Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 2 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

Characterization And Antifugal Susceptibility Of Different Fungal Strains Isolated From Hospital Environment

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ABSTRACT

Nosocomial fungal infections pose a growing global public health risk, necessitating immediate attention and effective management. Due to the limited availability of treatment options, it is crucial to comprehend the emerging epidemiological trends, mechanisms, and risk factors associated with these infections. To address this issue, a research study was conducted at PIC Peshawar, one of the largest cardiology hospitals in Peshawar. A total of 17 samples were collected using dry cotton tip swabs from various hospital equipment. These samples were then cultured on Sabouraud Dextrose agar media (SDA) for a period of 7 days. Sample identification was achieved using Bromophenol blue as a staining agent, while antifungal susceptibility testing was

performed using Fluconazole. Direct microscopy was employed to isolate and identify 8 strains. These strains included Rhizopus sp., Penicillium spp., Aspergillus spp.,

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Cladosporium sphaerospermum, Geotrichum candidum, Alternaria alternata, Scedosporium prolificans, and Scytalidium sp. Among these isolated colonies, Rhizopus sp., Aspergillus nidulans, and Aspergillus niger exhibited susceptibility to fluconazole, whereas the remaining colonies, Penicillium spp., Cladosporium sphaerospermum, Geotrichum candidum, Alternaria alternata, Scedosporium prolificans, and Scytalidium spp., displayed resistance to fluconazole. In our study, the highest fungal growth was observed in the pediatric ward (kids ward), indicating a need for targeted interventions in that area. The most frequently isolated fungal species included Cladosporium sphaerospermum and Aspergillus spp.

Key Words: Nosocomial Fungal Infections, SDA, Bromophenol Blue, Susceptibility.

INTORUDUCTION

Hospitals are enclosed spaces where there is a greater risk of cross-infection between the workers and patients. Nosocomial infections dramatically increased morbidity and mortality rates, according to studies conducted in European hospitals, and many of these infections are spread by inhalable pathogens. A number of routine tasks performed in medical institutions might accelerate the dispersion of bio-aerosols, which can cause the transmission of infectious diseases. So, the methods to prevent and control hospital-acquired infections must now include the assessment and control of microbial contamination in healthcare facilities. In order to tackle this difficult assessment, various studies have already noted the critical relevance of using passive sampling techniques. These techniques enable the characterization of contamination over a longer time frame. (Viegas, Twarużek et al. 2020). In recent years, under the influence of various factors, the probability of invasive fungal infection in hospitals is increasing, especially in patients in intensive care units, whose immune function is relatively low, and they are more prone to invasive fungal infection. In general, the characteristics of invasive fungal infections vary greatly depending on the patient's condition. In the intensive care unit, most of the patients have a variety of basic diseases, the rate of fungal infection is much higher than that of the general ward, and the mortality rate is between 38% and 68% (Ding et al., 2020). Fungal nosocomial infections continue to be a serious problem among hospitalized patients causing increasing morbidity, mortality and healthcare costs. A study done at a tertiary care center in India reported 15% invasive fungal infections in ICU, including invasive aspergillosis, invasive candidiasis and mucormycosis. Most of the fungal nosocomial infections are due to exogenous acquisition of the fungus which shows a marked tendency to colonize hospital environments. These fungal pathogens flourish on their surfaces and form biofilms causing plethora of hospital acquired infections. The presence of biofilms proves to be therapeutic challenge and thus complicates the clinical scenario. The major implicated agent involved in spread of fungal infections are the hands of healthcare workers (HCWs) which are vulnerable to colonization and infection by fungal pathogens, especially with Candida species. Recent studies have observed, Candida parapsilosis represents the most frequently isolated fungus from the hands of healthy people and healthcare workers thus highlighting the importance of hand washing to prevent the horizontal transmission of this pathogen (Keri et al., 2021). The distribution and viability of fungal contamination in a given environment

