

Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

Biorisk management in local mycology laboratories in Egypt

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ARTICLE INFO

Article history: Received 19 January 2025 Received in revised form 3 February 2025 Accepted 6 February 2025

Keywords

Biosafety cabinet Active air sampling Environmental swab

ABSTRACT

Background: Microbiological laboratories safeguard public health by detecting and responding to biological risks; nevertheless, they can also transmit infection to lab workers and the general population. Aim of the study: Measure the contamination level of laboratory and biosafety cabinet (BSC) air, as well as the contamination level of working benches and BSC surfaces in three local mycology labs in Egypt. Method: Detect the level of fungal and bacterial contamination in the air and surface of the lab, BSC, and randomly working benches at local mycology labs in Egypt using sterilized cotton swabs and active air sampling (SpinAir®) and compare the level of contamination. Result: The most frequent bacteria isolated from the laboratory air and the surface was Staphylococcus aureus in lab C (49.16%) and (57.28%), respectively. Aspergillus niger was the commonest fungus isolated from air in lab B (48.78%) and surface in lab C (42.37%) with significant difference across labs (p = 0.045). However, in BSC air samples, the commonest bacteria isolated was Coagulase-negative staphylococci (43.18%) in lab A, while Staphylococcus aureus was the most common bacterium isolated from the surface (57.7%) in the same lab. Aspergillus flavus (63.6% in lab B) was the most often isolated fungus from BSC air samples and surface (69.2% in in the same lab). Conclusion: G +ve bacteria and aspergillus spp were the most common isolated organisms from air and surface of labs and BSC. The level of contamination decreased after commitment with bio risk policy

Introduction

On the front line of identifying emerging and re-emerging infectious illnesses are microbiological laboratories. When handling hazardous microorganisms, laboratories must take responsible measures to manage the risks to their safety and security [1]. Globally, microbial contamination in hospitals and labs is growing, and detecting these contaminants may help treat some laboratory-acquired illnesses (LAI) [2].

Fungi are responsible for 9% of LAIs, according to a review of 3291 cases, aerosols of these fungal spores produced in different ways during pipetting and spills are likely the most frequent source of laboratory-associated fungal infections. Allergy (asthma, rhinitis), irritation (eyes, nose, skin) and toxic reactions (mycotoxins) are all linked to fungal exposure [3].

It can lead to tissue invasion and potentially fatal infections in those with weakened immune systems. The primary sources of opportunistic mold

DOI: 10.21608/MID.2025.345537.2470

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infections are Aspergillus species, particularly *Aspergillus fumigatus*, which causes invasive aspergillosis (IA), which typically starts as a respiratory tract infection before spreading [4].

So, in this work we will detect and compare the level of fungal contamination of air and surfaces of working benches at local mycology labs in Egypt and in biosafety cabinet in these labs.

Material and Methods

This exploratory study was conducted at 3 local mycology labs in Egypt to measure the level of fungal and bacterial contamination at these labs and was approved by the Research Ethics Committee at the Faculty of Medicine, Ain Shams University in the period between March 2023 to March 2024.

Study procedure

Base line study: BSC and bench surface swabs and air sampling by (SpinAir®, IUL S.A., and Barcelona, Spain) were taken to detect level of fungal and bacterial contamination in BSC and randomly working benches at local (anonymous) mycology labs in Egypt and compare the result of contamination between them.

1-BSC and bench surface sample:

The standard swabbing method was used to collect surface samples [5]. Random samples were taken twice a month from randomly selected work benches and BSC at three distinct mycology labs (whose names were not disclosed).

The swabbed surfaces by sterilized cotton swabs were standardized using a template with a surface area of 100 cm². The used cotton swabs were submerged in 10 mL of peptone saline then pushed against the tube wall to remove excess liquid. Each swab was labeled with the date, time, and code of swabbed surface. Threefold serial dilutions were made after shaking the test tube. Using the spread plate technique, 1 mL of each sample were pipetted onto pre-made agar dishes that contained blood agar and Sabouraud's dextrose agar (SDA) (HIMEDIA) with chloramphenicol

The bacteria and fungus were identified using both conventional biochemical reactions and microscopic inspection after incubation at 37 $^{\circ}$ C for 48 hours and at 25 $^{\circ}$ C for 5–7 days.

