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# Correlation Between Sedimentation Plate and Surface Swab in the Isolation of Fungi from the Hospital Wards

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**Abstract:** Periodic monitoring of fungi in the hospital wards is undertaken by various hospitals worldwide. The objective of this study was to compare the effectiveness of the sedimentation plate method with surface swab in the isolation of various fungi from some wards of Sultan Qaboos University Hospital (SQUH).Moistened swabs were used to swab surfaces of some articles and equipments found in some wards and the hospital kitchen. The swabs were subsequently inoculated onto Sabouraud dextrose agar. All growths were examined and identified. *Penicillium* and *Aspergillus niger* had distribution frequencies of 23.8 and 23.8%, respectively by sedimentation and 20 and 33.3%, respectively by surface swab. The total isolates by sedimentation for medical and kitchen were 44 and 56%, respectively by sedimentation, while 17.1 and 60% were by surface swab, respectively. The ICU had isolation rate of 22.8% by surface and no growth by sedimentation. *Fusarium* and *Cladosporium* species were isolated by surface method from medical and ICU, but could not be isolated by sedimentation method. *A. candidius* was isolated by sedimentation, but no growth was shown by surface method. It was concluded that no single method can isolate all the fungi from sources surveyed. It is recommended that the two methods be used in parallel when monitoring the degree of fungal load in the wards and hospital kitchen. However, it is necessary that this work be repeated with larger samples and at different periods of the year so as to evaluate conclusively the effectiveness of the two systems.

**Key words:** Sedimentation · Surface · Swab · Fungi · Sabouraud · Isolation

## INTRODUCTION

Sultan Qaboos University Hospital (SQUH) is a 380 bed hospital certified by ISOBS 9001, 2000 and regularly cleaned by 3 sets of hospital staff on 24 hour duty. The hospital has never had any major outbreak of hospital acquired infection since its inception 20 years ago, though some antibiotic resistant bacteria like *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia, Escherichia coli*, *Klebsiella pneumoniae, Acinetobacter baumannii* and *Staphylococcus aureus* are often times isolated from long staying patients.

Since its certification by ISO BS 9001 2000 four years ago, the hospital has resolved to monitor and sustain its cleanliness through scrupulous supervision by the infection control team of the hospital and through persistent checks on the microbial loads, especially bacteria and fungi circulating in various wards of the hospital.

The nature of fungi readily found in some articles and equipments of hospitals worldwide include *Aspergillus fumigatus, A. niger, A. flavus, A. nidulans, A. terreus, A. versicolor and A. clavatus* [1, 2]. Other isolates include *Penicillium* species, *Cladosporium, Rhizopus* and *Fusarium* while culture swabs of some patients and medical staff were found to grow *Candida albicans, C. glabrata, C. parapsilosis, C. tropicalis* and *C. krusei* [3-5].

The origin of some of these fungi were attributable to defects in the ventilation or air conditioning systems, human activities such as walking and vacuuming within the wards, dust, air movements, water used in the wards, plants and flowers brought into the wards, food like biscuits as well as building works around the hospital [6-10]. Some of these fungi, especially *Aspergillus* species were associated with allergic bronchopulmonary aspergillosis in immunocompromised patients like asthmatics and patients with haematological malignancies

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[11-13]. In these patients, the fatality rate was up to 75% [3]. However, the correlation between the concentration of fungal spores in the wards and the risk of patients developing serious infections remains unclear [14]. Nonetheless, the need to bring the fungal load in the hospital wards to very low or non existent remains the gold standard.

The objective of this study was to compare, in less complicated, but cost effective manner, the application of sedimentation system (exposed plate) and surface swab methods in the isolation of some fungi from various locations, articles and some equipments in some wards and from the hospital kitchen. It is envisaged that the outcome of the investigation will help decide which of the two methods can be used in the isolation of fungi in the absence of the air sampling system.

## MATERIALS AND METHODS

**Specimen Collection:** Three wards (Intensive care Unit (ICU), Surgical and Medical wards were selected at random for the investigation. Humid atmosphere is known to favour the growth and spread of fungal spores hence the hospital kitchen was included in the study since it is known to be always warm and humid.

**Media:** Sabouraud dextrose agar (SDA, Oxoid, UK) made selective for fungi by the addition of chloramphenicol (0.4µg/mL) was used as isolation medium.

**Surface Swabs:** Moistened swabs were used to swab surfaces measuring approximately 1cm$^2$ of ward benches, baby incubators, fridges, walls, ward floor tiles, windowsills, telephones, air conditioning vents, wash hand sinks, patients' warm blankets, phlebotomy hand trolleys, medical staff overalls and patients beds.

