wide range of bacteria and viruses, including those commonly found in pharmaceutical manufacturing environments. In India, the use of QAT-based disinfectants has become a standard practice in pharmaceutical manufacturing, especially in facilities producing sterile products such as injectables, ophthalmics, and biologics. Indian regulatory bodies, including the Central Drugs Standard Control Organization (CDSCO), mandate regular environmental monitoring and disinfection protocols to maintain compliance with Good Manufacturing Practices (GMP) (Uppal et al., 2020; Sharma et al., 2023). However, despite widespread adoption, there is limited published research on the specific challenges faced by Indian pharmaceutical facilities, such as seasonal variability in microbial loads and its impact on disinfection efficacy. Moreover in the India high temperatures and humidity levels, especially during the monsoon season, can promote the growth of bacteria and fungi in cleanroom environments (Chawla, H., et al., 2023). Therefore, effective disinfection strategies are necessary to ensure that microbial contamination remains under control, regardless of seasonal variations.

This study aims to fill that gap by evaluating the efficacy of QAT-based disinfectants across pharmaceutical facilities in India, focusing on microbial contamination. By analyzing data collected during different seasons and from various facility types, this research seeks to provide a deeper understanding of the effectiveness of QATs in real-world conditions and offer recommendations for optimizing disinfection practices.

METHODOLOGY

This study aimed to evaluate the efficacy of Quaternary Ammonium Compound (QAT)-based disinfectants in reducing microbial loads in pharmaceutical manufacturing facilities across different

regions of India for two years. The methodology involved selecting suitable study sites, implementing disinfection protocols, and conducting microbial monitoring through air and surface sampling methods.

Study Sites

Three pharmaceutical manufacturing facilities were selected for this study, representing various geographical regions and climatic conditions in India. The facilities varied in size and production capacities, including sterile and non-sterile operations. These facilities were chosen based on their adherence to Good Manufacturing Practices (GMP) and their consistent use of QAT-based disinfectants as part of their cleaning protocols.

*Cleaning with QAT-Based Disinfectant:*The disinfectant evaluated in this study is a commercially available QAT-based solution commonly used in the pharmaceutical industry. It contains a blend of Quaternary Ammonium Compounds formulated for use on non-porous surfaces. The concentration of the disinfectant used was based on the manufacturer's recommendations, typically in the ratio 1:15 (v/v). The diluted disinfectant solution was used for surface sanitisation of the area. After sanitisation, the room was left undisturbed for an additional 30 minutes, to ensure effective sanitisation. Microbial samples were collected periodically at different locations for two years. This method provided a quantitative assessment of airborne microbial contamination throughout the year.

*Microbial Monitoring Methods:*Microbial monitoring was conducted using two primary methods: the settle plate method and the air sampling method. These methods were chosen to assess the microbial load in the clean room air.

*Settle Plate Method:*Settle plate method (Pasquarella, C. *et al.,* 2000) was used with few modifications, involving exposing sterile agar plates. Soyabean Casein Digest Agar (SCDA) plates were exposed for four hours in critical areas of sterile manufacturing facility and for one hour in non-sterile production area to allow the settling of airborne microbes. After exposure, the plates were closed and incubated in a microbiological incubator at 20-25°C for 72 hours followed by a second incubation at 30-35°C for 48 hours, to detect both bacterial and fungal growth. Colony Forming Units (CFUs) were counted manually and recorded as CFU per plate, representing the microbial load present in the cleanroom environment.

*Air Sampling Method:*Air sampling method (Pitt, J.I. and Hocking, A.D. 1999) was used with few modifications, by positioning air sampler at approximately one meter above the floor to simulate typical human exposure levels. Air samples were collected from multiple locations within each cleanroom, including areas close to return risers of air handling units and critical work zones. Soyabean Casein Digest Agar (SCDA) plates were placed inside the impaction type air sampler, and air samples were drawn for a duration of 10 minutes. The samples were incubated at 20-25°C for 72 hours and then at 30-35°C for another 48 hours. CFUs were counted, and the results were expressed as CFU per cubic meter of air.