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is mostly influenced by building characteristics, comprising the availability of water and nutrients for fungal growth and survival, building occupancy, and the outdoor environment (Sudharsanam et al., 2012). In Healthcare environments, fungal presence also depend on the medical activities performed, number and types of patients, cleaning frequency and procedures and the type of ventilation systems (Qudiesat et al., 2009; Jung et al., 2015; Marchand et al., 2016). All these variables determine fungal dissemination and proliferation and influence the indoor sources of contamination in air, water, and surfaces (Sudharsanam et al., 2012). There is a significant rise in morbidity and mortality of infections caused by Candida. Candida spp. infections are currently ranked fourth among nosocomial infections which are difficult to diagnose and refractory to therapy. Given the differences in susceptibility among various spp., identification of Candida spp. is an important step that leads to the selection of a suitable antifungal (Kean et al., 2019). Given the increasing burden of nosocomial fungal infections, their association with hospital environments, and the growing challenge of antifungal resistance, a comprehensive understanding of fungal contamination and species distribution in healthcare settings is essential. **Therefore, the present study aimed to investigate** the prevalence, species distribution, and antifungal susceptibility patterns of fungal isolates recovered from hospital environments with the goal of generating evidence to support improved infection control strategies and clinical management.

METHODOLOGY

Sample Collection:

It was a laboratory based cross-sectional study carried out in the department of Microbiology of Sarhad University of Science and Information Technology Peshawar as a part of research project. Duration of our study was 4 months (Jan 2023 – April 2023). 17 samples were collected from PIC using dry cotton tip swabs from different hospital equipments from five different wards including: (1) surgical ward: breathing mask, oxygen flow metre, suction jar, (2) kids ward: flow metre, oxygen mask, imbobag, stretcher, (3) CCU: vent circuit, ventelator humidifier, hand wash sink, (4) surgical ICU: ECG machine, cardiac monitor, (5) emergency: stage pump, ECG leads, stethoscope, vent filter, sterile fluid. Dry sterile cotton tip swabs were rubbed on the surfaces transferred to SUIT in sterile tubes and subsequently inoculated on SDA agar media.

Isolation of Fungus:

The plates were kept in the incubator for 5 – 7 days at 27o C until colonies appeared on the plates. After 7 days of incubation, based on appearance, colony morphology was observed.

Antifungal Susceptibility Testing

SDA media was prepared by dissolving 35g of media in 550ml of distilled water. The mixture was heated, autoclaved, and allowed to cool. Each plate was then filled with 25ml of the media and left to solidify completely. In parallel, the powder from fluconazole capsules was dissolved in 1ml of distilled water. Once the media had solidified, holes were made in the centre of each plate using the tips of a micro-

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pipette. Next, 1mm of the fluconazole solution was added to each plate, and the plates were covered with plastic wrap. The prepared plates were then incubated for 4 days at 27°C. After the incubation period, the zone of inhibition was observed for each sample.

RESULTS

Sample Collection and Isolation

Fungal growth pattern from different areas with time is shown in Table-1.

Table 1. Time wise fungal growth pattern from different sites

Total number of samples	Site of sampling	Visible hyphal growth after days		
		Day 1	Day 4	Day 7
Surgical Ward				
1	Breathing mask	No growth	No growth	No growth
2	Oxygen flow meter	---	Growth	Fungal growth
3	Suction jar	---	No growth	Fungal growth
Paeds Ward				
4	Flow meter	---	No growth	Fungal growth
5	Oxygen mask	---	Growth	Fungal growth
6	Imbobag	---	Growth	Fungal growth
7	Stretcher	---	No growth	Fungal growth
Critical Care Unit (CCU)				
8	Vent circuit	---	No growth	No growth
9	Ventilator humidifier	---	Growth	Fungal growth
10	Hand wash sink	---	No growth	Fungal growth
Surgical ICU				

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11	ECG machine	---	No growth	Fungal growth
12	Cardiac monitor	---	Growth	Fungal growth
Emergency				
13	Stage pump	---	Growth	Fungal growth
14	ECG leads	---	No growth	No growth
15	Stethoscope	---	No growth	No growth
16	Vent filter	---	Growth	Fungal growth
17	Sterile fluid	---	Growth	Fungal growth

Detail of growth frequency from different inocula is given in Table 4.2. The overall number of isolates observed was 22. The highest frequency of growth (80%) was observed from the sample collected from oxygen flow meter from surgical ward. The minimum growth (20 – 40%) was noted for samples from suction jar (surgical ward), flow meter (kids ward), stretcher (kids ward), hand wash sink (CCU) and ECG machine (surgical ICU).

