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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)

(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 ± 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 ± 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 ± 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 ± 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 (Duhr 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

Agar and three from Sabouraud Dextrose Agar were used for each spot selected, that was 18 plates per direction per time, and 36 plates per day. Sedimentation method (open plate technique) was used for collecting fungi and bacteria. The plates were placed on the ground level, and their lids were opened at the same time in all spots and exposed to air for 15 min. The plates were then collected and incubated at 37 °C for 24 h and 28 °C for 7 days for bacteria and fungi, respectively. Temperature and relative humidity were recorded during sampling using the BT-1 Pro, Remal Temp (www.RemalCare.com).

2.3. Microorganism's identification

2.3.1. Bacteria

Bacterial strains were sub-cultured on Blood and MacConkey agar media and differentiated to gram-positive and gram-negative bacteria. Bacterial strains were then identified using PHOENIX 100 system at the Microbiology Laboratory, Ohud Hospital, Al-Madinah Al-Munawwarah, Saudi Arabia. *S. aureus* strains were differentiated into coagulase-negative and coagulase-positive. Moreover, methicillin-resistant *Staphylococcus aureus* (MRSA) and other strains of *S. aureus* were also differentiated. Total colony forming unit (CFU) per m^3 was calculated according to Abdul Hameed and Habeeballah (2013).

2.3.2. Fungi

Fungi were sub-cultured on Sabouraud Dextrose Agar, and were morphologically identified into genus level using Barnett and Hunter (2003) identification key. Moreover, 59 representative isolates were DNA extracted and sequenced. DNA Extraction was performed using cetyltrimethyl ammonium bromide (CTAB) DNA extraction protocol and was assessed for quantity and purity. The DNA samples were sequenced using the internal transcribed spacer (ITS) at the LGC genomics, Germany to differentiate within species and among genera. Sequences were received as FASTA files and BLASTn via the NCBI database (www.ncbi.nlm.nih.gov). Sequences of collected isolates and reference sequences were aligned and dendrogram was executed with 1000 bootstrap values using the Molecular Evolutionary Genetics Analysis (MEGA version 6) software (13). Isolates sequences were submitted at the Gene bank and accession numbers were received.

2.4. Statistical analysis

Descriptive statistics including means and standard deviations, Analysis of Variance, and grouping using Tukey's method were conducted via Minitab 17 statistical software (Minitab 17 Statistical Software (2010). State College, PA: Minitab, Inc. (www.minitab.com)).

3. Results

3.1. Bacteria

A total of 4.3×10^6 CFU/m³ was estimated for the four week-collecting period. Thirty five genera and fifty eight species were identified. The most recovered genera were *Staphylococcus*, *Micrococcus*, *Bacillus*, and *Dermacoccus* accounting for 32.47%, 18.18%, 12.85%, and 11.23% of the total count, respectively (Table 1). *S. aureus* accounted for 19.7% of the genus, and *S. aureus* MRSA was recovered with low frequency (0.206%) (Table 1). Two species of *Micrococcus* were recovered: *luteus* and *lylae* with 17.98% and 0.206%, respectively. Seven species of the genus *Bacillus* M. were recovered: *Bacillus pumilus* (4.43%), *Bacillus circulans* (3.66%), *Bacillus megaterium* (2.73%), *Bacillus licheniformis* (1.36%), *Bacillus subtilis* (0.464%), *Bacillus cereus* (0.103%) and non identified
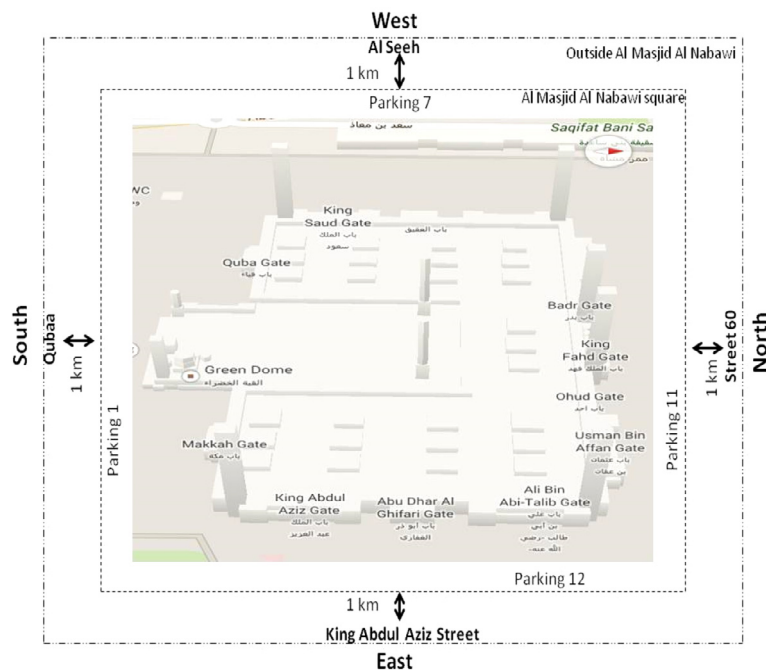

