

Microbiological detection of bacteria and fungi in the autopsy room

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Abstract: It is obviously known that the autopsy staff are under higher risk of infectious diseases than the other staff in the hospital. Inappropriate infrastructure and ventilation system installed in autopsy room could also increase such risk efficiently.

The aim of this study is to determine the presence of pathogenic bacteria and fungi in the autopsy room air, investigate the factors affecting the presence and the number of colonies of these microorganisms, and determine the extent of occupational risk in such scope.

The samples for the study were obtained from the autopsy room of Morgue Department of Turkish Council of Forensic Medicine. Samples were taken from the indoor air during, before and after autopsy by means of settle plates and air sampler in summer and spring seasons. Blood Agar and Sabouraud Dextrose Agar were used for isolation of bacteria and fungi, respectively.

Fourteen bacterial and 26 fungal species were cultured from the autopsy room air. Most frequently isolated bacteria were coagulase negative staphylococcus, *Micrococcus* spp., *Bacillus* spp., and diphtheroid bacillus for the gram positive, and *Acinetobacter* spp., *Proteus mirabilis*, and *Eschericia coli* for the gram negative groups. Most frequently isolated fungi were *Penicillium* spp., *Alternaria* spp., and *Aspergillus flavus*. When data obtained in the spring and summer was evaluated, it was found that the number of bacteria and fungi colonies grown in samples that were taken by using both methods, was significantly higher at the time of the autopsy than those taken pre and post- autopsy sessions. It was also determined that the autopsy room air had been contaminated with bacteria in 4 of 38 study days and with fungi in 18 of 38 study days.

This study could make a contribution not only in the training of autopsy personnel but also in detection of preventive measure to be taken against infections as well as to establish a common database for similar national and/or international research studies.

Key words: Autopsy room, autopsy room personnel, occupational diseases, infection, bacterium, fungus

Autopsy staff like forensic pathologists, technicians and observers could be infected through many ways while performing autopsies [1-10]. Transmitting infectious agents could be hazardous by direct inoculation and aerolization. The risk of infectious disease for the autopsy personnel increases especially in cases when the antemortem clinical and social history of the corpses are not clearly known [1-5]. The autopsy personnel could be infected by various infectious agents with high fatality, such as streptococcal sepsis, tuberculosis, AIDS, viral hemorrhagic fever [1, 3-7,11-15].

A limited number of studies has been carried out to review the microorganisms in the air quality of autopsy room [8,16,17].

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In this study, it was aimed to investigate the presence of bacteria and fungi that might be pathogen at indoor air quality of autopsy room. We also targeted to investigate the factors affecting the presence and the colony number of such microorganisms e.g. the season, temperature and humidity of indoor air, air conditioning and ventilation system, number of persons in the room, number of autopsies performed, number of tables used in the autopsy room and putrefaction level of the corpse.

2. Material and Method

This study was planned by the Forensic Medicine Department of Medical School of Dokuz Eylul University (DEUMF). The necessary permissions were taken from the Ethical Committee of DEUMF Clinical and Laboratory Research (No:154) and CFM (No:115). The samples were examined in the laboratories of Microbiology and Clinical Microbiology Department.

Sampling was done in the autopsy room of in the Department of Morgues of Council of Forensic Medicine (CFM) in İzmir as one of the biggest autopsy centers in Turkey. The autopsy room was 3 meters in height, 9 meters in length and 6 meters in width. The room had a single door, the floor and the walls had been tiled up to the ceiling, and there were five windows of which each one measured in size of 60x60 cm on each wall. The autopsy room was equipped with 10 refrigerators where the corpses can be stored and 3 autopsy tables made of stainless steel. The general air conditioning of the autopsy room was provided by the general ventilation system of the building as well as an air conditioner (without HEPA filtration) with single unit installed in the room (Alarko, APH 1259, 44.000 BTU) (Figure 1).

The autopsy room where almost 1300 autopsies have been performed annually is also used by three medical schools for the practical trainings of pre- and post-graduate forensic medicine.

The sampling in the air quality of autopsy room was done for 19 days during the spring months (April-May) and the summer months (June-July) (38 days in total). Such sampling could not be applied during the fall and winter months because of some financial insufficiencies in the project.