Microbial identification

Biochemical analysis: Microbial identification was performed using BioMérieux's API identification system for manual identification of Gram-positive and Gram-negative bacteria, to the species level. The test kits contains strips with 20 compartments containing dehydrated biochemical reagents. Pure culture was prepared from the test plate, a single isolated colony from a pure culture was suspended in sterile distilled water. The API20E Biochemical Test Strip was utilized, with each compartment filled to capacity containing the bacterial suspension. Sterile oil was added to specified compartments (ADH, LDC, ODC, H_2S, and URE). The loaded API Test strip was placed in a tray with drops of water, sealed, and labeled with identification informations and incubated at 37°C for 18- 24 hours. After inoculating and incubating the API test strips, results from the biochemical tests were recorded and entered into the APIWEB platform. It compared the test patterns with its extensive database, providing potential identifications along with probability scores. The highest-scoring matches were documented as the identified microbial species, ensuring accurate and efficient identification.

*VITEK Automated Identification System:*The VITEK system is an automated platform that offers rapid and accurate identification of a wide range of microorganisms, including bacteria and fungi. A single colony of the isolated microorganism was suspended in sterile saline solution to match a specified turbidity standard (0.5 McFarland) using a densitometer. The prepared suspension was then loaded into a VITEK identification card, which contained micro-wells with various biochemical substrates. Different cards, such as VITEK GN (for Gram-negative bacteria) and VITEK GP (for Gram-positive bacteria), were used

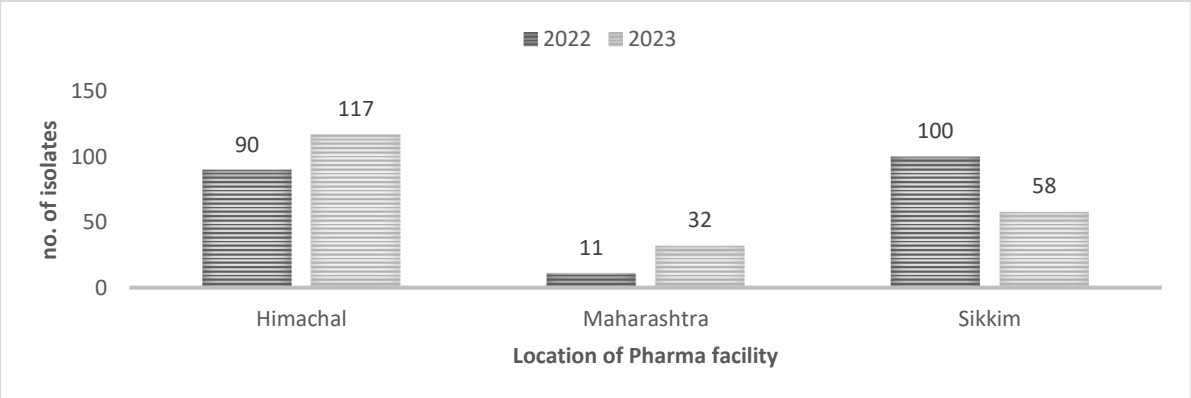
depending on the type of microorganism. The inoculated card was placed into the VITEK machine, where automated reading, incubation, and interpretation of results were conducted. The system used advanced algorithms and a large database to match the biochemical profile with known microorganisms. The VITEK system provided species-level identification results within 6-18 hours, displaying the probable microorganism and a confidence level for the identification.
Either of the two identification systems was used, as results obtained from either system is comparable as both identification systems are from same manufacturer, Biomeriux.