2-BSC and environmental air: (SpinAir®) was set up to sample 1000 L of air every collection at a flow rate of 100 L/min for 10 minutes .The basic idea is that air was drawn in through the sampling port, hit the agar plate and the microorganisms were forced

to adhere to its surface by the direct effect of the generated air on the petri dish [6].

3-Culture media and Identification: SDA supplemented with 10 mg/L chloramphenicol was used to isolate fungi, while blood agar supplemented with 5% sterile blood which allows aerobic bacteria to grow, Negative plates were maintained in order to identify fungi that grow slowly for up to 15 days [7].

Initially, colonies were identified through macroscopic appearance and shape, followed by microscopic examination and finally biochemical reaction testing. Fungi examination was made by a wet mount with a lactophenol-cotton blue solution and then examined under a microscope [8].

Calculation of microorganisms in surface samples and air samples:

To determine the surface total aerobic colony count (ACC) this formula was used: NA = N*v*d

NA: total ACCs per cm² (CFU/cm²) and N: the number of colonies in a plate.

V: the original volume (mL), d: dilution factor, A: swabbed area (100 cm²) [9].

To calculate **microorganisms** in air samples: Concerning solid agar impactor samplers this formula was used: C / (R * P) = N

N: number of colonies collected per cubic foot of air sampled (CFU/m³)

C: the number of colonies on culture plates.

R: the cubic feet per minute of airflow rate (L/min)

P: the sampling period's duration, in minutes (min) [10].

Intervention plan: The following recommendations were provided to the labs to improve their bio risk management performance

- A biohazard sign should be posted on the lab door, entry requirements, emergency contact information and any occupational health requirements.
- Lab doors should be kept closed during work and were closed and locked when unoccupied
- 3. Protective clothing should be removed prior to leaving when applicable.
- Protective footwear should be used when indicated to prevent cross contamination and disposed or decontaminated after use

- Spill kit should be available and lab personnel should be trained on how to use it
- 6. HEPA filters should be certified annually.
- 7. A smoke test should be done to ensure that the airflow patterns within a controlled environment meet the necessary standards.
- 8. Centrifuge should be available inside BSC
- Solid waste should be autoclaved prior disposal Recommendation
- Lab workbenches require special attention as they are in direct contact with all Lab activities.
- 11. Disinfection of workbenches before and after each session, create and implement policies for safe handling of hazardous materials.

Post intervention plan:

The same methodology was applied following the intervention.

Statistical analysis

Data was analyzed using SPSS V.26. Quantitative data was presented using mean whereas categorical data was given as frequency (count) or relative frequency (%).

Result

In this study 7 species of bacteria and 11 species of fungi were detected.

Regarding environmental air samples, 32 air samples were taken in each lab

- Staphylococcus aureus (S.aureus) had the highest incidence in labs A, B, and C before and after intervention and Bacillus spp. was detected only in labs A and B.
- Aspergillus niger (A. niger) was the most common fungus isolated from air in lab A and B, while Aspergillus flavus (A. flavus) was the commonest fungus isolated from air in lab C as seen in the table (1).

Regarding BSC air samples, 32 BSC air samples were taken from each lab.

Coagulase-negative staphylococci (Cons)
were the most prevalent in lab A and lab B
but wasn't detected in lab C and S.aureus
was the second most common in lab A and
lab B in comparison to lab C, where was
the only bacteria detected in BSC air.

- The intervention was effective in modifying overall bacterial profiles (*p*-value = 0.0001).
- Aspergillus flavus: was the most prevalent fungus in lab A and B pre and post intervention.
- A.niger: was detected in lab A and in lab B pre intervention and post intervention only detected in lab B.
- *Penicillium:* was detected in labs A and B only pre intervention and wasn't detected in any labs post intervention.
- No fungi were detected in BSC air in lab C before and after intervention.

Regarding bench samples, 32 bench swabs samples were taken from each lab

- Lab C had the highest prevalence of *S.aureus*, while labs A and B had higher prevalence of *Cons*.
- The intervention was effective in changing the overall bacterial profiles (*p*-value = 0.006).
- *A.niger* had the highest incidence in lab C before and after the intervention.
- Penicillium was found in lab B the most, followed by lab A and lab C prior to intervention.
- Candida was found in lab C only before and after intervention.
- Overall Fungus: p-value = 0.05: Significant, as seen in **table (2)**.

Regarding BSC surface samples, 32 BSC swabs samples were taken from each lab.

