Original Article

Air born bacteria evaluation in the kitchen air of restaurants in Babol City

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(Received: 16 Jan. 2019; Revised: 3 Mar. 2019; Accepted: 8 Jun. 2019)

Abstract

Background and Purpose: Bioaerosols enter into human body through various ways (inhalation, ingestion or skin absorption). The aim of this study was to investigate the type and density of the predominant bacteria in two seasons - cold and warm - in the kitchen air of restaurants.

Materials and Methods: This descriptive cross-sectional study was conducted on the air of restaurants' kitchen in Babol. Sampling was performed by using a personal sampling pump with impinger tank using a flow rate of 4 l/min during 50 minutes. Counting the colonies in the air was determined in terms of CFU/m^3 and the type of grown bacteria was identified using different tests, such as Gram staining and biochemical methods. Temperature and humidity were recorded at the time of sampling, too. Totally, 120 samples of bacteria were taken from the indoor air of kitchens with and without air conditioning.

Results: The results showed that the highest and lowest densities of bacteria in cold season were 15 CFU/m^3 and 63.7 CFU/m^3, and in warm season, they were 19.6 CFU/m^3 and 80 CFU/m^3, respectively. The predominant bacteria were Gram-positive bacteria in the air of kitchen but Bacillus and Micrococcus were the most frequent.

Conclusion: The results showed that due to humidity and the temperature (warmer), bacterial density was higher in summer than winter. The concentration of bacteria in the kitchen and restaurant was also less than WHO recommendation and guide values ACGIH (up to 500 CFU/m^3), respectively. So, air quality was found to be good and acceptable in terms of the restaurant kitchen.

Keywords: Bacteria; Air Pollution; Restaurant; kitchen

Citation: Asgharzadeh F, kermani M, **Jonidi Jafari A*,** Taheri Otaghsara S, Geraili Z. Air born bacteria evaluation in the kitchen air of restaurants in Babol City. Iran J Health Sci. 2019; 7 (2): 39-48. DOI: 10.18502/jhs.v7i2.1063

1. *Introduction*

Lifestyle has changed over the world, and as a result, people`s living has transferred from outdoors into indoors like homes and workplaces where nowadays people spend most of their time (1). Air is an essential need of human, and it contains various particles and microorganisms, so that people inhale many suspended particles via breathing in any environment. These particles have a wide range of particles and organic matters of microorganisms and the type, size, and concentration of these particles are dependent on the living and working environment of people (2). Human inhales 20 cubic metric air and microorganisms in it during 24 hours. Non-pathogenic microbes make no problem but some microorganisms are pathogenic and endanger the human health (3). Microorganisms are the most important measurement of biological aerosols. Sampling and analysis of microorganisms transmitted through air have been considered in recent years for some reasons, such as indoor air pollution, the issue of bioterrorism, health effects of bioaerosols like respiratory and infectious diseases, acute effects, allergy, and cancer (4). Microorganisms are everywhere in our environment: water, soil, air, animals, and humans. The microorganisms in the air are known as airborne microorganisms or bioaerosols (5). Bioaerosols either free or attached to other particles can be suspended in the air (6). In the past, most people just considered the air pollution in the outdoor environment, which affect human health; however, public welfare and health are also affected by physical, chemical, and biological air of indoors. The air quality of indoors is not easy to determine and control, therefore the poor air quality of these environments affects the health of workers and people who settle in these environments (7). Indoor Air Quality (IAQ) has been proposed over the last two centuries because of concerns, and it has become an important topic. Salavato recommended that biological aerosols are not available in Indoor Air. The major sources of biological aerosols in home buildings are human and animal metabolic activity products, infectious agents, allergens, fungi, bacteria in humidifiers, and bacteria in cooling devices (8). However, according to WHO recommendation and guide on values of ACGIH, the mean count of bacterial and fungal particles in indoor and outdoor environments, such as restaurants, are in the range of guide line value of 500 CFU/m^3(9). IAQ is affected by multiple factors, such as the type of source, external conditions, the structure of the building and materials, type of activity and the amount of ventilation (10). Activities, such as talking, sneezing, coughing, walking, washing, etc., can produce the biological particles (11). Bioaerosols enter into human body via various ways (inhalation, ingestion, or skin absorption), and make various health effects which include communicable diseases, acute toxic effects, allergy, and cancer. Inhalation is the most important transmission route of these microorganisms into the body. Respiratory infection and reduced lung function are created by health effects caused by bioaerosols (12). Unlike chemicals, exposure to bioaerosols does not have any health threshold due to the type of microorganisms, entrance way, and difference in individual immune response (13). Although several studies have been performed to determine the air born bacteria of indoor and outdoor (14, 15), few of those have evaluated indoor and outdoor Air born bacteria in the kitchen of restaurants.