Each article/equipment was swabbed once and in some cases twice weekly for three months, usually in the morning, before cleaners embark on morning duty. The swabs were subsequently inoculated onto SDA. The plates were sellotaped to avoid aerial contamination, incubated at 25°C for 7 days and examined for growth every other day.

**Sedimentation (Exposed or Settled Plate):** The sampling technique involved using two Sabouraud plates per exposure with their lids open and about 3 meters apart. The plates were placed on a four feet high table and exposed to air for an hour in the Surgical, Medical, Intensive Care Unit and the hospital kitchen. After exposure, the lids were put in place, sellotaped and the plates incubated for one week at 25°C. The sampling was done weekly within the period.

**Macroscopic Examination and Identification of Isolates**
**Sedimentation Plate:** Growth on each plate was examined macroscopically and microscopically.

**Swab Plates:** Macroscopic examination took the same pattern as in sedimentation plate.

**Identification:** Macroscopic examination involved among other things determining the shape of the conidial structures, presence or absence of colour development coupled with the size and texture of the conidia while microscopic examination and identification of isolates were based on conidial structures (shape) using lactophenol cotton blue.

## RESULTS

Table 1 shows the types of surfaces sampled and the nature of fungal isolate. The distribution frequencies of the isolates showed that *Penicillium* and *A. niger* were 10/42 (23.8%) and 10/42 (23.8%) respectively by sedimentation technique while by surface method, *Penicillium* was 7/54 (13%) and *A. niger* 18/54 (33.3%) (Figure 1). The total isolates by sedimentation was 11/42 (44%) for the medical ward and 14 (56%) for the kitchen while by surface swab method, they were 6 (17.1%) and 21/54 (60%) respectively (Fig. 2). No fungus was isolated from the surgical ward by either of the methods. ICU had a total of 8/54 (22.8%) by surface method none by sedimentation (Figure 2).

## DISCUSSION

Most fungal infections in the hospitals emanate from spores generated from air conditioners, ventilation systems, dust, wash hand sinks, flowers brought into the hospital by visitors, water used in the hospitals, staff clothes, building activities around the hospital and shoes worn by hospital staff [7-9]. In this experiment, two methods were applied to assess the nature of fungi prevalent in ICU, Medical, Surgical and the kitchen of SQUH. The experiment was not to determine the bacteria

Table 1: Types of objects sampled from each ward and fungi isolated

| Ward | Object | Samples | Fungal isolates | No of isolates |
|---|---|---|---|---|
| ICU | Sinks | 4 | Penicillium | 2 |
| | Walls of isolation rooms | 4 | Cladosporium | 1 |
| | Baby incubator surfaces | 4 | Penicillium | 2 |
| | Air conditioner vent | 2 | Cladosporium | 2 |
| | Medication fridge | 2 | Cladosporium | 1 |
| | Others: window frame, reception bench, telephone, warmer machine | 10 | Negative | nil |
| Sub total | | 26 | | 8 |
| Medical | Phlebotomy trolley | 1 | Penicillium | 1 |
| | | | A. flavus | 1 |
| | Toilet floor | 1 | Fusarium | 2 |
| | | | A. flavus | 1 |
| | | | C.uniguttulans | 1 |
| | Others: toilet door handle, medication bench, corridor wall, patients beds | 6 | Negative | Nil |
| Sub total | | 8 | | 6 |
| Surgical | Toilet door handle, phlebotomy trolley, medication preparation bench, sink, window frame, CPR trolley, | 9 | Negative | Nil |
| Sub total | | 9 | | nil |
| Kitchen | Fridge van | 1 | A. niger | 2 |
| | Fridge wall surface | 4 | A. niger | 5 |
| | | | Cladosporium | 1 |
| | Salad preparation sink | 1 | A. niger | 2 |
| | Shower head | 1 | A. niger | 3 |
| | Clean food tray | 1 | A. niger | 1 |
| | Door frame | 1 | A. niger | 2 |
| | Food trolley | 2 | A. niger | 3 |
| | | | Penicillium | 2 |
| Sub total | | 10 | | 21 |
| Total | | 54 | | 35 |