RESULT

The study aimed to assess the efficacy of QAT-based disinfectants in reducing microbial contamination in cleanroom environments of pharmaceutical facilities located in Himachal Pradesh, Sikkim, and Maharashtra during the years 2022 and 2023. Microbial samples were collected from multiple critical areas, including Near Autoclave, Near Riser, Center of the Room, Near Shifter Machine, Inside the Garment Cubicle, Near Entry-Exit Door, Under Left and Right Guards of the Filling Machine, Under AHU Return Room, Under LAF (Laminar Airflow), Inside the Garment Cubicle, and Under Supply etc. The results provided insights into the reduction of microbial loads across different facilities and seasons.
Table 1: Average microbial identification from all three pharmaceutical facility from 2022-2023

Himachal Pradesh				Maharashtra			Sikkim			
Month	Jan-April	May-August	Sept - Dec	Jan-April	May-August	Sept-Dec	Jan-April	May-August	Sept-Dec	
2022	23	20	47	3	2	6	25	64	11	
2023	82	20	15	4	11	17	23	4	31	

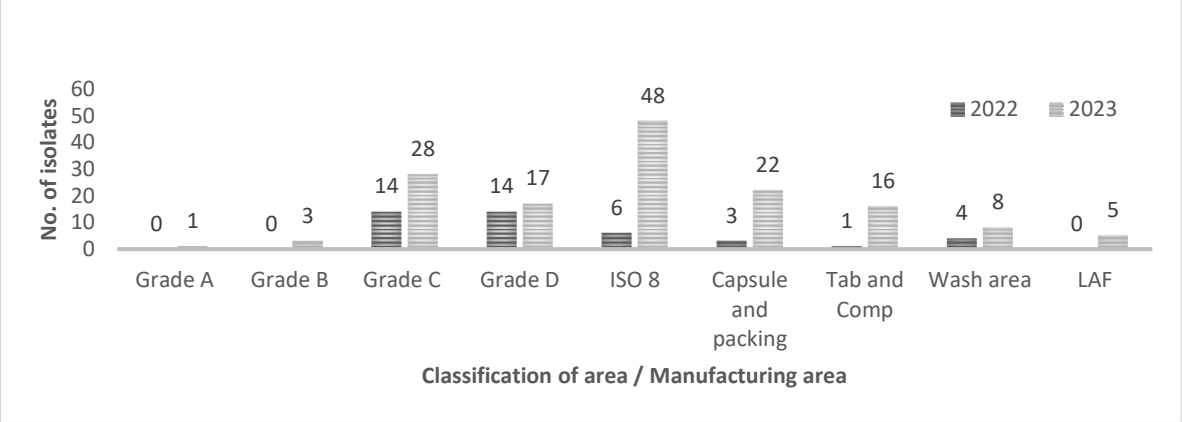
The findings revealed notable seasonal fluctuations in microbial diversity across the three pharmaceutical facilities, with distinct trends observed between 2022 and 2023. In the Sikkim facility during 2022, the highest microbial diversity were recorded during the monsoon season, followed by summer, with the lowest levels observed in the winter months. However, this trend shifted in 2023, as illustrated in Table 1, reflecting a dynamic microbial environment.
In contrast, both the Himachal Pradesh and Maharashtra facilities exhibited a different pattern in 2022, with peak microbial counts occurring during the winter season, followed by summer and monsoon. This unexpected spike in winter microbial loads suggests a potential influence of operational or environmental factors unique to these sites. Similarly, the Maharashtra facility experienced an overall increase in microbial loads in 2023, with winter continuing to register the highest contamination levels. The year-on-year increase in microbial exposure across all facilities, as depicted in Figure 1, underscores a general rise in contamination levels in 2023 compared to 2022.
Figure 1: comparative analysis of microflora from three pharmaceutical facilities from the year 2022-2023