Nowadays, food preparation premiss are occasional in our lifetime and people have to pass a significant part of their time in restaurant. Therefore, good air quality in restaurants not only protects people's health, but also enhances their enjoyment of food (16). The kitchen is one of the most crucial areas that harbors and transmits infection (17). Given the importance of bacteria in indoor pollution and few studies in this area in Iran, the aim of this study was to increase the information on the type and bacterial density in the kitchen of restaurants which can be considered as the most important centers because of preparing food.

2. *Material and methods*

Ten restaurants were selected to be studied in different parts of Babol city. Restaurants were selected because they are public places in which different people are served. All selected restaurants were traditional ones. Two season study was conducted during winter and summer in 2014-2015. The sampling from existing bacteria in the kitchen with the measurement of humidity and temperature was performed at the time of sampling. Sampling was done by using a personal sampling pump with impinger tank using a flow rate of 4 liters per minute during 50 minutes at a constant height of 1.5 meters above ground level (human respiratory height), with a distance of 60 cm from walls and barriers. Totally, 120 bacterial samples (60 with air conditioning and 60 without air conditioning) were taken from the kitchen indoor air (18, 19). The samples were collected from 11 to 13 o'clock at the height of kitchen activities. Before sampling, the tools were calibrated with flow rate and all equipment was sterilized, too. Sampling was conducted using the NIOSH (00239181) instruction-based exposure to liquid in the tank of impinger, and then, the samples were transferred to the laboratory at 4 degrees Celsius.

After transferring, the samples were filtered with a 0.45 micrometer (µm) filter. Then, the filters were transferred to the culture medium of Tryptic soy agar and Blood agar containing antibiotic Cycloheximide (concentration in 500µg/l) to prevent the fungal growth. They were then incubated for 24-48 hours at 35 degrees Celsius, and then examined for bacterial growth. Afterward, the number of bacterial colonies grown on the culture medium was counted and recorded in terms of CFU / m^3. CFU / m^3 were calculated based on the following formula.

The number of colonies/ sampling time × Flow rate sampling × (0.001).

The kind of grown bacteria was determined using different tests, such as Gram staining and biochemical detection methods including catalase, oxidase, DNAse, sugars, and urea's tests, resistance to bacitracin and novobiocin disc (20). Temperature and humidity of the air were measured to determine the relationship between the colonies and environmental conditions. Proposed maximum microbial load for IAQ was found to be 0-500 CFU / m3 according to the suggested standards for indoor air quality (21).