Table 2. Growth frequency of different isolates

S. No.	Inoculated samples	No. of colonies seen	Frequency of growth
1	1	0	0
2	1	1	80%
3	1	1	20%
4	1	1	20%
5	1	2	60%
6	1	2	40%
7	1	1	20%
8	1	0	0
9	1	2	60%
10	1	1	20%
11	1	1	20%
12	1	1	60%

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13	1	3	60%
14	1	0	0
15	1	0	0
16	1	1	60%
17	1	5	60%

The composite total number of inocula and the isolated colonies are summarized in Table 3. All the areas chosen for sampling showed fungal growth accept; breathing mask (surgical ward), vent circuit (CCU), ECG leas (E.R) and stethoscope (E.R).

Table 3. Overall isolated fungal colonies

S. No.	Total inoculum	No. of colonies
1	17	12

Morphological features and assigned codes

Morphological and microscopic features of colonies of isolated fungi along with sample ID is presented in Table 4. Morphological features for Rhizopus sp. showed that they were opaque, white and black in colour with smooth surface and irregular in shape (Fig. 1). Penicillium showed grey colour with circular shape (Fig. 2).



Fig. 1. Rhizopus sp.

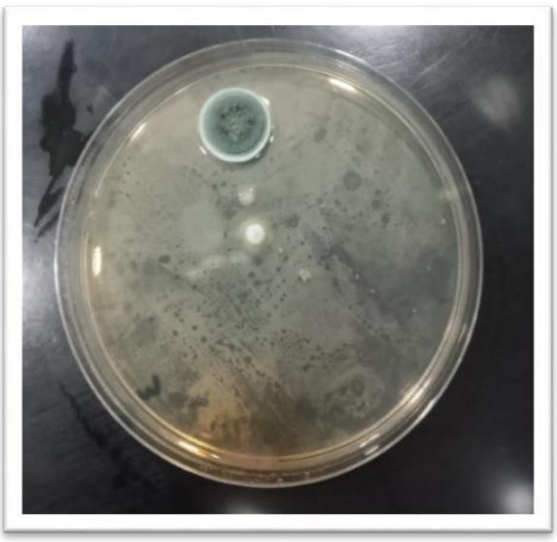


Fig. 2. Penicillium

Geotrichum candidum white in colour with D – shape (Fig.3). Cladosporium sphaerospermum dark green in round shape. Aspergillus fumigatus grey with circular shape (Fig. 4). Aspergillus niger bluish with irregular shape and Aspergillus nidulans in light green colour with circular shape (Fig. 5). Cladosporium sphaerospermum dark

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green in colour with round shape and Alternaria alternata light green with D – shape (Fig. 6). Scedosporium prolificans brown in colour with circular shape and smooth surface, Scytalidium sp. green in colour with circular shape, and Cladosporium sphaerospermum black with circular shape and rough surface (Fig. 7).



Fig. 3. Geotrichum candidum

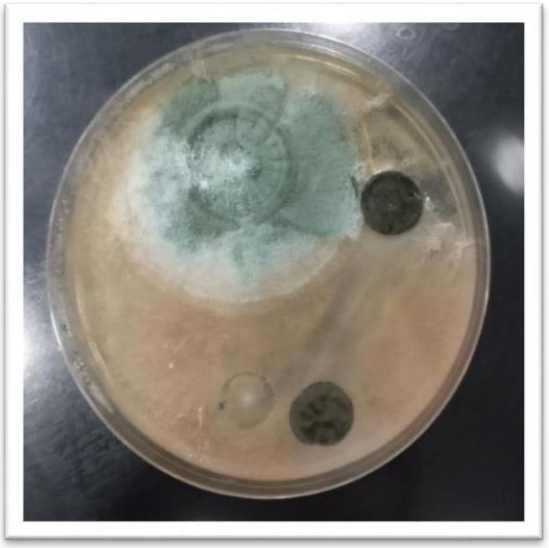


Fig. 4. Cladosporium sphaerospermum and A. fumigetus



Fig. 5. Aspergillus niger and A. nedulans



Fig. 6. C. sphaerospermum and Alternaria alternata

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Fig. 7. Scedosporium plorificans, Scytalidium sp., and C sphaerospermum

