

### Microbiological Quality Assessment of Indoor Air in Medical College in Saudi Arabia

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#### Abstract

To evaluate the microbial load indoor airs, is one of the most important parameter of the environmental factors quality, it has become necessary and show how safe the indoor air in the surrounding environments. This study was aimed to assess the microbiological quality of indoor air inside Mohammad Al-Mana Collage for Medical Sciences (MACHS) – Nursing Building – KSA. Samples were collected from each selected floors; (basement, ground, first and second floor), selected room; (corridors, laboratories, offices, classrooms and toilets).

Results shows that there are positive growth of microbial load in all samples in selected floors and rooms, all sample have growth of bacteria and fungi, the colony counts was high in basement and first floor comparing to other floors and in toilet comparing with other selected rooms. Statics shows that there were no significant differences between bacterial loads among the selected floors, while there were differences between selected rooms, it was seemed to be free of gram negative bacteria, and only toilets have gram negative bacteria. Significant differences were also noted among sampling time between 8: am and 4 pm, Afternoon samples were more contaminated with more microbes comparing to the morning. Microbe which were isolated from indoor air samples include: *Staphylococcus aureus*, *Bacillus* spp. *gram negative bacilli*, *E.coli*, and, *Aspergillus* species, *Penicillium* species, *Candida* species. The concentration of indoor bacterial aerosol observed in this study which was lower than the standard.

**Keywords:** Evening; Indoor air; morning; Microbial load; sample

#### Introduction

Bacteria and other pathogenic agents which may cause disease for human are living in the same environmental conditions with human, indoor air considered as essential factors for the human growth, in some cases, this air may be contaminated with one or more of those agents, this be able to cause disease for human [1].

Indoor air may have contained quantity of microorganisms, but this should be lower than the outdoor levels. One of the most common source of microbial contamination of indoor air is, people, dust, and the ventilation system itself [2] and may be affected by temperature, light, nutrients availability [3]. Human may have considered the main source of microbial contamination of indoor air by different aspects, sneezing one of the most common way and mechanism for spreading the microbes in air [4], such as pneumonia and other respiratory diseases. Fungi also may found in indoor air

which may cause the allergic diseases and may considered more serious than bacteria in air borne diseases [5].

Data by [5] indicate that bacteria and fungi found in Poland in university environments and include *Micrococcus* spp., *Enterococcus* spp., *Staphylococcus* spp., *Serratia* spp., *Bacillus* sp. and *Klebsiella* spp. Fungi: *Aspergillus* sp., *Fusarium* sp., *Alternaria* sp., *Penicillium* sp. and *Cladosporium* sp. It was reported by [6], that *Escherichia* genus was dominant in toilets with some species of *Cladosporium*, *Alternaria*, *Mucor*, *Rhizopus* and *Epicoccum* sp.

According to Naga K, et al. the main source of airborne infection in classrooms was, pupils of normal flora, uniforms, bags, sandals, as well as sneezing, coughing, talking and laughing, in addition to other materials such as cupboards, books and files. In addition to the house keeping facilities also playing Avery important roles in spreading infection such as; sweeping dusts by mops, using same clothes or cleaning materials which can aerosolize the practices of microbes and microorganisms [3].

Naga K et al. indicated that microbial contamination usually occur in universities and especially in class room, laboratories and offices, which are controlled by several factors; rate of populations or visitors in the place, air exchange, temperature, relative humidity, type of ventilation, and number of windows in each room [7].

According to Kumari NK, et al [8]; keeping healthy environments controlled may reduce disease transmission of pathogenic agents and considered as one of the key agendas in controlling diseases in schools.

It is important to understand the microbial community within public areas and, in particular, within school buildings as poor health in children impacts on wider society. Against this backdrop, the determination of indoor microbial density is necessary, and it is especially important in such populated areas like school settings.