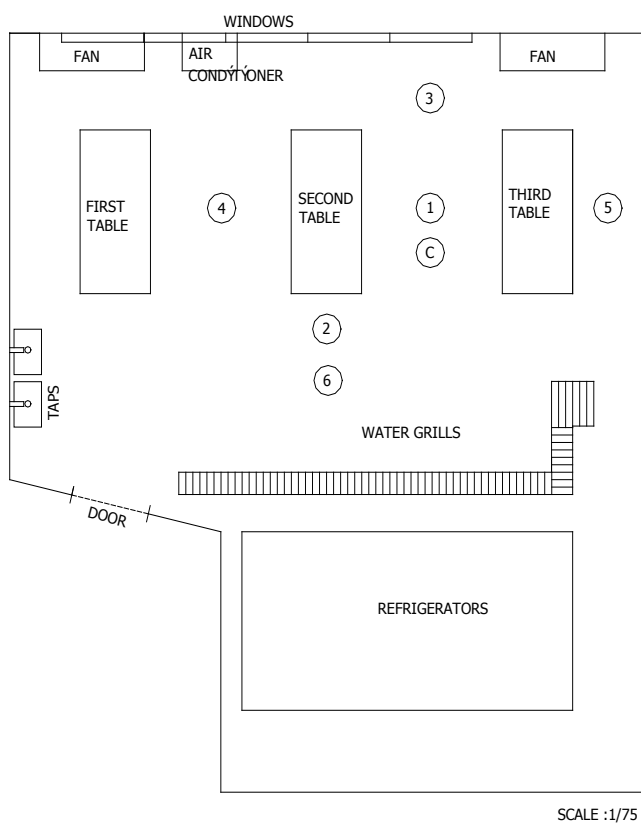
The arrangement of the petri dishes, air sampler, air conditioner and ventilation system arranged in the autopsy room and the number of tables used in the course of autopsy were evaluated by a microbiologists according to the criteria based upon the Index of Microbial air Contamination (IMA) rules (Figure 1).

Sampling was performed in 38 days from three autopsy tables, only one autopsy table was used on 4 days; 2 autopsy tables on 9 days and all 3 autopsy tables on 25 days. Since any other place was not allocated for the organ dissection in the autopsy room, this process was carried out on the autopsy table or the corp.

Two different methods were used for air sampling;

2.1. Absorption method by means of a device “AIR IDEAL” (bioMérieux, Marcy l’Etoile, France) air sampler was used for sampling. Filter covers that could be sterilized were used for each sampling. Samples were taken

Figure 1. The layout plan of the autopsy room and the place of air sampler (C) and petri dishes opened in the autopsy room



1-6 Petri Dishes placed 1 meter height
C- Air Sampler

from the predetermined places (Figure 1). According to this method, cfu/m³ (colony forming unit per cubic meter) index was used to determine the number of the colonies.

2.2. Settle plate method

Petri dishes with diameter of 9 cm were put by the microbiologists at predetermined places in the autopsy room according to 1/1/1 rule (petri dishes that will be left open for one hour, placed at an height of 1 meter from the floor and at a distance of 1 meter from the wall or any object) accepted by IMA index as a basis. According to this method, cfu (colony forming unit) index was used to determine the number of the colonies [18,19].

Air sampling was done by using these two methods pre, during and post autopsy when the room was completely cleaned up. The temperature and the humidity of the room, time of the sampling, whether or not the air conditioner and ventilation system work, and the number of the persons in the room were recorded during the sampling done pre and post autopsy.

In addition to the above mentioned items, some important factors such as the number of the autopsies done at the time of sampling, the number of the tables used in the autopsy room and the putrefaction level of corpse(s) that have been undergone autopsy (mild putrefaction: non-diffuse localized color changes on the skin; moderate putrefaction e.g. green skin color, localized bullous changes on the skin, epidermolysis; severe putrefaction e.g. green-black skin color, diffuse epidermolysis, diffuse bullous formation, wormy corpse) were kept in records at the time of sampling done during the autopsy.

By the purpose of insulating the bacteria in the air, the blood agar was used for 456 samples obtained through settle plate method and for 114 samples obtained through air sampler; and eosin methylene blue (EMB) agar was used for the passage of those considered to be gram negative among the bacteria grew in blood agar. By the purpose of insulating fungi, Sabouraud Dextrose Agar (SDA) was used for 456 samples obtained through settle plate method and for 114 samples obtained through air sampler (1140 petri dishes in total). Petri dishes containing blood agar were incubated at 37°C for 48 hours and petri dishes containing SDA were incubated at 26°C for 72 hours. The definition and colony counting of the microorganisms that have grown in the petri dishes at the end of time were achieved by a microbiologist.

2.3. In determination of contamination level of autopsy room

According to IMA rule, the value of 75 colonies that had been determined by means of settle plate method was considered as threshold level for dirtiness [18,19]. Any value that was determined in connection with fungal dirtiness could not be found in scope of international literature. A national study that was carried out previously suggested that the surgery rooms had to be considered as very contaminated [20] since fungus over 30 colonies determined by means of settle plate method. However, this value of 30 colonies was the number of fungi that was detected per square meter using the placement of the petri dishes at 1.5 to 2 m² in the surgery room for 20 minutes. In case this value was converted into IMA value using mathematical formulas, we found the number of 19 fungus colonies thus accepted this level as threshold value.