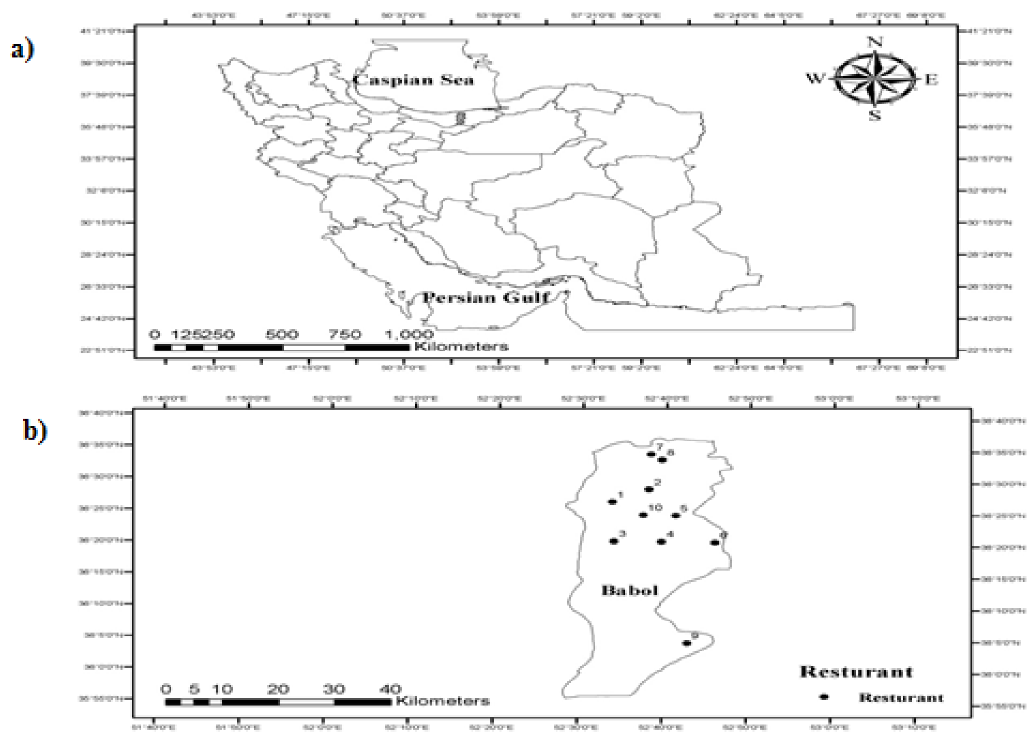

Fig 1. Geographic illustration of Babol urban area (a), sampling stations (b).

3. Results

The average, minimum and maximum density of bacteria as CFU / m^3, of indoor air of kitchen in restaurants in winter and summer, are shown in Table 1. Based on the sampling carried out on 10 restaurants, the highest density of bacteria belonged to the restaurant with code 6, and the amount of density equaled 63.7 CFU/m^3 and 80 CFU / m^3 in winter and summer, respectively. Moreover, the lowest density of bacteria was observed in the restaurant with code 1, with the amounts 15 CFU /m^3 and 19.6 CFU /m^3 in winter and summer, respectively.

Table 1. Average density of bacteria in the cold and hot season in restaurant kitchen (CFU/m^3)

Restaurant code	Cold season			Warm season		
	Min	Max	Mean±SD	Min	Max	Mean±SD
1	15	30.2	22±7.92	19.6	35	26.7±7.76
2	28.8	55	43.6±13.90	29.4	55	44.5±13.88
3	31.8	45	37.26±6.88	30	65	46.4±17.60
4	25	40	32.26±7.51	29.4	45	38.4±8.07
5	20.4	35	26.8±7.46	54	50	55.6±11.53
6	48	63.7	57.63±8.43	80	68.9	74.6±5.55
7	35	45	35.5±9.26	47	55	47.6±7.11
8	25.5	35	31.8±5.48	40	60	51.3±10.01
9	24.5	37.6	30.9±6.55	31.8	45	42.6±9.82
10	20	28.8	24.76±4.44	30	45	37.6±7.63

Table 2. Average density of bacteria in the cold and warm season in restaurant kitchen (CFU/m^3) (without air conditioning)

Restaurant code	Cold season			Warm season		
	Min	Max	Mean	Min	Max	Mean
1	20	40	28.8±10.77	24.5	45	35±10.26
2	33.6	60	51.2±15.24	49	70	59.3±10.50
3	45	50	47.6±2.50	35	70	53±17.51
4	45	35	40.8±5.18	44.1	56.1	50.1±6
5	30.6	45	36.9±7.37	52.8	79.5	64.1±13.81
6	62.4	78.4	72.4±8.74	79.5	95	86.5±7.85
7	37.1	60	49±11.48	51	70	60.7±9.50
8	35.7	50	43.5±7.25	50	70	60.7±10.02
9	39.2	43	41±1.90	47.7	66.3	56.39.37
10	35	43.2	38±4.54	45	60	53.3±7.63

The average, minimum and maximum density of bacteria as CFU / m^3, of indoor air of kitchen in restaurants without air conditioning in winter and summer, are shown in Table 2. Based on the sampling carried out on 10 restaurants, the highest density of bacteria belonged to the restaurant with code 6, with the amounts 74.4 CFU/m^3 and 95 CFU / m^3 in winter and summer, respectively. Moreover, the lowest density of bacteria was observed in the restaurant with code 1, with the density level of 20 CFU /m^3 and 24.5 CFU /m^3 in winter and summer, respectively.