- Lab A had the highest pre-intervention 15(57.7%) and post-intervention 5(71.4%) rates of *S.aureus*. The larger percentage after intervention can be explained by reducing the number of other bacteria, with *S.aureus* accounting for the majority. This shows that, while the intervention had some benefits, it may not have targeted *S.aureus* as successfully as other pathogens.
 - Streptococcus spp. was found exclusively in lab A and wasn't detected in all labs following intervention
 - Cons were the most prevalent pre intervention in lab B and was detected in lab A only post intervention.

- Bacillus spp. was only detected in labs A and B and wasn't detected in all labs following intervention
- E.coli was detected only in lab A 1(3.8%) pre intervention and wasn't detected in all labs following intervention
- A.niger was the most common fungus in Lab A 9 (47.7%) and post-intervention was with 1 (100%), while A.flavus was the commonest fungus in Lab B 9 (69.2%) pre intervention and wasn't detected in all labs following intervention
- Aspergillus fumigatus (A. fumigatus) was only detected in lab A 1(5.3%) and 0(0%) in all labs following intervention.
- Penicillium, Mucor, and Alternaria were discovered pre-intervention in Lab A only (2(10.5%), 1(5.3%) and 2(10.5%)) respectively.
- Candida: was only detected in lab A 1(5.3%) and in lab B it was 2(15.3%) and

- wasn't detected in all labs following intervention.
- Corynebacterium diphtheriae Spp, Klebsiella spp, Aspergillus terres, Lichtheimia corymbifera, Cladosporium, and Chaetomium were not found on the BSC surface of the 3 labs
- The intervention had a significant positive impact, lowering mean fungal contamination in both air and surfaces, particularly within the BSC (more than 90% reduction in air and almost 97% in surface), where both air and surface CFU levels were close to 0. This demonstrates that the intervention program was highly effective.

The intervention reduced airborne bacterial contamination by 66.7% in the lab and 88.4% in the BSC. The mean bacterial bench surface CFU was reduced by 57.4%, while the BSC surfaces showed a stunning 86.5% reduction (**Tables 3, 4**).

Table 1. Types and frequency of bacteria and fungi isolated from environmental air samples pre and after intervention.

	LAB A	LAB B	LAB C	LAB A*	LAB B*	LAB C*	Chi	<i>p</i> -
							square	value
Bacteria								
S.aureus	46(35.4%)	68(43.87%)	118(49.16%)	27(45%)	18(43.9%)	47(63.51%)	5.00	0.08
ConS	31(23.8%)	24(15.87%)	45(18.75%)	20(33.3%)	15(36.58%)	10(13.51%)	6.84	0.03
Strept spp.	18(13.84%)	19(12.25%)	66(27.5%)	2 (3.33%)	4(9.75%)	17(22.972%)	0.75	0.68
Bacillus spp.	18(13.84%)	28(18.06%)	0(0%)	9(15%)	3(7.31%)	0(0%)	4.92	0.027
Corynebacterium diphtheriae	5(3.8%)	8(5.16%)	0(0%)	2(3.33%)	1(2.43%)	0(0%)	0.78	0.37
Spp.								
(Escherichia coli) E coli	8(6.2%)	5(3.2%)	0(0%)	0(0%)	0(0%)	0(0%)	0	1
Klebsiella spp.	4(3.1%)	3(1.9%)	11(4.583%)	0(0%)	0(0%)	0(0%)	0	1
Total	130(100%)	155(100%)	240(100%)	60(100%)	41(100%)	74(100%)	6.47	0.039
Fungus				L				1
Aspergillus terres (A.terres)	2(2.63%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0	1
A.niger	26(34.21%)	20(48.78%)	12 (33.33%)	6 (46.1%)	2(13.3%)	8(47.1%)	6.18	0.045
A. fumigatus	2(2.63%)	4(9.76%)	0(0%)	0(0%)	2(13.3%)	0(0%)	0.889	0.59
A.flavus	14(18.42%)	11(26.83%)	17 (47.22%)	3(23.1%)	9 (60%)	9 (52.9%)	3.1	0.20
Penicillium spp.	14(18.42%)	3(26.83%)	6(16.67%)	2(15.4%)	1(6.6%)	0(0%)	1.507	0.64
Mucor	3(3.95%)	1(2.44%)	1(2.78%)	1(7.69%)	0(0%)	0(0%)	0.6	0.74
Alternaria	2(2.63%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0	1
Lichtheimia corymbifera	7(9.21%)	1 (2.44%)	0(0%)	0(0%)	1(6.66%)	0(0%)	3.938	0.04
Cladosporium	5(6.58%)	1 (2.44%)	0(0%)	1(7.69%)	0(0%)	0(0%)	0.194	0.9
Chaetomium	1(1.32%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0	1
Total	76(100%)	41(100%)	36(100%)	13(100%)	15(100%)	17(100%)	6.5	0.038

p-value > 0.05: Non-significant; p-value < 0.05: Significant; p-value < 0.01: Highly significant * Lab after intervention.