Table 4. Morphological features of isolated fungal species

S. No	Sample ID	Colony colour	Surface	Opacity	Shape of colony	Spore production	Elevation
1	Sample 1	---	---	---	---	---	---
2	Sample 2	White	Smooth	Obvious	Irregular	Yes	Raised
3	Sample 3	Grey	Smooth	Obvious	Circular	Yes	Raised
4	Sample 4	White	Smooth	Opaque	D – shape	---	Flat
5	Sample 5 – a	Dark green	Rough	Obvious	Circular	Yes	Flat
6	Sample 5 – b	Grey	Smooth	Opaque	Circular	Yes	Raised

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7	Sample 5 – a	Bluish	Smooth	Opaque	Irregular	Yes	Raised
8	Sample 6 – b	Green	Smooth	Obvious	Circular	Yes	Flat
9	Sample 7	White	Smooth	Opaque	D – shape	---	Flat
10	Sample 8	---	---	---	---	---	---
11	Sample 9 – a	Dark green	Smooth	Obvious	D – shape	Yes	Raised
12	Sample 9 – b	Light green	Smooth	Obvious	Circular	Yes	Raised
13	Sample 10	Dark green	Rough	Obvious	Circular	Yes	Flat
14	Sample 11	Brown	Smooth	Opaque	D – shape	---	Flat
15	Sample 12	Green	Smooth	Obvious	D – shape	Yes	Raised
16	Sample 13 – a	Brown	Smooth	Obvious	Circular	Yes	Raised
17	Sample 13 – b	Black	Rough	Obvious	Circular	---	Flat
18	Sample 13 – c	Green	Smooth	Obvious	Circular	Yes	Raised
19	Sample 14	---	---	---	---	---	---
20	Sample 15	---	---	---	---	---	---
21	Sample 16	White	Rough	Opaque	D – shape	Yes	Raised

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22	Sample 17 – a	Grey	Smooth	Opaque	Circular	Yes	Raised
23	Sample 17 – b	Green	Smooth	Obvious	Circular	Yes	Raised
24	Sample 17 – c	Black	Rough	Obvious	Circular	Yes	Flat
25	Sample 17 – d	White	Rough	Obvious	Circular	---	Raised
26	Sample 17 – e	Yellowish white	Smooth	Opaque	Circular	---	Raised

Microscopic identification of the isolated colonies

Microscopically identified colonies are mentioned in Table 5. Mostly found isolates were of Cladosporium sphaerospermum, observed in 7 colonies, and Aspergillus spp. found in 4 isolates. 3 colonies contained Geotrichum candidum. The remaining colonies contained Alternaria alternata, Scedosporium prolificans, Scytalidium sp., Rhizopus sp. and Pnicillium sp.



Fig. 8. Rhizopus sp.

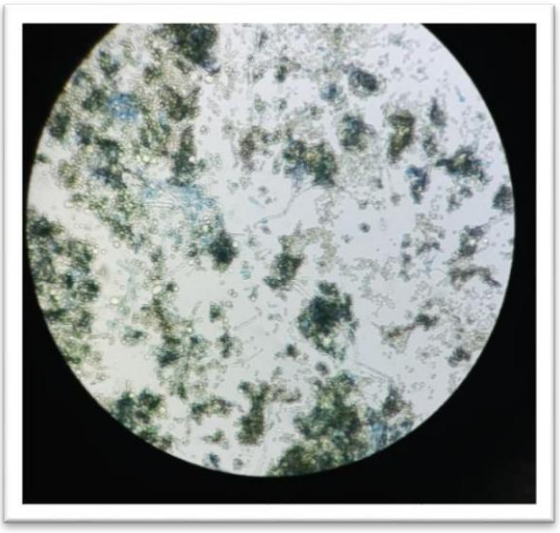


Fig. 9. Penicillium sp.