The microbial analysis conducted in Himachal Pradesh facility provided a detailed characterization of the microbial flora across various cleanroom areas. The results revealed a predominant presence of Gram-positive, cocci-shaped bacteria, with a significant dominance of *Staphylococcus* species (*Staphylococcus*

epidermidis, Staphylococcus hominis, Staphylococcus warneri, Staphylococcus cohnii, Staphylococcus haemolyticus, and Staphylococcus lentus). Additionally, the microbial profile indicated that the presence of *Kocuria* species was notable, with *Kocuria kristinae, Kocuria varians,* and *Kocuria rosea* being frequently isolated. Comparative analysis of microbial loads between 2022 and 2023 showed a clear increase in contamination levels across various cleanroom. Specifically, areas associated with capsule and tablet packing, compounding, washing, and laminar air flow (LAF), exhibited much lower microbial counts in 2022 compared to the heightened levels recorded in 2023. A significant surge in microbial load was observed in the ISO 1SO 8 area during 2023, which registered the highest contamination levels, followed closely by the Grade C areas.

Figure 2: Comparative analysis of microbial load from various areas from Himachal Pradesh facility



The microbial analysis conducted at the Maharashtra facility across various cleanroom grades revealed a diverse microbial ecosystem, characterized by the presence of Gram-positive cocci, rod-shaped bacteria, and a notable occurrence of spore-forming fungi and molds. The microbial community was comprised of species from several bacterial genera, with *Bacillus megaterium* emerging as a significant rod-shaped bacterium, known for its robust spore-forming capabilities. Among the cocci-shaped bacteria, *Staphylococcus* species were well-represented, including *Staphylococcus xylosus, Staphylococcus lentus, Staphylococcus cohnii,* and *Staphylococcus epidermidis*. Additionally, the presence of *Kocuria kristinae* and *Kocuria rhizophila* highlighted the diversity within the gram-positive microbial population, both species being known for their environmental resilience. *Roseomonas mucosa*, a pink-pigmented bacterium typically found in moist environments, was also isolated, underscoring the facility's susceptibility to moisture-driven microbial contamination. *Micrococcus* species, including *Micrococcus lylae* and *Micrococcus luteus*, were identified as well, contributing to the bacterial diversity in the facility's cleanroom environment. Equally noteworthy was the detection of spore-forming fungi, with *Aspergillus fumigatus* and *Aspergillus terreus* and mold like *Stachybotrys* species, being consistently observed in both 2022 and 2023 (Table 2).

Table 2: Microbial analyses from Maharashtra from 2022-2023

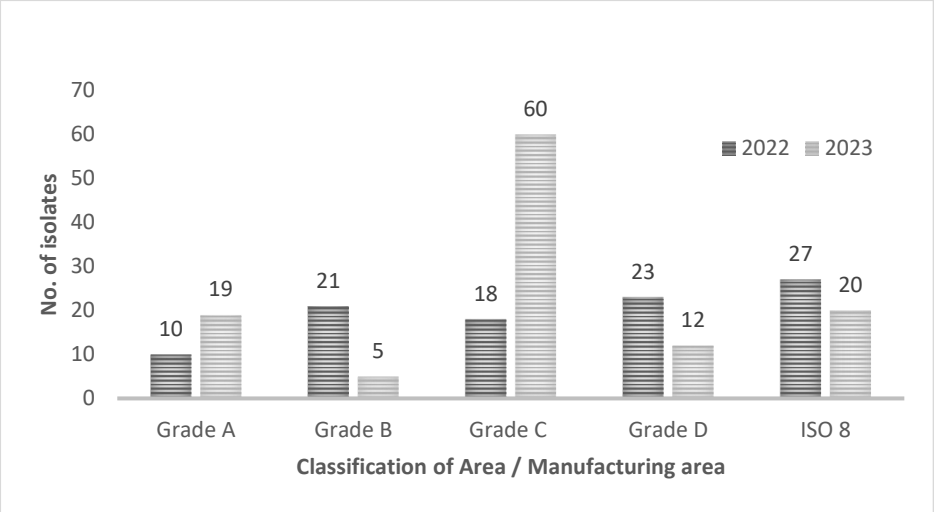
Ref Sample description	Nature	Cell shape	Name of Isolate
Infront of ECF112	Fungi	NA	*Aspergillus fumigates*
Infront of ECF112	Fungi	NA	*Stachybotrys sp.*
Infront of ECF112	Fungi	NA	*Aspergillus terreus*
Centre of the room	Gram Positive	Cocci	*Micrococcus luteus*
Centre of the room	Gram Positive	Cocci	*Staphylococcus lentus*
Centre of the room.	Gram Positive	Rods	*Bacillus megaterium*
Air lock ESPD-101	Gram Positive	Cocci	*Staphylococcus lentus*
Air lock ESPD-101	Gram Positive	Cocci	*Micrococcus lylae*
Near ER-109	Gram Positive	Rods	*Bacillus megaterium*