Figure 1 Sketch for the different locations of Al-Masjid Al-Nabawi from which the samples were collected.

Table 1 Percentage of the identified bacteria isolated from Al-Masjid Al-Nabawi during the pilgrimage of 2013.

Bacteria	%	Bacteria	%
Acinetobacter baumanii	0.103	*Lysinibacillus sphaericus*	2.47
Acinetobacter lwoffii/hemolysis	0.721	*Mannheimia haemolytica*	0.052
Acinetobacter species	0.567	*Micrococcus luteus*	17.98
Aerococcus viridans	0.206	*Micrococcus lylae*	0.206
Alcaligenes faecalis	0.052	*Moraxella* sp.	0.618
Arcanobacterium haemolyticum	0.412	*Non-coagulase Staphylococcus*	8.04
Arcanobacterium pyogenes	3.25	*Oerskovia xanthineolytica*	0.361
Bacillus cereus	0.103	*Pantoea agglomerans*	0.258
Bacillus circulans	3.66	*Pediococcus pentosaceus*	0.155
Bacillus coagulans	0.052	*Proteus mirabilis*	0.618
Bacillus licheniformis	1.36	*Proteus vulgaris*	0.824
Bacillus megaterium	2.73	*Providencia rettgeri*	0.052
Bacillus pumilus	4.43	*Providencia stuartii*	0.618
Bacillus sp.	0.052	*Pseudomonas aeruginosa*	0.052
Bacillus subtilis	0.464	*Pseudomonas oryzihabitans*	0.052
Brevibacillus brevis	4.64	*Pseudomonas putida*	0.155
Burkholderia cepacia	0.258	*Pseudomonas stutzeri*	0.258
CDC group-Vb3	0.67	*Rothia mucilaginosa*	0.103
Cedecea davisae	0.052	*Salmonella typhi*	0.103
Corynebacterium matruchotii	0.052	*Staphylococcus aureus*	19.7
Corynebacterium urealyticum	0.464	*Staphylococcus aureus* MRSA	0.206
Cronobacter sakazakii complex	0.206	*Staphylococcus auricularis*	0.36
Delftia acidovorans	0.258	*Staphylococcus capitis* ssp *capitis*	1.39
Dermacoccus nishinomiyaensis	11.23	*Staphylococcus carnosus*	0.155
Edwardsiella ictaluri	0.155	*Staphylococcus epedermidis*	0.309
Enterobacter cloacae	1.22	*Staphylococcus equorum*	1.18
Escherichia coli	0.017	*Staphylococcus hominis*	0.152
Escherichia vulneris	0.361	*Staphylococcus kloosii*	0.309
Klebsiella pneumonia	0.103	*Staphylococcus lentus*	0.052
Kocuria arosa	1.8	*Staphylococcus scuiri*	0.412
Kocuria varians	1.49	*Staphylococcus* sp.	0.206
Kytococcus sedenta	1.34	*Streptococcus pneumonia*	0.017
Leifsonia aquatica	0.017	*Suttonella indologenes*	0.258

Bacillus sp. (0.052%). Other bacterial genera recovered included *Brevibacillus brevis* (4.64%), *Arcanobacterium* sp. (3.66%), *Kocuria* sp. (3.29%), *Lysinibacillus sphaericus* (2.47%), *Kytococcus sedenta* (1.34%), *Enterobacter cloacae* (1.22%), and *Proteus* sp. (1.44%). The remaining genera accounted for less than 0.6% frequency (Table 1).