Table 2. Types and frequency of bacteria and fungi isolated from bench surface samples.

	LAB A	LAB B	LAB C	LAB A*	LAB B*	LAB C*	Chi	<i>p</i> -
							square	value
Bacteria								
S.aureus	30 (39.47%)	20(48.78 %)	358 (57.28%)	5(26.3 %)	6(75%)	200(74.07 %)	8.276	0.015
ConS	36(47.37%)	15(36.59%)	57(9.12%)	8(25.00 %)	2(25 %)	29(10.74 %)	5.756	0.05
Strept spp.	2(2.63 %)	2(4.87 %)	160(25.6	1(25.60 %)	0(0%)	51(18.89	0.776	0.67
Bacillus spp.	4(5.26%)	3(7.31%)	3(0.48%)	4(21.01%)	0(0.00 %)	1(0.37 %)	1.925	0.38
Corynebacterium diphtheriae Spp.	1(1.31%)	1(2.4%)	0(0%)	1(5.26%)	0(0%)	0(0%)	2	0.36
E coli	1(1.31%)	0(0%)	9(1.44%)	0(0%)	0(0%)	3(1.1%)	0.325	0.85
Klebsiella spp	2(2.6%)	0(0%)	38(6.08%)	0(0%)	0(0%)	5(1.85%)	0.262	0.87
Total	76(100%)	41(100%)	625(100%)	19(100%)	8(100%)	289(100%)	10.044	0.006
Fungus		I.	-1					
A. terres	8(17.7%)	12(27.9%)	30(5.08%)	0(0%)	0(0%)	10(3.8%)	6	0.04
A.niger	15(33.3%)	5(11.62%)	250(42.37%)	0(0%)	3(30%)	110(42.3%)	6.715	0.03
A. fumigatus	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	-	-
A.flavus	5(11.1%)	9(20.9%)	90(15.25%)	4(36.36%)	5(50%)	15(5.77%)	7.919	0.01
Penicillium spp.	10(22.2%)	22(51.16%)	0(0%)	5(45.45%)	2(20%)	0(0%)	3.917	0.04
Mucor	2(4.4%)	0(0%)	0(0%)	1(9.09%)	0(0%)	0(0%)	0	1
Alternaria	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	-	-
Lichtheimia corymbifera	2(4.4%)	1(2.3%)	0(0%)	0(0%)	0(0%)	0(0%)	0	1
Cladosporium	3(6.6%)	0(0%)	0(0%)	1(9.09%)	0(0%)	0(0%)	0	1
Chaetomium	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	-	-
Candida	0(0%)	0(0%)	220(37.28%)	0(0%)	0(0%)	125(48.07%)	0	1
Total	45(100%)	49(100%)	590(100%)	11(100%)	10(100%)	260(100%)	7.559	002

p-value > 0.05: Non significant; p-value < 0.05: Significant; p-value < 0.01: Highly significant * Lab after intervention.

Table 3. Mean of fungal CFU in mycology labs before and after intervention.

Labs	Air sample ¹	Air sample ¹ *	Surface sample ²	Surface sample ² *	BSC AIR sample ¹	BSC AIR sample ¹ *	BSC surface sample ²	BSC surface sample ² *
		21.3	228	90.6	18.6	•	10.6	0.33

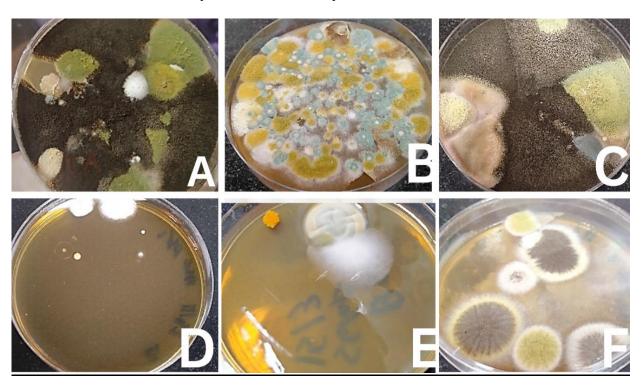
Table 4. Mean of bacterial CFU in mycology labs before and after intervention.