Centre of the room	Gram Positive	Rods	*Bacillus megaterium*
Centre of the room.	Gram Positive	Rods	*Bacillus megaterium*
Near ECF-102	Gram Positive	Cocci	*Micrococcus spp*
Near ECF-102	Fungi	NA	*Aspergillus fumigates*
Centre of the room.	Gram Positive	Cocci	*Staphylococcus xylosus*
After cross over bench.	Gram Positive	Cocci	*Micrococcus spp*
Near cyclone separator.	Gram Positive	Rods	*Bacillus megaterium*
After crossover bench.	Gram Positive	Cocci	*Staphylococcus lentus*
After crossover bench.	Gram Positive	Cocci	*Staphylococcus xylosus*
Centre of the room	Fungi	NA	*Aspergillus fumigates*
Centre of airlock.	Gram Positive	Cocci	*Kocuria kristinae*
Air lock ESPD-101	Gram Positive	Cocci	*Staphylococcus lentus*
Centre of the room	Fungi	NA	*Aspergillus fumigates*
Front of material entry door.	Fungi	NA	*Aspergillus fumigates*
After cross over bench.	Gram Positive	Cocci	*Staphylococcus cohnii*
After cross over bench.	Gram Positive	Cocci	*Kocuria rhizophila*
Ref Sample description	Nature	Cell shape	*Name of Isolate*
Centre of the room.	Gram Negative	Rods	*Pseudomonas stutzeri*
Near pendant.	Gram Negative	NA	*Roseomonas mucosa*
After cross over bench.	Gram Positive	Cocci	*Micrococcus luteus*
After cross over bench.	Fungi	NA	*Stachybotrys species*
Centre of the room.	Fungi	NA	*Aspergillus fumigates*
Centre of the room.	Fungi	NA	*Aspergillus fumigates*
Centre of the room.	Fungi	NA	*Aspergillus fumigates*
Centre of the room.	Gram Positive	Cocci	*Staphylococcus xylosus*
Centre of the room.	Gram Positive	Cocci	*Micrococcus lylae*
Front of material entry door.	Fungi	NA	*Stachybotrys species*
Near riser after crossover bench.	Gram Positive	Cocci	*Staphylococcus haemolyticus*
Near riser after crossover bench.	Gram Positive	Cocci	*Staphylococcus epidermidis*
After cross over bench.	Gram Positive	Rods	*Brevibacterium epidermidis*
After cross over bench.	Gram Positive	Cocci	*Staphylococcus epidermidis*

The microbial profiling conducted in the Sikkim facility revealed a diverse array of both Gram-positive and Gram-negative bacteria, encompassing both cocci and rod-shaped forms. This diverse microbial community predominantly included *Staphylococcus* species, with *Staphylococcus cohnii, Staphylococcus hominis, Staphylococcus epidermidis,* and *Staphylococcus capitis* emerging as the most prevalent. In addition to

Staphylococcus, the microbial spectrum included *Micrococcus* species and *Kocuria kristinae* and *Kocuria varians*, both of which are resilient environmental contaminants often found in cleanrooms. The presence of these Gram-positive bacteria highlights the difficulty in completely eradicating microbial populations even with rigorous disinfection protocols. Of particular concern was the identification of several Gram-negative species, including *Brevundimonas vesicularis, Pseudomonas stutzeri, Rhizobium radiobacter,* and *Enterobacter aerogenes*. In 2023, the Grade C cleanroom at the Sikkim facility exhibited the highest microbial load, followed by ISO classified area and Grade A. In contrast, during 2022, the greatest microbial contamination was observed in Grade ISO, followed by Grade D and Grade B.