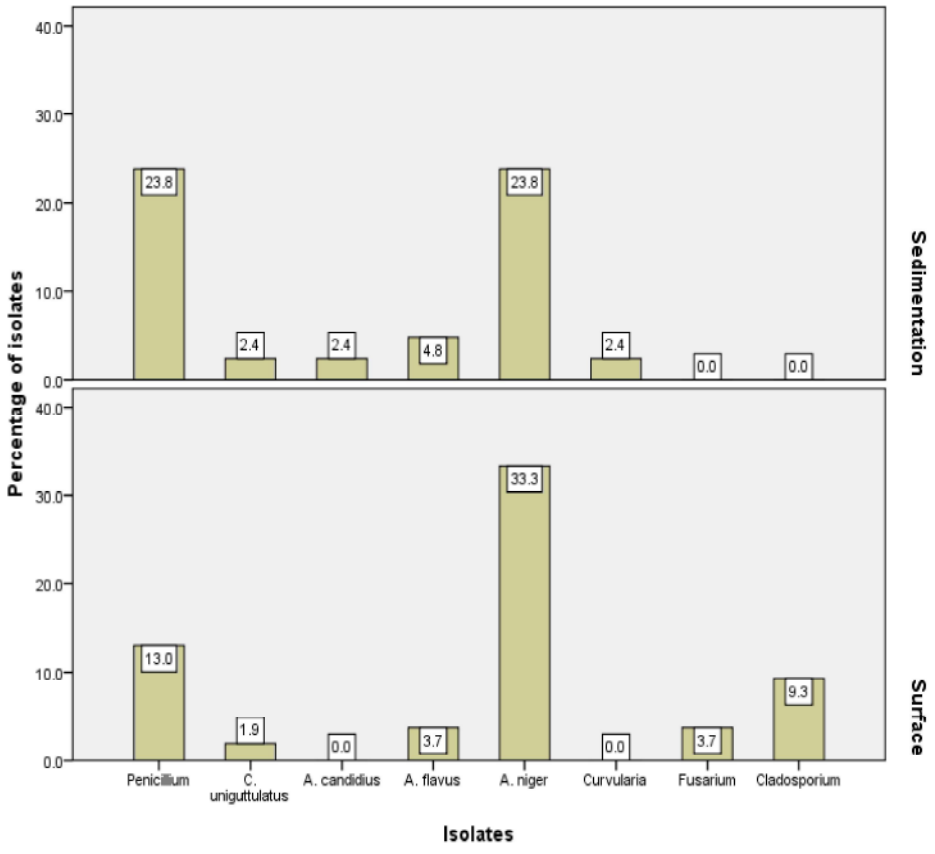


Fig. 1: The percentage (%) distribution of fungal isolates using plates and surface swabs