2.4. Statistical method

Data were analyzed with SPSS (Statistical Package for the Social Sciences) 11.0 statistical package program. In comparison of continuous data of “dependent groups”, t-test was used for parametric conditions, whereas “Wilcoxon test” was used for non-parametric conditions. “Mann-Whitney U test”, which was a non-parametric test, was used in comparison of continuous data of independent groups. “Mc Nemar test” was used in comparison of discrete data of dependent groups, whereas Chi-square test was used for the comparison of independent groups. A *p* value lower than 0.05 was considered significant [21].

Considering the settle plate method as threshold standard (as maximum acceptable IMA value, the values i.e. 75 cfu for bacteria and 19 cfu for fungi were taken), ROC (Receiver Operating Characteristics) curve related to the air sampler was used. ROC curve is the method used to determine the appropriate cut-off points for optimal sensitivity and specificity as well as to evaluate the diagnosis capability of a medical test [21].

3. Results

The temperature and humidity of the autopsy room, the number of the people in the room and the number of the autopsies according to the seasons are given on Table 1. The temperature of the autopsy

room pre- autopsy ($p= 0.000$), while performing the autopsy ($p= 0.004$) and post- autopsy ($p= 0.039$), were significantly higher in the summer than the values found in the spring. The humidity recorded in the autopsy room was significantly low in the summer (because of air conditioner) as compared to the value found in the spring ($p=0.009$). The amount of fungi grown detected using air sampler device when the temperature was above 24°C was observed significantly lower than those grown when the temperature was 24°C or below ($p= 0.004$). The mean humidity value of the room at the time of the autopsy was 56%. The humidity values recorded at both periods were analyzed based upon the values as 56- below and values above 56. It was determined that humidity had no effect on the amount of bacteria and fungi grown by two methods during each sampling season ($p>0.05$).

Table 1. Temperature and Humidity values of autopsy room, the number of autopsies, and the number of staffs present in the room during autopsy

Season	Mean \pm SD	23.3 \pm 1.7	55.0 \pm 5.1	23.2 \pm 1.7	57.7 \pm 4.7	23.0 \pm 1.5	63.4 \pm 5.3	3.4 \pm 1.2	12.8 \pm 8.1
	SPRING	Minimum	20.0	51.0	20.0	53.0	20.0	58.0	1
	Maximum	26.0	65.0	25.0	66.0	25.0	74.0	5	32
SUMMER	Mean \pm SD	26.5 \pm 1.6*	53.2 \pm 5.3	24.8 \pm 1.3*	53.9 \pm 3.6*	24.1 \pm 1.2*	62.6 \pm 5.2	3.2 \pm 1.5	6.1 \pm 3.2
	Minimum	23.0	43.0	23.0	47.0	22.0	53.0	1	4
	Maximum	29.0	63.0	26.0	62.0	26.0	75.0	5	16

* $p<0$

Table 2. Bacteria that grew in the air of autopsy room and the number of petri dishes that they grew in