Figure 3: Comparative analysis of microbial load from various areas from Sikkim facility



Data Analysis

Data collected from the settle plate and air sampling methods were analysed to determine the variation in microbial load after disinfection. Statistical analysis of certain predominant flora of the microbial load across seasons and facilities were evaluated.

Table 3: Statistical analyses of predominant flora

Name of the Micro organism	Himachal	Maharashtra	Sikkim	Chi-Square	P-Value	Conclusion
Aspergillus	2(1%)	9(20.9%)	9(5.7%)	30.7896	0.0000	Significant
Bacillus	7(3.4%)	5(11.6%)	4(2.5%)	FishersExact-P (0.0302)		Significant
Brevundimonas	1(0.5%)	1(2.3%)	10(6.3%)	10.791	0.00454	Significant
Kocuria	19(9.2%)	3(7%)	18(11.4%)	Fishers Exact P(0.604)		Not Significant
Micrococcus	2(1%)	5(11.6%)	1(0.6%)	23.42	0.0000	Significant
Staphylococcus	171(82.6%)	18(41.9%)	94(59.5%)	39.64	0.0000	Significant

The table 3 presented above highlights the growth rates of various microorganisms—Aspergillus, Bacillus, and Micrococcus—in three distinct regions of India: Maharashtra, Himachal Pradesh, and Sikkim. The data, supported by the significant P-values obtained from Chi-square and Fisher's Exact tests, indicate a notably higher growth rate for these three microorganisms in Maharashtra compared to Himachal Pradesh and Sikkim. This suggests that the environmental conditions in Maharashtra are more conducive to the proliferation of Aspergillus, Bacillus, and Micrococcus. Moreover, the analysis reveals that the microorganism Brevundimonas exhibits a significantly higher growth rate in Sikkim in comparison to both Himachal Pradesh and Maharashtra. These findings imply that Sikkim offers a more favorable environment for the growth of Brevundimonas. In contrast, the growth rate of Staphylococcus is considerably higher in Himachal Pradesh when compared to Sikkim and Maharashtra, with a confidence level of 95%. This suggests that Himachal Pradesh provides the optimal conditions for the growth of Staphylococcus. Finally, the study shows no significant difference in the growth rates of the microorganism Kocuria among Maharashtra, Himachal Pradesh, and Sikkim. This is supported by the insignificant P-value (0.604) obtained from Fisher's Exact test, indicating that the growth rates of Kocuria are relatively consistent across all three regions. By highlighting these variations, the table underscores

the unique environmental and geographical influences on microorganism growth in different regions of India.