#### This study aimed to

- Evaluate the microbiological quality of indoor environments of Mohammed Al-Mana College for medical science Sciences. Al-Khobar, Eastern Province, Kingdom of Saudi Arabia.

FLOOR	Corridor	Laboratory	Office	Classroom	Toilet
BASEMENT					
GROUND					
FIRST					
SECOUND					

**Table 1:** selected floors and rooms as study area in MACHS.

#### Microbiological analysis

##### Sample collection

Samples were collected from each selected floor/ room by taking 4 dishes from each place at different sampling time morning and afternoon.

The bacterial culture plates were incubated at a temperature of 37°C for 48 hours in an incubator while the fungal culture plates were incubated at a temperature of 37°C for 5-7 days. Bacterial and fungal Colony Forming Units (CFU) were enumerated. Afterwards, the colonial morphology of the different colonies formed were noted and identical colonies were sub-cultured into Nutrient Agar (NA), Blood Agar BA, Macconkey Agar MAC, Chocolate agar CHO and Sabouraud Dextrose Agar (SDA) plates, incubated appropriately and stored for further identification and characterization.

##### Identification of bacterial and fungal isolates

Each bacterial colony or fungi was identified using standard methods (including: colonial morphology, microscopy and

- Asses and identify the microbial load in the air samples in different room and labs in the college.

## Materials and Methods

### Study area

The study was conducted in Nursing Building- Mohammad Al Manaa Collage for Medical Sciences MACHS, Khobar- KSA, from 6-17 October 2019, at;

- **Tow sampling time** (8:00-10:00 am) and (2:00 - 4:00 pm),
- **Four selected floors**; (basement, ground, first and second floor) and
- **Selected rooms**; (office, class room, corridor, laboratory and toilet).

It experiences constant high temperatures and relative humidity throughout the year with a diurnal temperature range of minimum 21-27°C and maximum 30-34°C, with a mean relative humidity value of 65-70 %. (Table 1), show the study area selected floor and room.

biochemical tests) as described by Cheesbrough M. et al 2006. Fungal isolates were identified based microscopic properties (using Lactophenol cotton blue staining) and macroscopic characteristics (with the aid of an Atlas of Mycology) as described by Rajesh B, et al. 2008. Data were presented using tables and graphs.

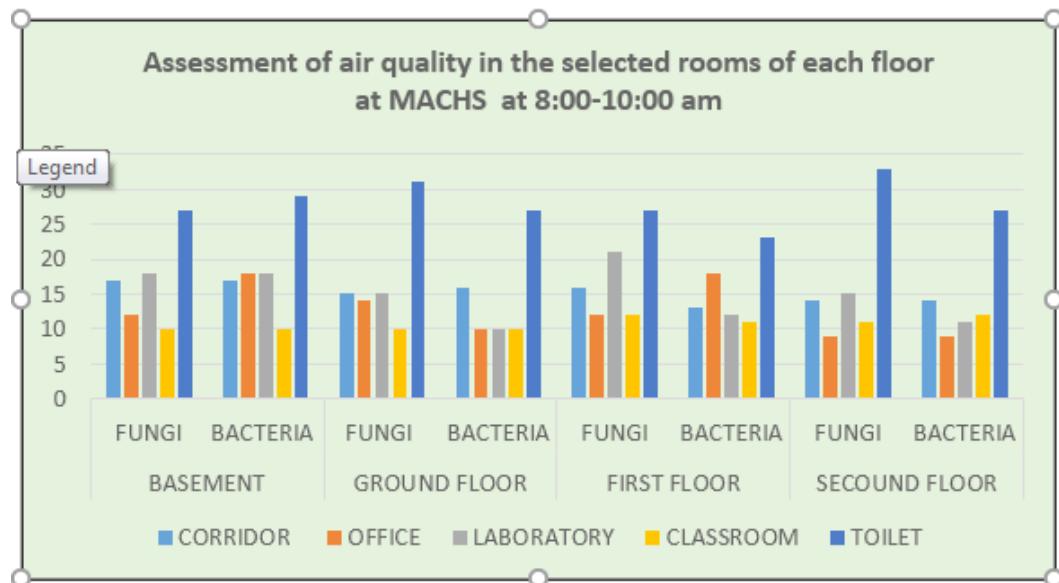
After incubation all cultures, at 37°C for 48hours, plates were observed in total number if forming colony (CFU), colony morphology and other microscopic examination were used to identification of bacteria according to the Bergey's Manual of Systematic Bacteriology [1,6]. Fungal colonies were identified as morphology of fungus and colony according to the manual of Barnet and Hunter 1972. The identification of fungal isolates was done according to standard methods.