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Fig. 10. Geotrichum candidum

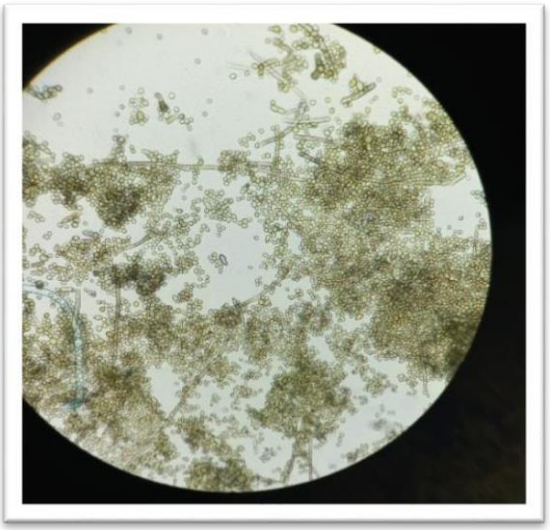


Fig. 11. Cladosporium sphaerospermum

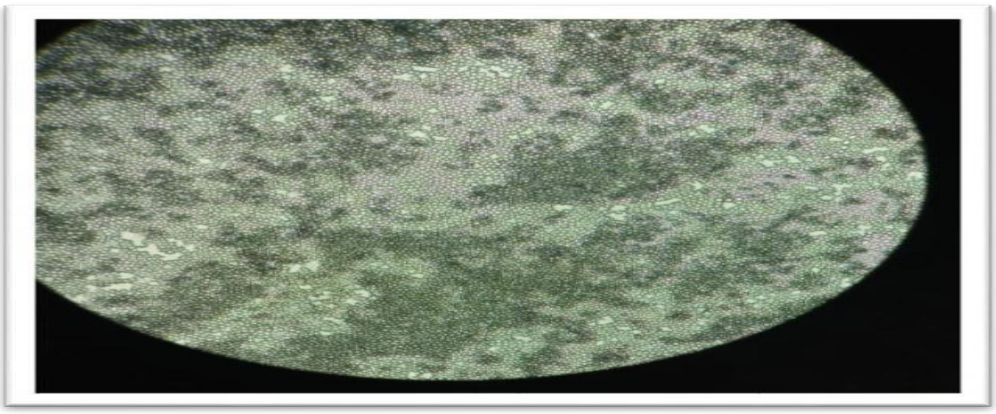
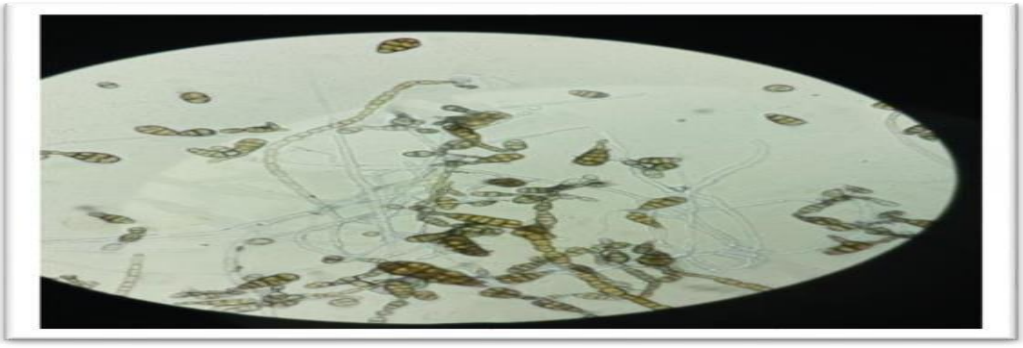


Fig. 12. Aspergillus sp.