According to the analysis of variance, significant differences were found among dates and among locations. However, no significant differences were obtained between the time of the day and the directions (Data not shown).

Week one (04/10/2013) had the highest bacterial CFU collected followed by week two. No significance was found between number of weeks three and four (Table 2). No significant differences were found between both times (1.00 and 8.00 pm) at which the samples were collected, nor among the different directions (North, South, East, West) (Table 2). Among locations and in general, bacterial CFU was higher from the outside locations (mean of the four outside locations 26.8) compared to the square (18.63) and the inner side of Al-Masjid Al-Nabawi (17.37). For the outside locations, Al-Seeh and Street 60 had higher mean bacterial CFU/plate compared to King Abdulaziz St and Qubaa. No differences were found among the square or the inner side locations of Al-Masjid Al-Nabawi for the mean bacterial CFU (Table 2).

3.2. Fungi

A total of 335 fungal isolates were recovered. Fifty nine isolates were molecularly identified and accession numbers (KP764857-KP764914) were received from the Genebank (www.ncbi.nlm.nih.gov). Out of the 59 isolates, seven genera and 17 species were identified (Fig. 2). *Aspergillus* species had the highest percentage (78%) with *Aspergillus oryzae* accounting for the highest percent (32%) (Fig. 3). *Aspergillus flavus* and *Aspergillus niger* were the next in frequency (18.6% and 15.2%, respectively). *Aspergillus nomius, Aspergillus tubingensis*, and *A. oryzae* accounted for 13.5%, 10.2%, and 8.5%, respectively. The remaining identified species of *Aspergillus* (*austroafricans, awamori,* and *minisclerotigene*) had ⩽2% of the total count of this genus (Fig. 3). The other fungal species identified (*Alternaria triticinia,*

Table 2 Mean of bacterial colony forming units (CFU) recovered from different directions, locations, dates, and times per 9 cm^2 in Al Masjid Al Nabawi during the pilgrimage of 2013.

Variable		Colony forming unit (CFU)		
		Mean	St. dev	Grouping
Date	04/10/2013	31.64	26.23	A
	11/10/2013	16.34	10.69	C
	18/10/2013	10.97	7.30	C
	25/10/2013	24.16	11.83	B
Time of the day	1.00	20.91	18.55	A
	8.00	20.85	16.61	A
Direction	West	23.48	25.50	A
	North	22.34	18.17	A
	East	18.98	13.43	A
	South	18.30	9.22	A
Location	Al-Seeh	40.31	37.90	A
	Street 60	32.44	25.82	AB
	King Abdulaziz St	19.50	14.77	BC
	Qubaa	15.15	7.15	C
	King Abdul Majeed gate	15.02	10.48	C
	Ali Ben Abi Taleb gate	16.96	13.42	BC
	Othman Ben Affan gate	17.00	10.29	BC
	Al Hijrah gate	20.52	11.68	BC
	Parking 7	17.21	13.78	BC
	Parking 11	17.58	9.34	BC
	Parking 12	20.48	12.31	BC
	Parking 2	19.23	7.64	BC

Emericella nidulans, *Estriata striata*, *Mucor circinelloides*, *Penicillium chrysogenum*, *Pminioluteum minioluteum*, *Rhizopus arrhizus, Rhizopus oryzae*, and *Syncephalastrum racemosum*) had less than 5% frequency (Fig. 3).

According to the descriptive statistics of the fungal isolates recovered, there was a significant difference between the samples collected in 18/10/2013 compared to the other two dates. However, no significance was found among the dates of 04/10/2013, 11/10/2013, and 25/10/2013 (Table 3). No significance was found between noon (1.00 pm) and evening (8.00) times, among directions, and among the 12 locations from which the samples were collected (Table 3).

Temperature and relative humidity during sampling ranged between 23.6–47.0 °C and 20–34%, respectively. The temperature at outside locations of Al-Masjid Al-Nabawi ranged between 31.0 and 47.0 °C while it was 27.4–38.0 °C and 22.8–28.0 °C for the Masjid square and the inner parts of the Masjid, respectively (Fig. 4). Relative humidity was higher in the inner side of the Masjid compared to the square and the outer sides (Fig. 4).