Statistical analyses were carried out with one way analysis of variance (ANOVA) and Tukey-Kramer Multiple Comparisons.

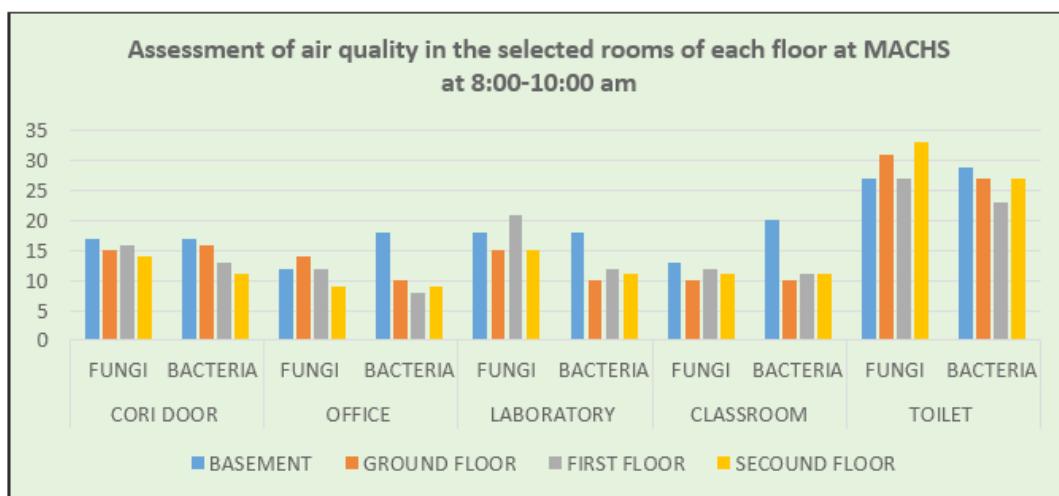
### Results

In order to do the Assessment for the microbial load and quality of the Indoor Air in MACHS (4 floors, 5 different rooms) were selected (Table 1).

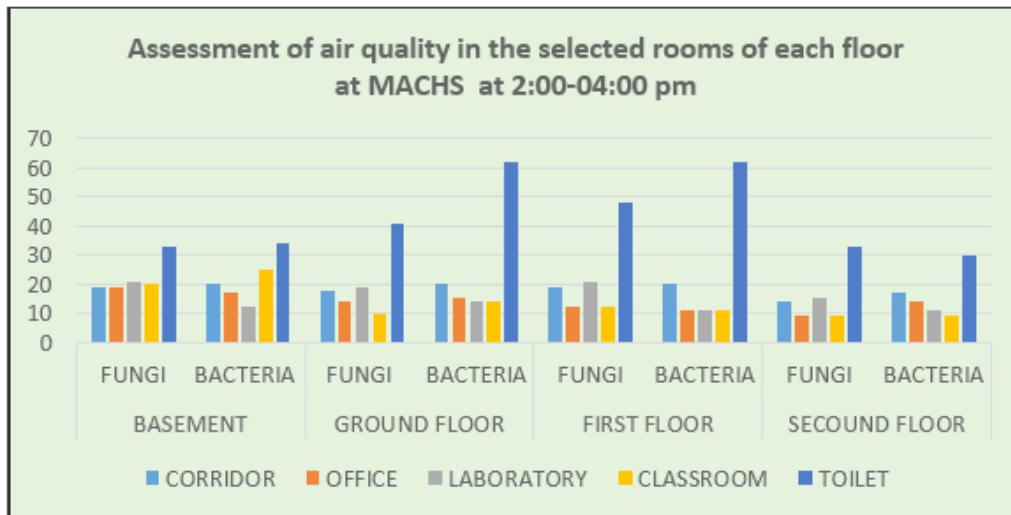
Data indicated that microbial load of indoor air were significantly different among the selected floors and rooms, figures 1-4 revealed the microbiological load of indoor air samples at both sampling periods, as well revealed frequency of the microbial species as isolated from the indoor air sampled.