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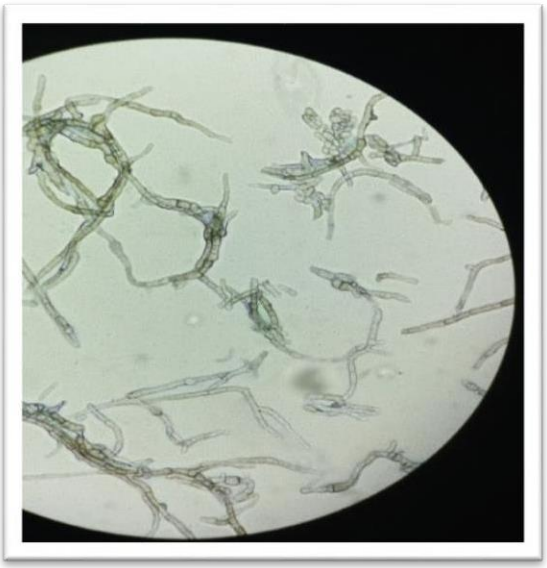


Fig. 13. Alternaria alternata

Fig. 14. Scedosporium prolificans

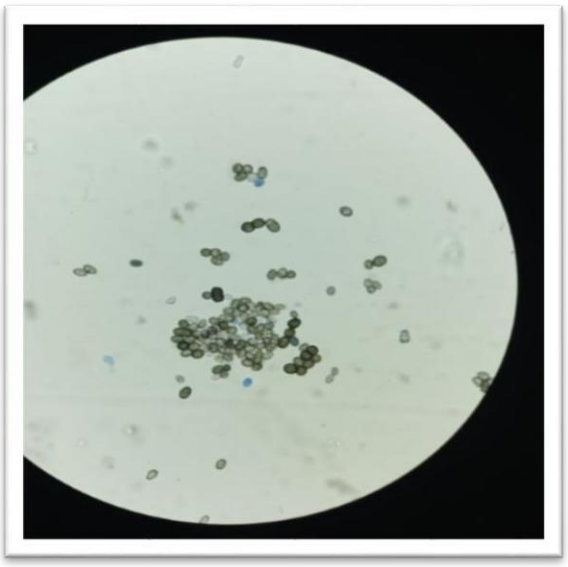


Fig. 15. Scytalidium sp.

Table 5. Microscopically identified fungal isolates

S. No.	Sample ID	Isolated fungal species
1	Sample 2	Rhizopus spp.
2	Sample 3	Penicillium spp.
3	Sample 4	Geotrichum candidum
4	Sample 5 – a	Cladosporium sphaerospermum

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5	Sample 5 – b	Aspergillus fumigatus
6	Sample 6 – a	Aspergillus niger
7	Sample 6 – b	Aspergillus nidulans
8	Sample 7	Geotrichum candidum
9	Sample 9 – a	Cladosporium sphaerospermum
10	Sample 9 – b	Alternaria alternate
11	Sample 10	Cladosporium sphaerospermum
12	Sample 11	Alternaria alternate
13	Sample 12	Cladosporium sphaerospermum
14	Sample 13 – a	Scedosporium prolificans
15	Sample 13 – b	Scytalidium spp.
16	Sample 13 – c	Cladosporium sphaerospermum
17	Sample 16	Geotrichum candidum
18	Sample 17 – a	Aspergillus niger
19	Sample 17 – b	Cladosporium sphaerospermum
20	Sample 17 – c	Cladosporium sphaerospermum
21	Sample 17 – d	Immature colony/ Unidentified
22	Sample 17 – e	Immature colony/ Unidentified

Antifungal susceptibility testing of the isolated samples

Antifungal susceptibility pattern is shown in Table 6. Fluconazole was used as an antifungal agent. Three samples were susceptible to fluconazole i.e. Rhizopus sp. (Fig. 16), Aspergillus nidulans (Fig. 17) and Aspergillus niger (Fig. 18). The remaining samples showed resistance against fluconazole.