4. Discussion

Studying air and microbial pollutants is an important step to understand the public health policies and to decrease the negative effect of these pollutants especially in crowded areas. Aerial microbial contamination is a critical human concern due to the possibility of transmitting pathogens (Peccia et al., 2011). Microorganism's survival in air depends on many factors including their source, dispersal in the air (Lighthart, 1997), and structure. For example, bacteria are more susceptible than other microorganisms, although some bacteria may be resistant such as *Bacillus* sp. (Fowoyo et al., 2014), and thus, can stay viable for a long time in the air (Dowd and Maier, 1999).

Hajj starts from day 8th to 12th of Thul-Hijja month of the Islamic calendar. Pilgrims must be in Makkah during that period. Before and after the actual Hajj days, pilgrims visit Al-Masjid Al-Nabawi at Madinah for worshipping and for visiting their prophet. According to the Central Department of Statistics and Information in Saudi Arabia (http://www.cdsi.gov.sa/2010-10-02-08-30-17/260-hajj1433), the total number of pilgrims in 2013 was 3,161,573 person;1,408,641 were from inside the Saudi Arabia Kingdom, and 1,752,932 were from outside the Kingdom. The statistical results showed that the pilgrims from outside the kingdom were from 107 nationalities, mainly Egyptians and Pakistanis. The 7th, 8th, and 9th day of Thul Hijja had the highest numbers when 88.5% of the pilgrims arrived at Makkah. Peccia et al. (2011) reported a link between the crowded area and adverse health effects. For that reason, in this study, time of sampling was chosen to represent variability in pilgrim's numbers. For instance, in the first, second and fourth week of collection dates, pilgrims were at their highest numbers and Al-Masjid Al-Nabawi was very crowded compared to the third week where around 90% of the pilgrims left for Makkah. Indoor and outdoor areas vary in their microorganisms content due to the variation in the temperature, humidity, and ventilation. Similarly, directions might also vary in microbe load due to wind direction, if occurred. Moreover, outside Al-Masjid locations varied due to the nature of the place from where the samples were collected (open or close area), and whether the area was crowded with

Figure 2 Dendrogram of the fungal isolates recovered from Al-Masjid Al-Nabawi during pilgrimage of 2013. The dendrogram was created using MEGA 6 software with 1000 bootstrap values.
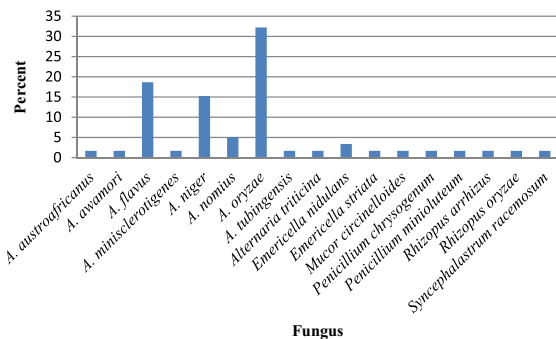
Figure 3 Percentage of the identified fungi isolated from Al Masjid Al Nabawi during the pilgrimage of 2013.

Table 3 Mean of fungal isolates recovered from different directions, locations, dates, and times per 9 cm^2 in Al-Masjid Al-Nabawi during pilgrimage of 2013.

Variable		Colony forming unit (CFU)		
		Mean	St. dev	Grouping
Date	18/10/2013	1.93	2.50	A
	25/10/2013	0.63	1.13	B
	04/10/2013	1.25	1.44	AB
	11/10/2013	0.85	1.23	B
Time of the day	1.00	1.15	1.55	A
	8.00	1.17	1.90	A
Direction	West	1.07	1.54	A
	North	1.18	1.91	A
	East	1.38	1.86	A
	South	1.03	1.61	A
Location	Al-Seeh	1.13	2.00	A
	Street 60	1.21	1.72	A
	King Abdulaziz St	1.79	2.10	A
	Qubaa	1.42	2.26	A
	King Abdul Majeed gate	0.58	0.88	A
	Ali Ben Abi Taleb gate	0.83	1.13	A
	Othman Ben Affan gate	1.75	2.59	A
	Al Hijrah gate	1.71	2.03	A
	Parking 7	1.38	1.69	A
	Parking 11	0.58	0.93	A
	Parking 12	0.58	0.88	A
	Parking 2	1.00	1.06	A

automobiles which is considered the most significant source of air pollution (Al-Jeelani, 1996). Therefore, the current study aimed to collect samples from different directions and locations.

