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## ORIGINAL ARTICLE

# Evaluation of aerial microbial pollutants in Al-Haram Al-Nabawi during pilgrimage of 2013



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**Abstract** Al-Madinah Al-Munawwarah is the second holiest site in Islam. The possibility of new emerging microbes is valid due to the increased number of pilgrims. The objectives of the current study were to estimate the numbers of fungi and bacteria inside and outside Al-Haram Al-Nabawi and to find whether new bacterial and fungal species have emerged compared to previous studies. Air samples were collected twice a day from 12 spots and four directions during the pilgrim year of 2013 for four consecutive weeks by using the sedimentation method. Thirty five genera and fifty eight species were identified. The most recovered bacterial genera were Staphylococcus, Micrococcus, Bacillus, and Dermacoccus with 32.47%, 18.18%, 12.85%, and 11.23%, respectively. Fifty nine isolates of fungi were molecularly identified. Aspergillus species had the highest percentage (78%). The other fungal genera identified (*Alternaria triticina*, *Emericella nidulans*, *Emericella striata*, *Mucor circinelloides*, *Penicillium chrysogenum*, *Penicillium minioluteum*, *Rhizopus arrhizus*, *Rhizopus oryzae*, and *Syncephalastrum racemosum*) had less than 5% frequency. In places such as Al-Haram Al-Nabawi, a large and crowded public (millions) exist especially during pilgrimages and Ramadan, thus, exposure to microorganisms is high. On the other hand, microorganism infectivity depends on many factors including their virulence, landing site, and person's immunity. For those reasons, many aspects should be considered to avoid aerosol contaminants.

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## 1. Introduction

The number of microorganisms accounts the highest if compared to other organisms in any ecosystem (Al-Sarrani et al., 2003). Microorganisms are found all around us; in air, soil, and water.

Air is composed of a mixture of gases, water vapor, microbes, and other solid substances. It also contains a number of other contaminants including natural (microbial)

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(Al-Sarrani and Al-Turk, 1997), radiation (Al-Ghorabie, 2005), and chemicals (Al-Jeelani, 2009). However, microbes are considered one of the most important contaminants, which get more interest day after day due to the increase in human population density on one hand and the deterioration of the immunity system on another hand (Kawther, 2002).

Sources of aerosol microbes are soil surface through winds, coughing, sneezing, plants, and water (Dowd and Maier, 1999). Air is not considered a natural habitat for growth and reproduction of microbes, it is only a carrier. The number of microbes in air varies according to the surrounding environmental conditions and the amount of dust that rose into air.

The interest of studying aerial microbial pollutants started in Paris in 1899. After that, many studies were conducted in many other countries to estimate and identify the aerial microbes (Al-Suwaine et al., 1999; Roure and Ramirez, 1970).

Al Madinah Al Munawwarah is the second holiest site in Islam. All visitors to Makkah (either during Pilgrimage or during Umrah) visit this city because it has Al Masjid Al Nabawi, where the tomb of their prophet is located. According to the Central Department of Statistics and Information (CDSI) of Saudi Arabia, millions of visitors enter the country each year, thus, Al Masjid Al Nabawi area became crowded mainly during Pilgrimage and Ramadan, the fasting holy month. Visitors come from all over the world, and transcontinental movement of disease or disease vectors becomes possible.

Recent studies were conducted to study air pollutants of different gases in the crowded areas such as Mekkah and Jeddah during Pilgrimage time (Al-Jeelani, 2009, 2013; Al-Ahmadi and Al-Zahrani, 2013) and correlate the pollutants with certain diseases such as cancer (Al-Ahmadi and Al-Zahrani, 2013). Other but few studies concentrated on studying the microbial pollution in the two Harams in KSA. In 1997, Al-Sarrani and Al-Turk studied the aerial microbial pollutants in Al-Haram Al-Nabawi and the surrounding area during the pilgrimage period for five consecutive weeks. Their study concluded that the numbers of fungal and bacterial colonies vary according to the locations (three random ones) of sampling, the component of the media used, and the time of sampling. Moreover, they identified the collected fungal and bacterial isolates using the morphological and biochemical tests.