**Figure 1:** Assessment of microbial load in indoor air in the selected rooms at MACHS at 8:00-10:00 am.

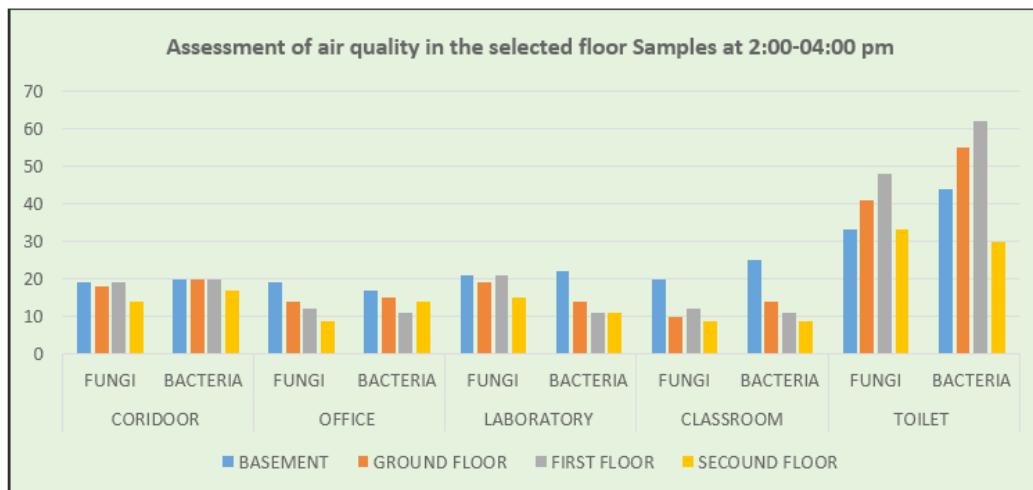


**Figure 2:** Assessment of microbial load in indoor air in the selected floors at MACHS at 8:00-10:00 am.



P<0.05 is considered statistically significant.

**Figure 3:** Assessment of microbial load in indoor air in the selected rooms at MACHS at 2:00-4:00 pm.



P<0.05 is considered statistically significant.

**Figure 4:** Assessment of microbial load in indoor air in the selected floors at MACHS at 2:00-4:00 pm.

The study also assessed the microbiological load, at different selected rooms in each floor, there were significant differences between selected floors ( $P<0.05$ ), basement and first floors were more contaminated by microbes, there was no significant difference ( $P<0.05$ ) between selected rooms except toilet which was more contaminated with bacteria and fungi in each basement and first floor, it was explained due to overloaded.

Data also revealed that there was significant difference ( $P<0.05$ ) between sampling time (8:00-10:00 am) and (2:00-4:00 pm), 2:00-4:00 pm was more microbial loaded in each selected rooms among selected floors.

pm), 2:00-4:00 pm was more microbial loaded in each selected rooms among selected floors.

Across sampling time, temperature was measured during both time; it was 22.5-25.5 °C at 8:00-10:00 am and 25-27.6 °C at 2-4 pm, the only factor which may affected the microbiological load in all samples was the overloaded, college in general used to be overloaded after 11 am especial in the basement floor where the training center is located and first floor where the main toilet for male prayer place is located.