Table 3. The average density of the dominant bacteria (CFU / m3) in winter and summer

Restaurant code	Bacillus spp		Micrococcus spp		Staphylococcus		pseudomonas		Moraxella		Microbacterium	
	cold	warm	cold	warm	cold	warm	cold	warm	cold	warm	cold	warm
1	5.06	13.3	15.13	8.36	NA	5.3	NA	NA	1.6	NA	NA	NA
2	11.53	23.03	26.4	14.83	NA	3.33	NA	3.26	5	NA	1.6	NA
3	10.1	19.86	18.6	11.56	NA	NA	5	9.93	NA	NA	NA	NA
4	18.73	23.4	10.2	10	NA	NA	NA	NA	NA	NA	3.33	3.33
5	6.7	30.3	16.76	16.86	NA	10.06	1.6	NA	1.6	NA	NA	NA
6	14.7	40.8	29.6	15.3	3.26	11.86	1.6	NA	3.26	NA	NA	NA
7	10.1	26.36	20.4	13.1	3.33	NA	1.6	NA	NA	NA	NA	NA
8	11.73	23.46	16.76	13.43	1.6	6.73	NA	NA	1.6	NA	NA	NA
9	8.16	17.1	16.26	15.4	NA	5.1	6.24	NA	NA	NA	NA	NA
10	6.66	18.33	16.5	10	NA	3.33	1.6	NA	NA	NA	NA	NA

As is shown in the table, the highest percentage of bacteria found in the air of kitchen in winter was Micrococcus 57.173%, Bacillus 29.08%, Pseudomonas 5.20%, Moraxella 3.82%, Staphylococcus 2.39%, and Mycobacterium 1.44%, respectively, and the highest percentage of bacteria found in the air of kitchen in summer was Bacillus 50.69%, Micrococcus 27.68%, Staphylococcus 9.88%, Pseudomonas 2.81%, Mycobacterium 0.71%, respectively. In this present study 6 dominant bacteria have been identified.

Table 4. Kitchen condition of dimension, ventilation, number of employees, and food served (Number per day)

Restaurant code	**Kitchen Dimension(m^2)**	**Ventilation condition**		**Sunlit condition**		**Food served (Number per day)**	**Number of employees**
		natural	artificial	shadow	sunlit		
1	70	×	×		×	100	7
2	45	×	×		×	110	5
3	70	×	×		×	150	9
4	70	×	×		×	150	10
5	50	×	×	×		90	4
6	30	×		×		100	4
7	40	×	×		×	100	5
8	50	×	×	×		200	8
9	70	×	×		×	150	7
10	60	×	×		×	130	9

According to the results in Table 4, it was observed that the smaller the kitchen space was, the weaker the ventilation became, which resulted in more concentration of bacteria.