Air sampling of microorganisms is a popular method to examine microbes because it allows direct toxicological evaluation (Yassin and Almouqatea, 2010). There are many sampling methods used to collect airborne microorganisms such as gravity deposition, impingement, impaction, and sedimentation (Griffin, 2007). In the current study, the sedimentation method was used. The sedimentation method gives a rough approximation of the numbers and types of airborne organisms (Atlas and Bartha, 1998), has shown lower false negative

results mainly for fungi (Yassin and Almouqatea, 2010), and yields data with higher standard deviation (Pitzurra et al., 1996), yet, it is considered a practical and of low cost (Atlas and Bartha, 1998).

Traditional identification of bacteria is based upon phenotypic and biochemical tests, however, limitations to its use are found. Some of these limitations are (i) inability to be used for uncultivable organisms, (ii) requirement for additional equipments and expertise bacterial groups, and (iii) some organisms do not fit into any genus or species when using biochemical tests (Woo et al., 2008). Moreover, traditional methods need time and effort. Automated methods such as PHEONIX 100 are a rapid, reliable, and accurate identification way for the identification and susceptibility testing of bacteria (Yvette et al., 2005; Snyder et al., 2008). PHOENIX system does not require additional reagents and off line tests and no major errors are produced (Yvette et al., 2005). Results of a study conducted by Duggal et al. (2012) showed that a 100% concordance was found in identification of gram-negative isolates and 94.83% for gram-positive isolates. The bacterial isolates recovered in this study were first sub-cultured on different media (Blood and MacConkey agar), then were separated based on their morphological characters, and representative isolates were analyzed using PHOENIX automated system.

For fungal identification, morphological characters were primarily used to differentiate among genera and among some species. Primary identification for the isolates showed that *Aspergillus* spp. accounted for the highest frequency. This genus has more than 180 species (Henry et al., 2000) and requires more advanced techniques to differentiate them. For that reason, representative isolates were sequenced using the internal transcribed spacer (ITS) gene region to verify and differentiate the closely related species. ITS has high rates of divergence (Ritland et al., 1993). It is considered a universal DNA barcode marker for Fungi (Schocha et al., 2012). It is used to infer closely related species and population phylogenies (King and Schaal, 1989) due to its increased sensitivity resulted from the existence of approximately 100 copies per genome (Henry et al., 2000). ITS 1 was found to be sufficient for *Aspergillus* differentiation from other molds like *Penicillium* (Gaskell et al., 1997).
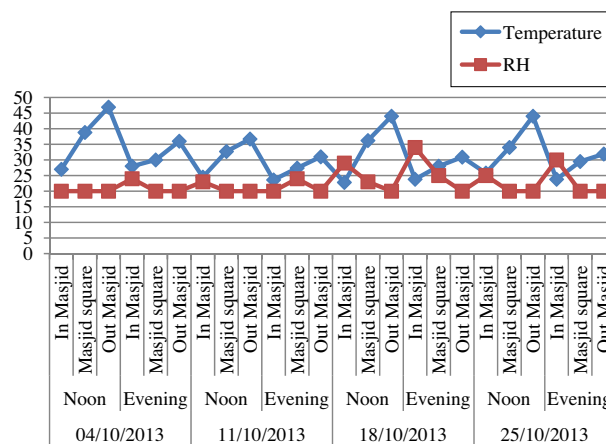

Figure 4 Temperature (°C) and relative humidity for the two times and the four collecting dates in Al Masjid Al Nabawi during the pilgrimage of 2013.

Airborne microorganisms, either molds or bacteria, rarely cause infections (Yassin and Almouqatea, 2010), however, they have an effect on elderly, children, and immune-compromised people, especially at crowded areas. The response to these microorganisms varies according to the immune system of those individuals and the type of microorganisms (ACGIH, 1999). For example, spores of some mycotoxin-producing fungi may have an effect only if it is inhaled in high concentrations (Yassin and Almouqatea, 2010).