No significant differences were found in the bacterial colony counts between the classrooms ( $P>0.05$ ). However, there were significant differences between the 8:00-10:00 am and 2:00-4:00 pm bacterial colony counts; as well as between the floors and room in bacterial colony counts ( $P<0.05$ ); but, there was no significant difference between the classroom, offices, and labs, but toilets were the highest in each floor ( $P>0.05$ ) (Figures 1-4).

## Discussion

Microbiological load in indoor air samples from each selected location indicated that there were significant differences between selected floors as well as selected rooms. It was found that there were significant differences between sampling times; (8:00-10:00 am) Bacteria colony rated from (73-92), (45-106) cfu in each selected floor and selected room respectively. Fungal count rated from (82-88), (46-118) for selected floor and selected room respectively. While at (2:00-4:00 pm) bacteria colony rated from (81-115), (58-191) for selected floor and room respectively.

Fungal count rated from (80-112), (51-155) for selected floor and room respectively.

Data indicated that a variations were found in the

concentration of bacteria and fungi among selected samples in each selected floors and rooms. This may be due to the overloaded of some sites and human density at the time of sample collection, these results were agreed with the results reported by [9,10], which were affected the sample collected at noon time, in addition to the bad ventilation in these sites which play an important role in microbial load in closed areas, which may affected the morning samples [9,11,12]. However, Due to good ventilation the bacteria and fungi counts were found less microbial loads in classroom, offices and corridors, while laboratories and toilets were the highest, toilet in each floor, they were the most contaminated site, (27-29), (27-33) for bacteria and fungi count respectively at 8 am, while it was, (30-62), (33-48) for bacteria and fungi count respectively at 2 pm.

These data were agreed with the results obtained by Stryjakowska- Sekulski *et al.*, [6] who found an elevated count in bacteria load in lecture rooms, and also he mentioned that ventilation in important factor in the determination of indoor air quality.

The bacterial genera and species isolated and characterized from all sample were *Staphylococcus aureus*, *Escherichia coli*; gram + ve, the fungal species isolated were; *Aspergillus sp*, *Penicillium sp.*, and *Candida sp* (Tables 2,3).

FLOOR	FUNGI				BACTERIA							
	Penicillium spp.	Aspergillus spp.	candida	Other	Gram-positive, cocci	Staphylococcus	Streptococcus	Gram-positive rods	Gram-negative cocci	Gram-negative rods	Shigella	e.coli
BASE- MENT	✓	✓	✓	✓	✓	✓	x	✓	x	✓	x	✓
GROUND	✓	✓	✓	✓	✓	✓	X	✓	X	✓	X	✓
FIRST	✓	✓	✓	✓	✓	✓	x	✓	x	✓	x	✓
SECOUND	✓	✓	✓	✓	✓	✓	X	✓	X	✓	X	✓

\* $P<0.05$  is considered statistically significant.

**Table 2:** Distribution of aero-flora in each FLOOR in MACH.

Rooms	FUNGI				BACTERIA							
	Penicillium spp.	Aspergillus spp.	candida	Other	Gram-positive, cocci	Staphylococcus	Streptococcus	Gram-positive rods	Gram-negative cocci	Gram-negative rods	Shigella	e.coli
Corridor	✓	✓	✓	✓	✓	X	X	✓	X	X	X	✓
Laboratory	✓	✓	X	✓	✓	X	X	✓	X	X	X	✓
Office	✓	✓	X	✓	✓	X	X	✓	X	X	X	✓
Classroom	✓	✓	X	✓	✓	X	X	✓	X	X	X	✓
Toilet	✓	✓	✓	✓	✓	✓	X	✓	x	✓	x	✓

\* $P<0.05$  is considered statistically significant.