4. Discussion

In all kitchens, the density of bacteria were lower than WHO recommendation and guidance ACGIH (guide line value of 500 CFU/m^3). According to the Environmental Health Unit of Health Department, 80% improvements in terms of restaurant kitchens were found in good conditions. And all kitchen windows to prevent the entry of insects and rodents were shadowed in the restaurants. The concentration of bacteria was found to be higher in summer than winter, which was due to the proper humidity, higher temperature, dusty conditions and higher human activities in summer (22, 23). Chan et al. tested bacteria in the air of two restaurants in four seasons, and represented the number of colonies identified in four seasons from spring to winter in the first restaurant to be 54, 137, 95, 37 CFU/m^3, respectively. In the second restaurant the number of colonies were 25, 103, 105, 463 CFU/m3, respectively (16). In their study, Dehdashti et al. reported the airborne bacteria concentrations in hospital kitchen to be 10/60 CFU/m3 (24), which was consistent with the results of the present study. In their study, Mushtaq et al. reported this amount to be 805 CFU/m3 as observed indoors of the kitchen (25). In another study conducted by Mentese et al., the concentration level of bacteria was found to be less in the kitchen, as most of the detected bacteria in the kitchen were Micrococcus (26). Gorny et al. reported that the density of bacteria was 88-4751 CFU/m3 in their study (27). JO et al., on the other hand, reported that the number of colonies was between 10 to 100 CFU/m3 (28), which were similar to the results of this study. The findings also showed that most of the observed bacteria included Micrococcus, Bacillus, Staphylococcus, Pseudomonas, Bacillus Moraxella, and Mycobacterium. The sources of the detected bacteria were usually from human and soil, and approximately the major groups of the detected bacteria were opportunistic pathogens and non-pathogenic bacteria (16). It should also be mentioned that Bacillus abundance is more in summer and Micrococcus abundance is more in winter. Bacillus is associated with outdoor sources, such as soil emissions, water, dust, air, feces, plants, sore, and abscesses (29). In the present study, due to the hot summer air, the kitchen windows were kept open to replace the air inside, so, the number of bacillus increased in summer samples. The presence of Micrococcus in the air of the kitchen implied inadequate ventilation (30). In the winter sample, Micrococcus were more than Bacillus due to less indoor air conditioning, because the weather was cold and windows were closed. Moraxella is a type of bacteria that is in the respiratory system, and is spread by respiratory diseases such as cold in the environment, and its incidence is higher in winter than in summer (16). The comparison of the types of bacteria detected in this study with the results of other similar studies showed that the kind of bacteria existed in the studied air were the most common types in the air of indoors. Chan et al. examined the characteristics of the bacteria in the air of two restaurants. The predominant bacteria in these restaurants were gram-positive ones including Micrococcus and Bacillus species. More detected bacteria represented species of bacteria transferred through the skin and respiratory tract by humans and soil. The number of counted colonies was also less in winter than in summer, and this amount was less than standard (500 CFU / m^3), too. Most of these bacteria were the causes of certain type of opportunistic disease (16). The results of the present study were also found to be consistent with that of Chan in terms of the type of detected bacteria (16). Gram-positive bacteria have a thick peptidoglycan layer on their cell wall which protects them against dryness, so they can survive on land. Carotenoid pigment in Micrococcus species can also protect them from damage by the ultraviolet (UV) radiation from sunlight. Bacillus can form endospore, allowing them to tolerate harsh (e.g., dry) environment, and easily disperse in the air. In addition, the bacteria isolated were usually of human and soil origins. The bacteria that come from human were mainly from the skin and respiratory tract and were released by occupants into the sites through shedding from the skin, sneezing or talking, whereas the soil bacteria were usually dispersed in the air by dust (16). In a study conducted by Yassin and Almouqatea in Kuwait, the concentration of bacteria and fungi was measured indoors and outdoors of 4 public places, such as kitchens, classrooms, laboratories, and recreational sites. In all places, the amount of bacterium was higher than fungus, and most species of detected bacteria in these environments were Diphtheroids, Staphylococcus haemolyticus, Acinetobacter, Kocuria Kristina, Micrococcus luteus, Ochrobactrum anthropi, and Staphylococcus hominis (31). The highest density of bacteria was found to be in the restaurant with Code 6, which had smaller area and more appropriate ventilation, and it has been in the shadows. The lowest density of bacteria, on the other hand, was in a restaurant with Code 1, which was properly ventilated and had bigger space and the sun visor, because sunlight has disinfection property and destroys some bacteria. Therefore, the proper design of ventilation and the location of kitchens were in the sense that the sun visor could prevent bioaerosols air pollution.

The results of the present study showed that the number and type of bacteria in the environment depends on the characteristics of the environment. The highest and lowest densities of bacteria in cold season were 15 CFU/m^3 and 63.7 CFU/m^3, respectively, and in the warm season, they were 19.6cfu/m^3 and 80 CFU/m^3, respectively. The density of bacteria in various restaurants was different, but the type of bacteria was similar in the environment. As can be seen in the results, the types of bacteria found in the kitchen have been identified. Gram-positive bacteria in addition to Gram-negative ones were more dominant. Also, Bacillus, that is resistant to drought in summer than in winter were identified. The restaurant with code 6 that had poor ventilation was more polluted. The effect of the amount and type of food on the density and type of bacteria was found to be none, and these two parameters did not influence the density of bacteria in two restaurants with Code 3 and 9, because only Pseudomonas bacteria were more recognized in cooked meat. The concentration of bacteria in the kitchen and restaurant was also less than the WHO recommendation and guide values ACGIH (up to 500 colonies per cubic meter of air), respectively. So the air quality was found to be good and acceptable in terms of the restaurant kitchen.

Acknowledgments

The authors wish to warmly acknowledge the invaluable cooperation and support from the Iran University of Medical Sciences.

Conflict of interest

The authors of this article declare that they have no conflict of interests.

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