In other studies conducted in Al-Masjid Al-Haram in Makkah, the fungal and bacterial pollutants were estimated and identified (cited by Abdul Hameed and Habeeballah (2013)); Kawther, 2002). Bacterial colony numbers were found to be higher than those of fungal colonies. It ranged between 33 and 320 for bacteria and between 7 and 122 for fungi (Al-Falih and Qahtani, 1998 (cited by Abdul Hameed and Habeeballah (2013))). In another study by Kawther (2002) the aerial microbial pollutants in Makkah during Ramdan, the fasting month of 1419 H, were estimated. In this study, many bacterial isolates were identified and the total bacterial CFU ranged between 42 and 285. On the other hand, a recent publication of Abdul Hameed and Habeeballah (2013) studied in detail the airborne microbial contamination of the holy mosque (Al-Haram mosque) by the gravitational method. Their study found significant differences in the bacterial CFU concentrations between directions, and most of the collected bacteria were belonging to gram-positive bacteria. For fungi, *Aspergillus* was the predominant fungal genera. They also concluded that the microbial concentrations are more

influenced by human activities rather than meteorological factors.

The possibility of new-emerging microbes is valid due to the increased number of pilgrims during the last 15 years since Al-Sarrani and Al-Turk study (1997). The objectives of the current study were to (i) estimate the numbers of fungi and bacteria inside and outside Al-Haram Al-Nabawi by using different types of media, and confirm the identity of these fungi by using molecular techniques, and (ii) to find whether new bacterial and fungal species have emerged compared to Al-Sarrani and Al-Turk study (1997).

## 2. Materials and methods

### 2.1. Media used

Four media were used for this study: Nutrient Agar (gms/L: peptic digest of animal 5, tissue 1.5, beef extract 1.5, yeast extract 5, sodium chloride 5, and agar 15) for collecting bacteria, Sabouraud Dextrose Agar (gms/L: dextrose 40, mycological peptone 10, Agar 15, final pH (at 25 °C)  $5.6 \pm 0.2$ ) for collecting fungal isolates, blood agar (gms/L: casein enzymic hydrolysate 14.0, peptic digest of animal tissue 4.5, yeast extract 4.5, sodium chloride 5.0, agar 12.5 with a final pH (at 25 °C)  $7.3 \pm 0.2$ ) and MacConkey Agar (gms/L: peptones (meat and casein) 3, pancreatic digest of gelatin 17, lactose monohydrate 10, bile salts 1.5, sodium chloride 5, crystal violet 0.001, neutral red 0.03, agar 13.5, pH after sterilization (at 25 °C)  $7.1 \pm 0.2$ ) for differentiating between gram-positive and gram-negative bacteria. Selective Mannitol Broth (SMB) (gms/L: proteose peptone 10, beef extract 1, sodium chloride 75, D-Mannitol 10, phenol red 0.025, agar 15, final pH (at 25 °C)  $7.4 \pm 0.2$ ) to differentiate between methicillin-resistant *Staphylococcus aureus* (MRSA) and other strains of *S. aureus*. *S. aureus* ferment mannitol and produce yellow colored colonies surrounded by yellow zones, while coagulase-negative strains produce pink to red colonies surrounded by red-purple zones of *S. aureus* (mannitol non-fermenters). Presumptive coagulase-positive yellow colonies of *S. aureus* were confirmed by performing the coagulase test tube, which is used to identify and differentiate *S. aureus* from coagulase negative staphylococci.

### 2.2. Sampling dates, times, and locations

Samples were collected each Friday during the pilgrim year of 2013 for four consecutive weeks starting from 04/10/2013 to 25/10/2013. The samples were collected twice daily, one at 1.00 pm (Duhur prayer time) and the other one was at 8.00 pm (Isha prayer time). Fridays and the two times (1.00 and 8.00 pm) were selected because the number of prayers was the highest.

Twelve spots representing the four directions (South, North, East, and West) were selected for sampling. From each direction three spots were selected (inside Al Masjid Al Nabawi, outside Al Masjid Al Nabawi (at the Masjid square), and outside the Haram by 1 km from each side. At the Masjid square, four locations were chosen: Parking 2, Parking 7, Parking 11, and Parking 12. The locations chosen for sampling outside the Haram were: Street 60, King Abdul-Aziz Street, Qubaa, and Al Seeh (Fig. 1). Three plates from Nutrient

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