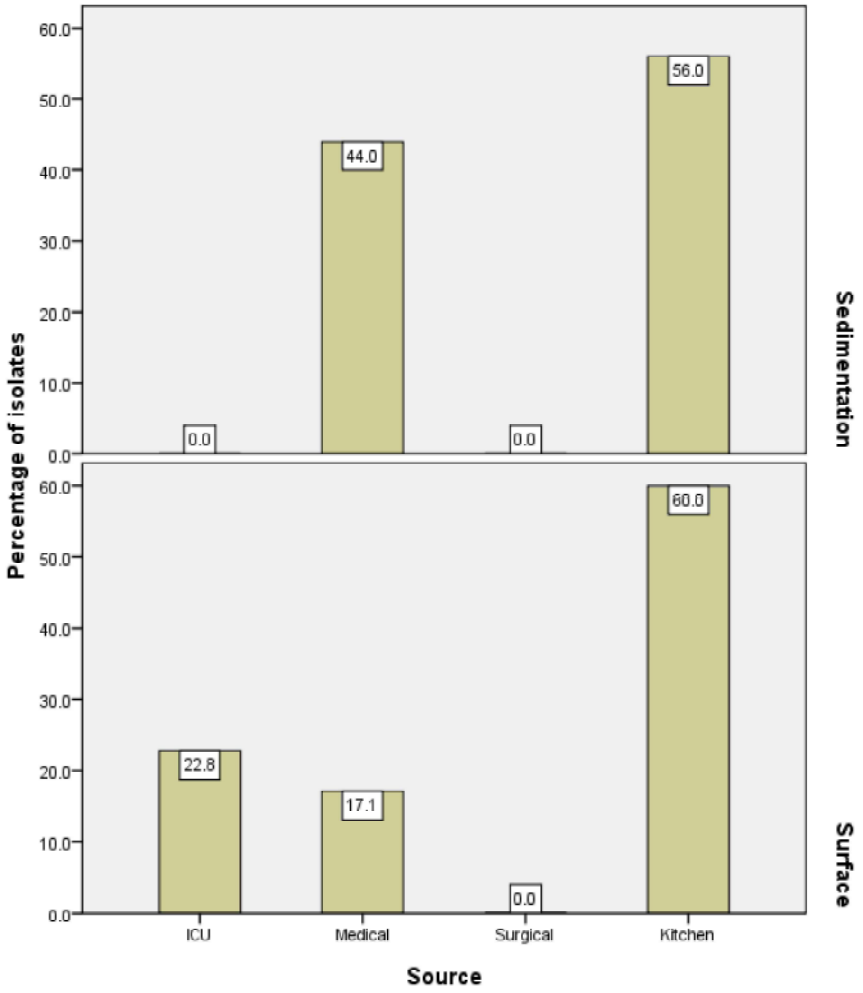


Fig. 2: The percentage (%) distribution of fungal isolates from different sources using sedimentation plates and surface swabs

carrying particle (BCP), the number of particles/colony forming units (CFU) per $m^3$ or the sedimentation rate ($CFU/m^2/hr$) but on the ability to isolate or not isolate the fungi present in the wards and some articles found in the wards using two simple and uncomplicated methods. The findings of the experiment are represented by Figures 1, 2, respectively. From the figures, no one method can be said to be better than the other for in Figure 1, *A. candidius* was not isolated by surface swab but it was isolated by the sedimentation method which also failed to isolate *Fusarium and Cladosporium* isolated by surface cultural method. *Curvularia* was not isolated by surface method but was isolated by sedimentation. In figure 2, no fungi were isolated in ICU by sedimentation plate but were isolated by surface swab. Results from the surgical ward were the same for the two methods. Though the experiments were run in parallel in which swab samples were taken the same day and nearly at the same time the exposure plates were done, differences in the isolation frequencies in the two systems are attributable to differences in the respective buoyancies, sizes of the fungal spores and number of particles per unit volume. It is likely that the fungal species that grew by sedimentation method were those circulating in the air at the material time, while surface swab grew those already settled on the surfaces. In this case, the combination of the two systems would be ideal for the isolation of the fungi circulating or have settled on surfaces in the wards. *Aspergillus* species (*A. niger, A. flavus, A. candidius*) were found more in the kitchen than in the wards because of its warm humid environment. Some workers found distribution frequencies of 65, 26.9, 19.8 and 16.2% for *Cladosporium, Fusarium, Penicillium* and *Curvularia,* respectively [1, 15, 16].

They observed that the differences in prevalent rates as reported by other investigators were due to differences in air movement, humidity, time of the day and from activities within the ward. In this investigation, *Cladosporium* and *Fusarium* were 9.3% and 3.7% respectively by surface method and negative by sedimentation plate while *Penicillium* was 23.8 and 13.0 by sedimentation and surface methods respectively. Kordbacheh [7] using surface culture found in ICU distribution frequencies of 27.5, 19.9 and 16.3% for *Penicillium* spp, *Cladosporium* spp.and *A. niger* respectively [7]. This finding contrasts with the result of their experiment. Some workers recommend that hospital wards and operating theatres should be constructed and used in such a way as to minimize the introduction, generation and retention of air-borne BCP inside them. These particles proliferate under good ambient temperature, high humidity and air pressure [17]. *Aspergillus fumigatus* spores were found to have a velocity of 1meter/hr in still air enabling it to withstand desiccation and remain indefinitely air-borne [18]. One of the fungi seen in this investigation was *C. uniguttulatus* found in the faeces of pigeons. These birds defecate around the windowsills of the wards. Though *C. uniguttulatus* was isolated, the hospital has not experienced any cryptococcosis outbreak but its isolation highlights the necessity for monitoring, on regular basis, the degree of cleanliness of hospital windowsills. Respiratory fungal infections, though rare in immunocompetent hosts, were found in immunocompromised individuals with asthma or allergy [3, 19-22]. With the continuing increase in the number of severely immunocompromised patients, it is observed that the use of filtered air in the wards can reduce the incidence of respiratory aspergillosis and thus, the fungal load in the wards [14]. The settled spores on articles and equipments in the wards if not removed immediately become nicks or nidus for growth and proliferation of more spores.

In conclusion, this work investigated the application of sedimentation (settled plate) and surface (cultural swabs) in the isolation of some fungi found in the hospital wards and kitchen of SQUH. The aim was to highlight which of the two methods could isolate fungi more than the other. Neither was found to be better than the other as their P values were 0.83 using students t-test. It is recommended that the two methods should be used in parallel when investigating the incidence of fungi in the wards.

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