Labs	Air sample ¹	Air sample ¹ *	Surface sample ²	Surface sample ² *	BSC AIR sample ¹	BSC AIR sample ¹ *	BSC surface sample ²	BSC surface sample ² *
	175.0	58.33	247	105.3	39.6	4.6	17	2.3

^{*} Lab after intervention

Is it the total? Yes, it is the total mean of the three labs before and after intervention.

 $\begin{tabular}{ll} \textbf{Figure1.A}, B and C showing environmental air sample on SDA media of lab A, B and C before applying recommendations and D, E and F post intervention air samples. \\ \end{tabular}$



¹ CFU/m3

 $^{2 \, \}text{CFU/cm}^2$

Figure 2. (A) *A.niger* colonies on corn meal media (B) *A.niger* by lacto-phenol cotton blue stain (C) corn meal media showing colonies of *A.niger*, *A.fumigatus* and *A. terres* (D) *A fumigatus*. By lacto-phenol cotton blue stain by Tape Touch Method (E) *A terrus* on SDA media (F) *A.terrus* by lacto-phenol cotton blue stain by Tape Touch Method.

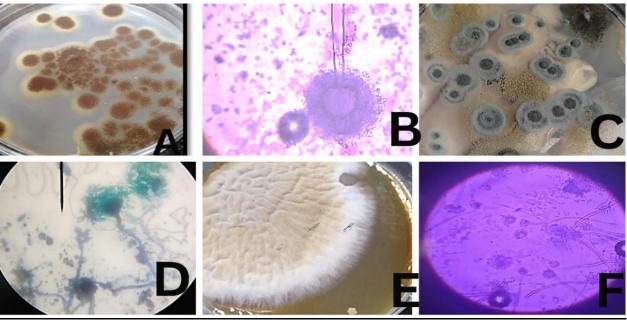
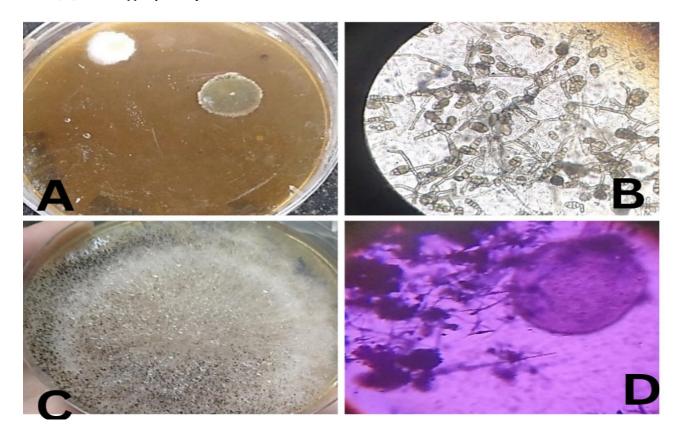


Figure 3. (A) *Alternaria* on SDA media (B) microscopic examination of *Alternaria* (C) *Mucor spp.* on SDA media (D) *Mucor spp.* by lacto-phenol cotton blue stain.



Discussion

A well-designed hospital laboratory could reduce the risk of worker injury while also providing precautions against laboratory pollution of the surrounding environment. Working with hazardous biological materials necessitates the adoption of a comprehensive bio-risk management policy that protects laboratory personnel and prevents the inadvertent or intentional leakage of hazardous chemicals from the lab [1].

Regarding environmental air samples:

In this study, *S.aureus* had the highest prevalence pre and post intervention. Similar result in Nigeria by **Ikon et al.** [11] who reported that *S.aureus* was (61.5%) in Laboratory Sections in Obong University.

The percentage of *Strept spp* in this study similar to **Mirhoseini & Bayani** [12] in Iran ,where *Strept spp* was 20% of bacterial contamination of air in dentistry clinics.

The percentage of *Bacillus spp* in this study was in accordance with **Ikon et al.** [11] who reported that *Bacillus spp* (11.5%) and in Malaysia **Yogeswaran et al.** [13] detected *Bacillus spp* was (11.11%) in research laboratories.