Reproduced Bacterium	BEFORE AUTOPSY			DURING AUTOPSY			AFTER AUTOPSY			TOTAL NUMBER OF PETRI DISHES	
	Reproduction	Number of petri dishes		Reproduction	Number of petri dishes		Reproduction	Number of petri dishes		SP	AS
		SP	AS		SP	AS		SP	AS		
CNS	Present	143 (%94.1)	38 (%100)	Present	148 (%97.4)	37 (%97.4)	Present	117 (%77.0)	36 (%94.7)	408 (%89.5)	111 (%97.4)
<i>Micrococcus</i> spp.	Present	86 (%56.6)	35 (%92.1)	Present	129 (%84.9)	37 (%97.4)	Present	67 (%44.1)	37 (%97.4)	282 (%61.8)	109 (%95.6)
<i>Bacillus</i> spp.	Present	93 (%61.2)	32 (%84.2)	Present	121 (%79.6)	32 (%84.2)	Present	75 (%49.3)	30 (%78.9)	289 (%63.4)	94 (%82.5)
Difteroid basil	Present	53 (%34.9)	24 (%63.2)	Present	73 (%48.0)	22 (%57.9)	Present	34 (%22.4)	25 (%65.8)	160 (%35.1)	71 (%62.3)
<i>Acinetobacter</i> spp.	Present	3 (%2.0)	1 (%2.6)	Present	9 (%5.9)	1 (%2.6)	Present	4 (%2.6)	2 (%5.3)	16 (%3.5)	4 (%3.5)
<i>P. mirabilis</i>	Present	1 (%0.7)	0	Present	6 (%3.9)	2 (%5.3)	Present	1 (%0.7)	2 (%5.3)	8 (%1.8)	4 (%3.5)
Alfahemolytic streptococ	Present	4 (%2.6)	1 (%2.6)	Present	3 (%2.0)	0	Present	1 (%0.7)	0	8 (%1.8)	1 (%0.9)
<i>E. coli</i>	Absent	0	0	Present	3 (%2.0)	2 (%5.3)	Absent	0	0	3 (%0.7)	2 (%1.8)
<i>K. pneumoniae</i>	Absent	0	0	Present	1 (%0.7)	2 (%5.3)	Absent	0	0	1 (%0.2)	2 (%1.8)
<i>Enterobacter</i> spp.	Absent	0	0	Absent	0	0	Present	2 (%1.3)	0	2 (%0.4)	0
<i>S. aureus</i>	Absent	0	0	Present	0	1 (%2.6)	Absent	0	0	0	1 (%0.9)
<i>Enterococcus</i> spp.	Absent	0	0	Absent	0	0	Present	0	1 (%2.6)	0	1 (%0.9)
<i>P. vulgaris</i>	Absent	0	0	Present	1 (%0.7)	0	Absent	0	0	1 (%0.2)	0
nonAnonB streptococ	Absent	0	0	Present	1 (%0.7)	0	Absent	0	0	1 (%0.2)	0

CNS: Coagulase negative staphylococci

SP: Settle plates

AS: Air sampler

Bacterium species grown in the air quality of autopsy room pre, during and post autopsy and the number of petri dishes grown are given on Table 2. Gram negative bacteria grew in air quality of autopsy room and the characteristics observed in such period are given on Table 3.

Fungi grown up in the air quality of autopsy room pre, during and post autopsy and the number of petri dishes grew are given on Table 4. According to the seasons, the numbers of bacterial and fungal colonies that have been grown up pre, during and post autopsy are shown on Table 5.

The effects of factors i.e. the season, the air temperature of autopsy room, humidity, air conditioner and ventilation system, the number of people in the room, the number of autopsy completed, the number of table used in autopsy room and the putrefaction level of corpse in autopsy on the number of colonies have been shown in Table 6.

Table 3. Gram negative bacteria grew in the present study

Date of autopsy	Period	Number of colony	Method	Number of person	Ventilation	Air conditioner	Number of autopsy	Putrefaction
<i>K. pneumoniae</i>								
21.04.	During autopsy	5	AS	20	Open	Closed	5	Absent
26.04.	During autopsy	4	SP(number 2)	14	Open	Closed	4	Absent
08.07.	During autopsy	1	AS	5	Closed	Open	4	Absent
<i>E. coli</i>								
21.04.	During autopsy	2	SP(number 2)	20	Open	Closed	5	Absent
26.04.	During autopsy	1	SP(number 1)	14	Open	Closed	4	Absent
27.04.	During autopsy	7	AS	14	Open	Closed	4	Absent
21.07.	During autopsy	9	AS	5	Open	Open	5	Absent
21.07.	During autopsy	23	SP(all of which)	5	Open	Open	5	Absent
<i>Acinetobacter spp.</i>								
27.04.	Before autopsy	10	AS	1	Closed	Closed		
27.04.	During autopsy	7	SP (number 1,2,6)	14	Open	Closed	4	Absent
27.04.	After autopsy	16	AS	2	Closed	Closed		
27.04.	After autopsy	29	SP(number 2,3)	2	Closed	Closed		
31.05.	During autopsy	7	SP (number 2,4)	32	Open	Closed	3	Absent
31.05.	After autopsy	1	SP (number 4)	1	Closed	Closed		
06.07.	After autopsy	1	SP (number 4)	1	Open	Open		
21.07.	Before autopsy	6	SP (number 2)	1	Closed	Open		
22.07.	Before autopsy	2	SP (number 2)	1	Closed	Open		
22.07.	During autopsy	10	SP(all of which)	5	Open	Open	3	Absent
22.07.	During autopsy	5	AS	5	Open	Open	3	Absent
<i>P. mirabilis</i>								
22.05.	During autopsy	5	AS	6	Open	Closed	4	Present
22.05.	During autopsy	4	SP (number 6)	6	Open	Closed	4	Present
22.05.	After autopsy	7	AS	2	Open	Closed		
22.05.	After autopsy	4	SP (number 2)	2	Open	Closed		
17.07.	During autopsy	25	AS	5	Open	Open	5	Present
17.07.	During autopsy	46	SP (all of which)	5	Open	Open	5	Present
29.07.	During autopsy	6	SP (number 3)	4	Closed	Open	1	Absent
<i>P. vulgaris</i>								
01.06.	During autopsy	1	SP (number 1)	27	Open	Closed	2	Present
<i>Enterobacter spp.</i>								
22.05.	After autopsy	2	SP (number 1,4)	2	Open	Closed		