**Table 3:** Distribution of aero-flora in each ROOM in MACH.

Results from this research indicated that *Aspergillus niger* was the most common mold in all samples while the least isolates was *cadida*. Gram positive bacteria was the most common among all samples, *Staphylococcus aureus* and *Escherichia coli* were detected as pathogenic genera but with the least count comparing with others (Table 2,3).

Similar results were reported by Udochukwuet *et al.*, [5] as *Aspergillus* (52.3%), was the most common genera of molds among all samples with *Penicillium* while, the least percentage frequency (4%) of fungi species was recorded as *Candida* sp. these findings were interpreted as a results of movement of microbes, and pathogenic among the collage atmosphere [5]. On the other hand, *Staphylococcus aureus* was recorded as the highest frequency (27%) of bacteria isolated while *Sarretiamarcenses* had the least percentage frequency (4%) of bacteria isolated [5].

Other research data reported that indoor air samples contained species of *Staphylococcus* and *Micrococcus*, except for *Difteroides* in Italian classrooms Maroni M, *et al* 1993 also it was reported by [13], that indoor air samples were loaded with *Staphylococcus auerus*, except for *Escherichia coli* and *Streptococcus D*. but disagrees with the same on *Streptococcus*, *Pseudomonas*, *Klebsiella*

and *Escherichia* as the dominant bacteria genera [8].

On the other hand, fungal counts were presents in the same rate of this study while it was found that indoor air contained molds genera: *Penicillium* and *Aspergillus* from the indoor air of some Norwich schools and Danish schools [14].

In further agrees with data of Stryjakowska-Sekulska M, *et al* *Penicillium*, Yeasts and *Aspegillus* as the most common fungal genera isolated, except for *Cladosporium* and Actinobacteria. Except for *Cladosporium spp.* and *Alternaria spp* as the dominant fungal isolates

Unfortunately, there is no uniform international standard on levels of maximum microbial load in indoor air samples [15] but according to many national standards and guidelines of bio-aerosol counts at 500 cfu/m<sup>3</sup> [16], none of the bacterial count reaches the bio-aerosol count 500 cfu/m<sup>3</sup>.

Furthermore (Table 4) shows the sensitivity test for both *Staphylococcus auerus*, and *Escherichia coli*, which indicated that both isolates are sensitive for all of antibiotics which may use as indicator that the concentration of both isolates are non-pathogenic.

Antibiotic	<i>Staphylococcus</i>	Antibiotic	<i>e. coli</i>	Antibiotic	<i>e. coli</i>
Penicillin	S	Amox/k clav	S	Gentamicin	S
cefoxitin	S	Amp/sub	I	Imipenem	S
oxacillin	S	Ampicillin	I	Levofloxacin	S
clindamycin	S	Cefazoline		Nitrofurantoin	S
vancomycin	S	Cefepime	S	Tetracyclin	S
rifampicin	S	Cefuroxone	S	Trimeth/sulfa	S
		ciprofloxacin	S		

**Table 4:** a culture and sensitivity report for a *staphylococcus* and *e. coli*.

Since the microbial counts are lower that the standards, all selected floors and rooms were considered as non-contaminated areas, but toilets in each selected floor need more hygienic conditions especially at afternoon periods which considered as overloaded period.

Air-conditioners unites filters need to be changed from time to time, while class room and office may need ventilation at least once a day.

The results also recommended further studies in the same area to determine the reasons of spreading the pathogenic agents among closed or opened areas.

## Conclusion

This study suggested that regardless of the time of day, indoor conditions may allow aerosols areas which may be considered as potential risk factors for spreading pathogenic bacteria or fungi.

The concentration of air flora in side MACHS was under the danger zone -according to the standards- but an immediate intervention is needed in order to control the quality of indoor air as human and environmental factors that stimulate the growth of microorganisms.

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## Conflict of interest

The authors declared no conflict of interests.

## Ethics requirements

This article doesn't contain any studies with human or animal subjects.

## Ethical number "SR/RP/16"

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