In this study, the most recovered bacteria genera were *Staphylococcus*, *Micrococcus*, *Bacillus*, and *Dermacoccus*. *Staphylococcus* sp. (32.47%) is efficiently transmitted through direct contact and less efficiently by air (John and Barg, 1999). Nose, clothes, skin, fingers, utensils play a role in dispersing *S. aureus* (Bassetti et al., 2005; Fowoyo et al., 2014). Knowing the transmission mechanism of this genus helps in implementing preventive strategies (John and Barg, 1999). Anyone who sheds at least $3.6\,CFU/m^3$ (Bethune et al., 1965) or $7.1\,CFU/m^3$ (Noble, 1962) of *S. aureus* into the air is considered a disperser. Decreasing *S. aureus* dispersal in the air is not affected by wearing a mask (Bassetti et al., 2005). Different species of *Staphylococcus* have different medical importance. *S. aureus* is a worldwide distributed species that is known to cause skin and soft tissue infection, furuncles and carbuncles (McCaig et al., 2006). Methicillin-resistant *Staphylococcus aureus* (MRSA) is associated with worldwide healthcare- and community infections. In Europe, it was reported to affect more than 150,000 patients annually, resulting in extra hospital costs (Kock et al., 2010). *Staphylococcus auricularis*, *Staphylococcus capitis*, *Staphylococcus carnosus*, *Staphylococcus epidermidis*, *Staphylococcus equorum*, *Staphylococcus hominis*, *Staphylococcus kloosii*, *Staphylococcus lentus*, and *Staphylococcus sciuri* are coagulase negative bacteria that are found in the normal skin flora and mucous membranes, and have recently received consideration as potential pathogens (Winn et al., 2006). These strains are important mainly for patients with immune deficiency and malignancy (Mayhall, 2004). Moreover, they started to gain resistance against many antibiotics (Winn et al., 2006).

Micrococcus spp. had 18.18% prevalence in this study. It comprises worldwide genera that are found on human skins, animals, plants, soil, water, dust, and air. They are considered as harmless saprophytes; however, they may become opportunistic pathogens for immunocompromised people (Bannerman and Peacock, 2007; Kocur et al., 2006). Transmission of this genus can be through contaminated objects (Harrison et al., 2003) or inhalation of contaminated aerosols. *Micrococcus luteus* has been reported to be the causal agent of intracranial abscesses, septic arthritis, pneumonia, endocarditis, and meningitis (Bannerman and Peacock, 2007).

The third recovered species was *Bacillus* sp. (12.85%). It is a spore-forming aerobic bacterium that is widespread in the environment including the air. It is associated with the production of a toxin due to food poisoning (Fowoyo et al., 2014), and has been known as opportunist pathogens. These organisms are able of causing serious human infections such as sepsis, endocarditis, meningitis, pneumonia, and surgical wound infections (Drobniewski, 1993).

The genus *Dermacoccus* was the fourth most recovered bacteria (11.23%). It contains two species, *Dermacoccus nishinomiyaensis* and *Dermacoccus abyssi* (Pathom-aree et al., 2006). *Dermacoccus* spp. are typically associated with terrestrial habitats, skin, soil, and cured meat products (Papamanoli et al., 2002).

In the present study Gram positive bacteria were more frequent than gram negative. This could be attributed to the cell wall structure of the gram-positive bacteria and the sensitivity of the gram-negative bacteria to air environment (Perrone et al., 2007). There were lower frequencies of other bacterial species (around 45) that were identified other than the major four high-frequency bacteria mentioned above.

Airborne fungi are similar all over the world, however, dominating genera vary according to the geographic location, human activity, and plant cover (Perrone et al., 2007). Fungi require enough moisture for their activity (Lacey and Dutkiewicz, 1994), and the moisture level dictates what fungal types reproduce to problematic levels (Nevalainen, 1993). Inhaling spores of airborne fungi or their mycotoxins may cause inflammation, allergies (Dutkiewicz, 1997), and pneumonia (Hansen, 1999).