AS: Air sampler

SP: Settle plate

124 autopsies consisting of 64 in the spring and 60 in the summer were performed in total. Only 1 autopsy was performed in the autopsy room at 4 of 38 days during the sampling time then more than 1 autopsies were performed in the rest 34 days.

There were totally 9 autopsies of putrefied corpses in 5 days during the spring and 2 days during the summer. The degree of putrefaction was mild in 1, moderate in 3 and severe in 5 of them. It was determined that the presence of putrefied corpses among the autopsies performed during the spring and summer time had no effect on the number of bacteria and fungi grown by means of using two methods ($p>0.05$). *P. mirabilis* was the only gram negative bacterium that grew on the autopsy days of putrefied corpses. The growth of *P. mirabilis* on the autopsy days of putrefied corpses was found statistically significant when compared to the days when autopsy of putrefied corpse was not performed ($p= 0.002$).

In order to determine the acceptable level of contamination with the number of colonies obtained by using these two methods in the study, maximum acceptable IMA value was considered as to be 75 cfu for bacteria and 19 cfu for fungi. The cut-off point (cfu/m³) for the values determined by means of air sampler was tried to be determined by means of ROC curve.

Table 4. Fungi that grew in the air of autopsy room and the number of petri dishes that they grew in.

Reproduced fungi	BEFORE AUTOPSY			DURING AUTOPSY			AFTER AUTOPSY			TOTAL NUMBER OF PETRI DISHES	
	Reproduction	Number of petri dishes		Reproduction	Number of petri dishes		Reproduction	Number of petri dishes		SP	AS
		SP	AS		SP	AS		SP	AS		
<i>Penicillium</i> spp.	Present	103 (%67.8)	32 (%84.2)	Present	134 (%88.2)	32 (%84.2)	Present	101 (%66.4)	35 (%92.1)	338 (%74.1)	99 (%86.8)
<i>Alternaria</i> spp.	Present	35 (%23.0)	9 (%23.7)	Present	50 (%32.9)	14 (%36.8)	Present	30 (%19.7)	16 (%42.1)	115 (%25.2)	39 (%34.2)
<i>Aspergillus flavus</i>	Present	20 (%13.2)	12 (%31.6)	Present	35 (%23.0)	14 (%36.8)	Present	9 (%5.9)	12 (%31.6)	64 (%14.0)	38 (%33.3)
<i>Aspergillus niger</i>	Present	7 (%4.6)	9 (%23.7)	Present	38 (%25.0)	19 (%50.0)	Present	8 (%5.3)	12 (%31.6)	53 (%11.6)	40 (%35.1)
<i>Aspergillus fumigatus</i>	Present	19 (%12.5)	11 (%28.9)	Present	27 (%17.8)	10 (%26.3)	Present	7 (%4.6)	7 (%18.4)	53 (%11.6)	28 (%24.6)
<i>Mucor</i> spp.	Present	5 (%3.3)	5 (%13.2)	Present	11 (%7.2)	9 (%23.7)	Present	5 (%3.3)	5 (%13.2)	21 (%4.6)	19 (%16.7)
<i>S. apiospermum</i>	Present	7 (%4.6)	6 (%15.8)	Present	10 (%6.6)	4 (%10.5)	Present	4 (%2.6)	2 (%5.3)	21 (%4.6)	12 (%10.5)
<i>Fusarium</i> spp.	Present	2 (%1.3)	2 (%5.3)	Present	6 (%3.9)	1 (%2.6)	Present	5 (%3.3)	1 (%2.6)	13 (%2.9)	4 (%3.5)
Maya benzeri mantar	Present	4 (%2.6)	1 (%2.6)	Present	6 (%3.9)	1 (%2.6)	Present	1 (%0.7)	3 (%7.9)	11 (%2.4)	5 (%4.4)
<i>C. sitophila</i>	Present	3 (%2.0)	2 (%5.3)	Present	4 (%2.6)	1 (%2.6)	Present	4 (%2.6)	1 (%2.6)	11 (%2.4)	4 (%3.5)
<i>Chrysosporium</i> spp.	Present	4 (%2.6)	2 (%5.3)	Present	4 (%2.6)	1 (%2.6)	Present	0	2 (%5.3)	8 (%1.8)	5 (%4.4)
<i>Trichoderma</i> spp.	Present	4 (%2.6)	3 (%7.9)	Present	3 (%2.0)	2 (%5.3)	Absent	0	0	7 (%1.5)	5 (%4.4)
<i>Aspergillus glaucus</i> group	Present	2 (%1.3)	0	Present	5 (%3.3)	1 (%2.6)	Present	1 (%0.7)	1 (%2.6)	8 (%1.8)	2 (%1.8)
<i>Acremonium</i> spp.	Present	2 (%1.3)	1 (%2.6)	Present	1 (%0.7)	1 (%2.6)	Present	0	3 (%7.9)	3 (%0.7)	5 (%1.1)
<i>Aspergillus versicolor</i>	Present	3 (%2.0)	0	Present	3 (%2.0)	0	Present	1 (%0.7)	0	7 (%1.5)	0
<i>Paecilomyces</i> spp.	Present	4 (%2.6)	1 (%2.6)	Present	0	2 (%5.3)	Absent	0	0	4 (%0.9)	3 (%2.6)
<i>Aspergillus nidulans</i>	Absent	0	0	Present	2 (%1.3)	1 (%2.6)	Present	2 (%1.3)	1 (%2.6)	4 (%0.9)	2 (%1.8)
<i>Bipolaris</i> spp.	Present	0	1 (%2.6)	Present	1 (%0.7)	1 (%2.6)	Present	1 (%0.7)	1 (%2.6)	2 (%0.4)	3 (%2.6)
<i>A. pullulans</i>	Present	0	1 (%2.6)	Present	2 (%1.3)	1 (%2.6)	Absent	0	0	2 (%0.4)	2 (%1.8)
<i>Rhizopus</i> spp.	Present	1 (%0.7)	0	Present	3 (%2.0)	0	Absent	0	0	4 (%0.9)	0
<i>M. sitophila</i>	Present	1 (%0.7)	1 (%2.6)	Present	1 (%0.7)	0	Absent	0	0	2 (%0.4)	1 (%0.9)
<i>Scopulariopsis</i> spp.	Present	0	1 (%2.6)	Present	1 (%0.7)	0	Present	0	1 (%2.6)	1 (%0.2)	2 (%1.8)
<i>Rhodotorula</i> spp.	Absent	0	0	Present	1 (%0.7)	0	Present	1 (%0.7)	0	2 (%0.4)	0
<i>Sporotrichum</i> sp.	Absent	0	0	Present	2 (%1.3)	0	Absent	0	0	2 (%0.4)	0
<i>C. tropicalis</i>	Absent	0	0	Present	0	1 (%2.6)	Absent	0	0	0	1 (%0.9)
<i>Verticillium</i> spp.	Absent	0	0	Present	1 (%0.7)	0	Absent	0	0	1 (%0.2)	0