In this study and prior research, G+ve bacteria was the highest prevalence which was explained by the high peptidoglycan content of their cell walls. Because they are more resilient to heat and pressure and have a longer lifespan in aerosolized environments, Additionally, *Staphylococci* can withstand drought.

The percentage of Aspergillus spp increased after intervention in this study, the higher percentage after intervention can be explained by reducing the incidence of other fungi to make Aspergillus spp. the highest percentage. This could imply that while the intervention was somewhat effective, it may not have targeted Aspergillus spp as effectively as it did other fungi.

The pre intervention result of *Aspergillus spp.* coincidence with **Sautour et al.** [14] in France who found *Aspergillus spp.* accounted for 53% of the isolates collected from the laboratory environment. but in Malaysia, **Yogeswaran et al.** [13] reported that *Aspergillus spp* (20%).

The significant prevalence of *Aspergillus spp*. in the current study indicates that molds existing in buildings may form sporulating microcolonies that release fungal spores in microenvironments with adequate building

materials, moisture, and temperature conditions [14].

The post intervention result of *Penicillium spp* was similar to **Ikon et al.** [11] who recorded that *Penicillium spp* occur at percentage of 14.3%, while **Sautour et al.** [14] reported that the most frequently recovered airborne fungi were *Penicillium spp*. (75 to 100%) in the new medical mycology lab.

The percentage differences between labs were caused by humidity levels, which affect the concentration of airborne fungus, frequent door use, and ventilation system leaks in older labs, all of which increase airborne fungus counts [8].

Regarding working bench surface swabs:

The pre intervention result of *S.aureus* was coincidence with **Alfy et al.** [16], in Egypt, where *S. aureus* was 44.4 %.

The high percentage of *S. aureus* can be explained by its persistence on inanimate items and survival in dry environments for a long time [8].

The post intervention result of *Cons* was similar to **Ghayoor et al.** [17] ,in Pakistan, where *Staphylococcus epidermis* was detected with percentage of 2 (25%) out of 22 bacterial contaminants,

The percentage of *Strept spp* was similar **Taheri et al.** [18] ,in Iran where *streptococcus viridans* in dental lab was 2.2%.

The percentage of *Bacillus spp* similar to ,**Ghayoor et al.** [17] as *Bacillus spp* was 1(14.25%). In contrast *was* 55.6 % in **Alfy et al.** [16]

The divergence in environmental contaminants can be defined as differences in lab work and experiment performance and geographical distribution.

Corynebacterium diphtheriae Spp was similar to **Ghayoor et al.** [17] ,who found that the percentage of Corynebacterium diphtheriae Spp was 0(0%).

According to the percentage Aspergillus spp was similar to **Sautour et al.** [14] who found Aspergillus spp count on bench was 82 CFU /plate and Penicillium spp count on bench was 6 CFU/plate as in our study where Penicillium spp

According to the percentage *Lichtheimia corymbifera* was similar to **Sautour et al.** [14] who found *Lichtheimia corymbifera* count on bench was 1 cfu/plate

Candida was similar to **Sautour et al.** [14] who found yeast count on bench was 61 cfu/plate

Unlikely, **Viegas et al.** [3] reported that *Candida* was the most prevalent 6×10^3 CFU/cm².

Conclusion

Aspergillus niger was the most frequent fungus isolated from environmental lab air and bench, but A. flavus was the most frequent fungus isolated from BSC air and BSC surface. Staphylococcus aureus was the most frequent bacteria isolated from environmental lab air and benches, but ConS was the most frequent bacteria isolated from BSC air and S. aureus from BSC surface.

The intervention significantly reduced the mean levels of bacterial and fungal contamination in the air and on surfaces, especially in the BSC.

Recommendation

Work benches should be cleaned and sanitized before and after each session, and procedures for handling hazardous items safely should be developed and put into place.

- More studies should be done to quantify how microorganisms are impacted by pressure, humidity, and seasonal changes.
- In order to foster a culture of superior biosafety procedures in labs, future research and initiatives should concentrate on specialized training, policy development, and continuous assessment.

Conflicts of interest

All authors declare no conflict of interest in this work.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability

All data generated or analyzed during this study are included in this puplished article.

Authors' contribution

All authors made significant contributions to the work presented, including study design, data collection, analysis, and interpretation. They also contributed to the article's writing, revising, or critical evaluation, gave final approval for the version to be published.

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