In this study, *Aspergillus* species had the highest percentage (78%) with *A. oryzae* accounting the highest recovered (32%). *Aspergillus* is a worldwide ubiquitous distributed fungus and its spores are common components of aerosols. It may disperse to short or long distances depending on environmental conditions (Bakerand Bennett, 2007). *Aspergillus* spores are found everywhere and pose no adverse health effects. However, it may cause animal diseases through mycotoxins production, allergies, and localized or systemic infections. In order to cause systemic infection in humans, immunosuppression is a pre-requisite. Aspergillosis is the disease this genus causes to animals and humans and it is non-contagious (Bakerand Bennett, 2007). *A. oryzae* and *A. niger* are not human pathogens, while *A. flavus* is the second leading cause of Aspergillosis in humans. The other fungi recovered in this study such as *Penicillium* were found in low frequencies, this could be due to high temperature conditions, low humidity, and solar radiation.

There were few similarly recovered bacterial species between the current study and Al-Sarrani and Al-Turk (1997) study. Those are *Pseudomonas aeruginosa*, *Pseudomonas stutzeri*, *M. luteus*, *Bacillus megaterium*, *B. cicculans*, and *Klebsiella pneumonia* for bacteria and *A. flavus*, *A. niger*, and *Alternaria alternata* for fungi. There are a few differences between Hajj 1997 and 2013: (1) the number of pilgrims has increased by at least one-third, (2) Hajj month was in April in 1997 but it was in October in 2013, (3) the expansion of Al-Haram Al-Nabawi surrounding areas, (4) introducing cool-mist humidifiers to the masjid square. These factors could explain the difference in the recovered species in both studies.

There were no significant differences in the mean CFU of bacteria and fungi recovered from the four directions (East, West, North, and South) in this study. This could be explained by the low wind speed (2 m/s) (https://weatherspark.com/history/32771/2013/Medina-Al-Madinah-Saudi-Arabia), and the careful sampling procedure applied at all the directions. The outside Haram locations, i.e. Al-Seeh and Street 60 (West and North, respectively), had the highest and significant bacterial CFU compared to the other locations (Qubaa and King Abdul-Aziz St). This could be due to the high density of pilgrim visitors who reside there. Moreover, no significant differences were found between indoors and outdoors (Masjid square) in the total mean CFU of bacteria or fungi (Tables 2 and 3).

Human pathogen is defined as 'a microbial or parasitic species that can infect and is capable of causing disease in humans under natural transmission conditions' (Woolhouse, 2006). In places such as Al-Haram Al-Nabawi and the Holy Mosque, a large and crowded public (millions) exist especially during pilgrimage 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 (Cox, 1987). For those reasons, many aspects should be considered to avoid aerosol contaminants. First, all visitors should concentrate on eating food that strengthens their immunity and start an antibiotic course before they arrive because many of the recovered bacteria in this study are susceptible to antibiotics (Szczerba, 2003). Second, visitors should stay away from construction work because it increases the amount of bacteria in the air (Adler et al., 2005; Loeb et al., 1995). Third, visitors should minimize the exposure time to the contaminants in order to decrease the probability of infection (Hameed and Habeeballah, 2013). This can be obtained by avoiding the crowded areas, wearing masks and following hygienic standards especially in toilets. Fourth, many of the bacteria are susceptible to a number of disinfectants like phenolic compounds, hypochlorites (1%), ethanol (70%), formaldehyde (18.5 g/L; 5% formalin in water), glutaraldehyde, and iodine (0.075 g/L) (Disinfection and Sterilization, 1993). An ongoing cleaning for the Al-Haram and the surrounding area, which is actually the situation all the time, is required.

In conclusion, many bacterial and fungal genera were isolated and identified from Al-Haram Al-Nabawi during the pilgrimage of 2013, however, most of these rarely cause infections. On the other hand, these microbes have an effect on elderly, children, and immune-compromised people, especially at crowded areas. For that reason, ongoing studies should be conducted for bioaerosol identification and prevalence in both Al-Masjid Al-Haram and Al-Masjid Al-Nabawi, and powerful detergents should always be used to minimize the affectivity of any possible infectious microbes, which is the technique currently followed.

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