Table 5. Comparison of the amount of bacterial and fungal colonies that grew via settle plate and air sampling method

	SETTLE PLATES								AIR SAMPLER							
	Before Autopsy		During Autopsy		After autopsy		p*		Before Autopsy		During Autopsy		After autopsy		p*	
	Spring	Sum-mer	Spring	Sum-mer	Spring	Sum-mer	Spring	Sum-mer	Spring	Sum-mer	Spring	Sum-mer	Spring	Sum-mer	Spring	Sum-mer
Bac-terium	9.1± 5.7	27.4± 22.1	51.1± 17.1	60.9± 65.7	21.6± 49.3	19.7± 21.6	†0.000 ‡0.004 ±0.952	†0.028 ‡0.002 ±0.184	222.4± 107	140.4± 68.1	459.1± 290.8	306.9± 190.6	291.7± 253.8	191.4± 129.4	†0.014 ‡0.064 ±0.494	†0.001 ‡0.005 ±0.080
Fun-gus	2.7± 1.7	16.7± 26.3	117.8± 271.6	99.3± 175.6	13.0± 28.4	9.2± 10.5	†0.000 ‡0.006 ±0.014	†0.004 ‡0.000 ±0.394	66.0± 40.0	95.2± 55.7	352.7± 456.3	298.9± 234.9	249.3± 282.3	124.4± 108.7	†0.001 ‡0.673 ±0.005	†0.002 ‡0.003 ±0.695