DISCUSSION

The present study highlights the critical importance of continuous assessment and optimization of Quaternary Ammonium Compound (QAT)-based disinfectants for microbial control in pharmaceutical cleanrooms. While QATs are widely recognized for their potent antimicrobial properties, emerging microbial resistance, environmental influences, and potential health implications demand a re-evaluation of their efficacy and safe use.The data from this study, particularly from 2023, suggests that shifts in environmental and operational conditions contributed to increased microbial challenges across all facilities. This reinforces the necessity of adaptive disinfection strategies that consider both seasonal variations and the specific microbial risks inherent to each facility. Across the study sites, *Staphylococcus* species were prevalent, a finding that aligns with their well-documented association with human skin and their ability to survive in cleanroom environments due to frequent personnel movement. The presence of these bacteria in high-traffic areas poses a significant challenge to maintaining the stringent microbial control required in pharmaceutical settings. As noted by Oruko et al. (2019) and Amulioto et al. (2021), *Staphylococcus* species have the ability to spread through contact and withstand standard cleaning protocols, further complicating efforts to ensure sterility. Similarly, *Kocuria* species, though typically less pathogenic, were found in substantial quantities, adding to the microbial burden. These organisms, like *Micrococcus* species, which were also detected, pose contamination risks, especially in environments that demand high levels of cleanliness (Understanding the Risks of Microbiological Contamination in Pharmaceutical Cleanrooms, 2023).The findings from the Maharashtra facility underscore the complexity of maintaining microbial control in environments where bacterial, fungal, and mold contamination coexist. The consistent detection of *Stachybotrys* and *Aspergillus* species, both of which thrive in damp, humid conditions, emphasizes the difficulties of regulating microbial populations in such facilities, particularly in regions prone to high humidity or temperature fluctuations. As discussed by Belizario et al. (2021) and Visconti et al. (2021), these fungi are not only resilient but also capable of forming biofilms and spore structures that are highly resistant to conventional disinfection methods. This persistence across multiple years suggests a critical need for pharmaceutical facilities to strengthen their disinfection protocols to specifically target such spore-forming organisms. The failure to adequately address these contaminants could result in compromised sterility, posing risks to both product integrity and patient safety (Kohli, 2019; Willison-Parry et al., 2019).Another key observation was the presence of *Pseudomonas stutzeri* and *Enterobacter aerogenes* in the Sikkim facility, two Gram-negative bacteria that present heightened contamination risks due to their ability to persist in damp environments and form biofilms. Pseudomonas stutzeri, in particular, is well-known for its opportunistic pathogenicity and its capacity to resist disinfection, making it a formidable challenge in pharmaceutical cleanrooms (Ghorpade et al., 2019; Li et al., 2023). Likewise, *Enterobacter aerogenes*, a member of the *Enterobacteriaceae* family, poses significant risks in environments where humidity is high, as it can spread rapidly and develop resistance to conventional disinfection practices. These findings are indicative of the diverse microbial threats that pharmaceutical cleanrooms face, where both Gram-positive and Gram-negative bacteria contribute to an intricate and dynamic contamination landscape (Selim et al., 2020; Amulioto et al., 2021).

The study also emphasized the importance of personnel training in the proper handling and application of QAT-based disinfectants. Ensuring that cleaning staff are adequately educated on the correct concentration, contact time, and application techniques is crucial for maintaining the efficacy of these compounds and preventing microbial resistance. As noted by DeLeo et al. (2020) and Arnold et al. (2023), empowering staff with a deep understanding of the mechanisms of action for QATs, as well as the risks associated with improper usage, is essential for optimizing disinfection outcomes. Ongoing education, coupled with regular performance assessments and refresher courses, will ensure that personnel are equipped to meet the evolving challenges posed by microbial contamination in pharmaceutical settings.

CONCLUSION

The findings from this study underscore the importance of continuous evaluation and monitoring of the efficacy of QAT-based disinfectants in pharmaceutical cleanroom environments. While these disinfectants have been widely adopted due to their potent antimicrobial properties, the emergence of resistant microbial strains and potential environmental and health impacts necessitate a careful re-evaluation of their use. The data presented in the discussion section highlights the complexity of microbial contamination in these controlled environments, where various bacterial and fungal species, including those associated with damp conditions and the ability to form resilient biofilms, persist despite disinfection efforts. This emphasizes the need for an enhanced and adaptive approach to disinfection, with a focus on targeting spore-forming organisms and implementing stricter microbial monitoring to ensure compliance with cleanroom standards. Ongoing training and education of personnel responsible for the handling and application of QAT-based disinfectants is crucial to ensure proper usage and prevent the development of further resistance. By addressing these challenges, pharmaceutical facilities can optimize the efficacy of QAT-based disinfectants and maintain the highest levels of microbial control to ensure product quality and patient safety.

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