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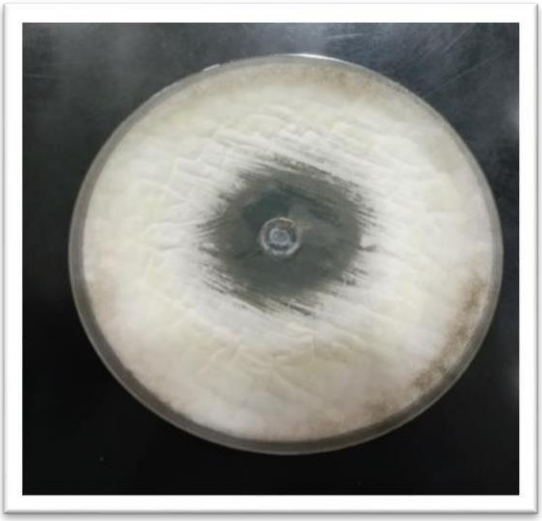


Fig. 16. Rhizopus sp.

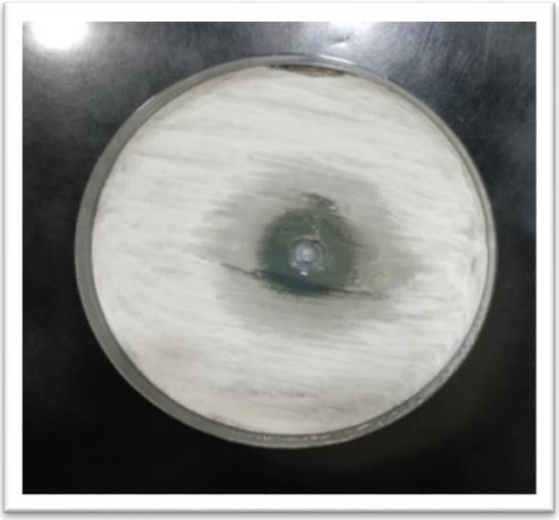


Fig. 17. Aspergillus nidulans



Fig. 18. Aspergillus niger

Table. 6. Antifungal susceptibility pattern in different fungal isolates

S. No.	Isolated fungal species	Susceptibility	Resistivity	Zone area
1	Rhizopus sp.	Susceptible	---	3cm
2	Penicillium sp.	---	Resistant	---

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3	Geotrichum candidum	---	Resistant	---
4	Cladosporium sphaerospermum	---	Resistant	---
5	Aspergillus fumigatus	---	Resistant	---
6	Aspergillus nidulans	Susceptible	---	2.2cm
7	Alternaria alternata	---	Resistant	---
8	Scedosporium prolificans	---	Resistant	---
9	Scytalidium sp.	---	Resistant	---
10	Aspergillus niger	Susceptible	---	1.5cm
11	Unidentified	---	Resistant	---
12	Unidentified	---	Resistant	---

DISCUSSION

This study conducted at PIC Peshawar, the objective were to identify fungi isolated from hospital equipment using morphological examination methods. Direct microscopy was employed which isolated and identified eight fungal species, which belonged to six different families. These species included: Rhizopus and Scedosporium prolificans from the Mucoraceae family, Penicillium and Aspergillus sp. from the Trichocomaceae family, Geotrichum candidum from the Endomycetaceae family, Cladosporium sphaerospermum from the Cladosporiaceae family, Alternaria alternata from the Pleosporaceae family, and Scytalidium sp. from the Chaetomiaceae family. It is worth noting that poor indoor air quality in hospitals can contribute to hospital- acquired fungal infections, sick hospital syndrome, and various occupational risks, as discussed by (SS Haider et al., 2017). The isolated fungal species were obtained from hospital equipment. It is important to note that exposure to airborne microorganisms in both occupational and residential indoor environments has been linked to a wide range of negative health effects, with significant implications for public health (S. Kotgire et al., 2020). This study aligns with previous research that reported the isolation of Aspergillus and Penicillium from various objects within hospitals (SS Haider et al., 2017). Conversely, our findings differ from those of (VC Keri et al., 2021), who identified Candida tropicalis, Candida parapsilosis, and Candida auris in their study. These studies offer significant contributions by shedding light on fungal contamination in diverse healthcare environments, highlighting the prevalence of particular fungal species, and examining their susceptibility to antifungal treatments. In line with our study, (OM Okolo et al., 2020) found similarities, as they isolated Aspergillus, Penicillium, and Cladosporium spp. from various hospital equipment in a tertiary hospital in Nigeria. However, in