*Wilcoxon

† Before autopsy- During autopsy

‡ During autopsy –After autopsy

± Before autopsy -After autopsy

Table 6. Factors which effect the number of colonies

FACTORS	SPRING		SUMMER	
	Number of the bacterial colonies	Number of the fungal colonies	Number of the bacterial colonies	Number of the fungal colonies
Number of the person	+(p=0.033) SP	+(p>0.05)	+(p>0.05)	+(p>0.05)
Number of the autopsy	+(p>0.05)	+(p>0.05)	+(p>0.05)	+(p>0.05)
Temperature	+(p>0.05)	+(p>0.05)	+(p>0.05)	+(p=0.004) AS
Humidity	+(p>0.05)	+(p>0.05)	+(p>0.05)	+(p>0.05)
Air conditioner	+(p>0.05)	+(p>0.05)	+(p>0.05)	+(p>0.05)
Ventilation system	+(p>0.05)	+(p>0.05)	+(p>0.05)	+(p=0.016)SP +(p=0.019)AS
Number of the table used	+(p>0.05)	+(p>0.05)	+(p>0.05)	+(p>0.05)
Putrefaction degree of the corpse	+(p>0.05)	+(p>0.05)	+(p>0.05)	+(p>0.05)

SP: Settle plate

AS: Air sampler

The cut-off value for the contamination was considered to be 258 cfu/m³ when the sensitivity was found as 86% and the specificity as 68% for the bacteria determined using air sampler whereas it was considered to be 126 cfu/m³ when the sensitivity was found as 79% and the specificity as 74% for the fungi determined using air sampler.

When the number of bacterial colony growth determined using settle plate method was considered, the number of uncontaminated days 'prior' to the autopsy was not significantly higher than those during the autopsy days (p>0.05). However, this value for fungal colony number was 17 times higher significantly. Also, the number of uncontaminated days 'after' the autopsy was 16 times higher significantly than the number of uncontaminated days 'at the time' of the autopsy (p=0.000).

When the number of bacterial colony growth by air sampler was considered, the number of uncontaminated days 'prior' to the autopsy was 4.75 times higher significantly than that of the 'at the time' of the autopsy days (p=0.003), whereas it was 10.5 times significantly higher in fungal colony number (p=0.000).

The number of uncontaminated days 'after' the autopsy in bacterial colony growth was 2.16 times higher significantly than the number of uncontaminated days 'at the time' of the autopsy ($p=0.035$), whereas it was insignificant in fungal colony numbers ($p>0.05$).

4. Discussion

Despite the levels of bacteria and fungi allowed to be present in the air (hazardous levels) that have been confirmed for hospitals and laboratories there is not a standard sampling method offered for autopsy rooms including frequency and location of sampling, incubation time and etc [19,20,22,23].

It was reported that the amount of normal bacterial flora, particularly such as CNS, diptheroid bacillus and *Micrococcus* spp., were increased with the number of staff present in the room. It was also stated that *S. aureus* does not commonly exist in dermal flora like CNS and *Micrococcus* spp., but is rarely present [16,24-28)].

In a study conducted by Obbard and colleagues [29] using air sampler, it was emphasized that the staff number was the key point used to determine the amount of bacteria in the air, and importance of humidity is increased according to the location from which the sample is obtained. In this present study, when the number of the colonies obtained using both methods is evaluated together, CNS, *Micrococcus* spp., *Bacillus* spp. and diptheroid bacillus were the most frequently insulated microorganisms respectively, and the number of staff and humidity had no significant effect on the number of colonies obtained via air sampler. However, we believe that it is difficult to determine the origin of these bacteria, since they can be present in antemortem flora of the cadaver.

In England in 1986 and 1987, Babb et al. had obtained samples from the breathing air of 30 autopsy rooms via air sampler, and demonstrated that *Staphylococcus epidermidis*, *Micrococcus* spp. and diptheroid bacillus were most commonly insulated organisms, and *S. aureus* was insulated in 11% of all samples. They also reported that the insulated gram negative bacteria were *Acinetobacter* spp., *Enterobacter* spp., *Proteus* spp., *Klebsiella* spp., *Citrobacter* spp., *Serratia* spp. and *E. coli* [16]. In a study performed by Newson and colleagues, they insulated gram positive coccus, particularly *S. epidermidis* and *Sarcina* spp., in addition to coliform bacteria and they determined that *S. aureus* was minimally grown by air sampler (17).

In this study, *Citrobacter* spp., and *Serratia* spp., which were the gram negative bacteria that had been insulated in the study conducted by Babb et al. [16], was not grown. *S. aureus* was insulated from 2.6% of the samples obtained via air sampler. *S. aureus* was minimally insulated in the present study in a similar way with the study conducted by Newson and colleagues [17].

In both studies, it was concluded that the bacteria originated from the skins of the staff members rather than those of cadavers, and it was demonstrated that the amount of bacteria was increased as the number of staff in the room increased [16,17]. In this study, significant similarity was observed between the bacterial colony amount and the increased staff number only in the spring by the settle plate method.