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contrast to our findings, (F Allaw et al., 2021) reported an inability to isolate the organism from environmental surfaces. These variations underscore the importance of considering contextual factors and implementing appropriate infection control measures to mitigate fungal contamination in healthcare settings.The study of fungal load in hospital environment is important because fungi are frequent causes of allergic and invasive nosocomial infections. (VC Keri et al., 2021) concurred with our study, confirming that electronic devices and stethoscopes did not exhibit any fungal contamination. Similar to our findings, most of the studies reviewed also reported the presence of Aspergillus spp. in the majority of their samples. Our study findings were further supported by another study conducted in South India. The results of the study by (Roy et al., 2020) also confirmed that the prominent fungal species identified were Cladosporium sp., Aspergillus sp., and Alternaria alternata. Furthermore, the study observed a seasonal pattern in the occurrence of these fungal species within the fungal flora. Similar to our study, Kenyatta National Hospital (Kenimak et al., 2020) also exhibited a significant prevalence of Aspergillus spp. The quality of air in operating units is influenced by a combination of environmental and behavioral factors. These factors include temperature, humidity, and the percentage of fresh air in circulation, the type of doors, controlled entry systems, and minimizing door opening. Together, these factors work concurrently to ensure and maintain air quality within the operating units. In line with our study, several other studies have also identified mycotoxins produced by various fungal species. Like our findings, these studies from (Kenimak et al., 2019) have commonly identified the fungal genera Aspergillus, Cladosporium, and Penicillium. The presence of various opportunistic yeasts in the NICU environment suggests that these surfaces have the potential to act as a means for the transmission of fungal pathogens. However, a study published in the Journal of Clinical Microbiology presented contrasting findings to our study. According to this study, a significant number of surfaces in the NICU exhibited the presence of several yeasts, including Saccharomyces cerevisiae, Cryptococcus albidus, Debaryomyces fabryi, and Candida albicans. Among these, S. cerevisiae was found to be the most predominant fungus, accounting for up to 100% relative abundance on certain surfaces. In contrast, our study findings indicated a higher prevalence of Cladosporium sphaerospermum and Aspergillus spp. compared to other fungal species. Furthermore, our study revealed that all isolated samples exhibited resistance to fluconazole, except for Aspergillus niger, Aspergillus nidulans, and Rhizopus sp. This finding is consistent with the findings of a study conducted by (Mohsin et al., 2020) and (Oliveira et al., in 2020), which identified that all isolated samples from hospitals causing fungemia were non-susceptible to fluconazole.

CONCLUSION

The investigation and identification of fungi in PIC Peshawar revealed the presence and abundance of economically important fungi. Notably, this study was the first of its kind in PIC to utilize staining techniques for accurate identification and differentiation of various fungi. The contamination of hospital environments with fungi and the subsequent risk of nosocomial fungal infections are significant concerns for patient safety and healthcare facilities. Previous studies have emphasized the high presence of pathogenic fungi on different surfaces, objects, and personal items,

Online ISSN 3007-3197
Print ISSN 3007-3189

indicating the potential for transmission. Our study primarily focused on the fungal bioload present in hospital equipments. In our study, the highest fungal growth was observed in the pediatric ward (kids ward), indicating a need for targeted interventions in that area. The most frequently isolated fungal species included Cladosporium sphaerospermum and Aspergillus spp. precise identification and characterization of fungal isolates are crucial for appropriate treatment and the implementation of effective infection control measures. Overall, this study underscores the importance of monitoring fungal contamination in hospital environments and implementing robust infection control measures to prevent nosocomial fungal infections.

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