It is suggested that *Bacillus* species commonly exist in the soil, in the air and in dusty environments in the nature. The relevant certain species existing in the intestinal flora of human and animals and *Bacillus* species other than *Bacillus anthracis*, could not cause an infection in the patients except for those with immune deficiency [27,30]. It is emphasized that diptheroid bacillus as well (*Corynebacterium* spp.) may exist in the soil, in the air, in the skin and in mucous membranes. However, these species, other than *Corynebacterium diphtheriae*, are not considered as pathogens for the patients except for those with immune deficiency [22,31,32]. Although both bacteria were insulated in this present study before-during and after autopsy by the 2 methods, detailed microbiologic examination could not be performed to determine their species.

Since gram negative bacteria other than *Acinetobacter* spp. are susceptible for dryness, they are not expected to exist in the air [20,30,31]. In the present study, it was observed that gram negative bacteria, such as *E. coli*, *K. pneumoniae* and *P. vulgaris*, were grown only during the sampling 'at

the time' of the autopsy. Gram negative bacteria growth in the samples of the autopsy room air 'at the time' of the autopsy has indicated that these bacteria might have originated from the cadaver.

It was expressed that *Acinetobacter* spp. was relatively resistant to dryness as compared with other gram negative bacteria [20]. We believe that the growth of *Acinetobacter* spp at each all sampling periods may be related to the above mentioned characteristics. It is conspicuous that *P. mirabilis*, which is one of the gram negative bacteria, has been grown 'at the time' of the autopsy only during the days when putrefied corpse autopsies have been performed.

In this study, the amount of bacterial colonies obtained in the spring and in the summer via both methods was significantly higher 'at the time' of the autopsy than those obtained 'pre' to and 'post' the autopsy. We believe that increased amount of microorganisms in the air at the time of the autopsy may be associated with the autopsy procedures.

M. tuberculosis is an important microorganism that can be hazardous at autopsy but we can not comment about this microorganism in our study. We did not aim to investigate fastidious slow growing microorganisms and *Mycobacterium tuberculosis* since different appropriate culture media was needed for these microorganisms. We could not incubate blood agar plates more than 48 hours as the surface of the plates were thoroughly covered by the colonies. Our interpretation of the results involves the bacteria that can be grown up on blood agar during 48 hours and the fungi on SDA after 72 hours.

It is explained that the fungi takes place in the putrefaction period of energy cycle in the nature [33]. In this study, the contamination with fungi, which was determined by using both methods within 5 of 7 sampling days including the days of putrefied corpses, could indicate that the contamination was resulted from putrefied corpses. We consider that isolation of many different fungi types (Table 3) may be related to seasonal and environmental factors, as well as to the differences between the places where the cadavers are present before the autopsy.

Airborne transmission may lead to aspergillosis in the patients with immune deficiency [22,34]. *Aspergillus* spores always exist in non-filtrated air [20]. *Mucor* spp., *Rhizopus* spp., *Acremonium* spp., *Fusarium* spp., *Pseudoallescheria boydii* (*Scedosporium apiospermum*), *Scedosporium* spp. and *Sporothrix cyanescens* have been reported to be other hospital acquired fungal infectious agents related to airborne transmission [18,22]. It is expressed that airborne fungal infections may lead to opportunist infections in immune deficient subjects and may trigger asthma attacks in immune competent subjects [22,34-36]. In this study, all of these fungi, except for *Sporothrix cyanescens*, were insulated in the autopsy room.

The active method performed by using air sampler and the passive method performed by using settle plate method are the 2 most commonly used techniques for sampling. However, there is no valid opinion concerning which method is more appropriate [18,19,37,38].

It is reported in the literature that there is a weak correlation between settle plate method and air sampling method, which gives quantitative results, and that there is not always compatible outcomes even between the different devices that give quantitative results [37,38]. In the present study as well, the amount of bacterial colonies determined pre autopsy using the settle plate method was significantly higher in summer as compared to those determined in spring, whereas the amount of bacterial colonies determined via air sampler was significantly higher in the spring as compared to those determined in the summer.

Based upon the statistically acceptable criteria as indicated by cfu/m³, obtaining samples quickly, and ability of determining rarely existing microorganisms in the air have been expressed as the advantages of active method performed by air sampler; since the equipment to be used for the requirements of sterilization and calibration is expensive and noisy thus affecting normal air flow in the environment, the sampling amount of air is limited obtaining different results from different types of device, disability to evaluate the amount of microorganism that is decreased, inactivation of a number of microorganism at the time of crushing into the media have been expressed as the disadvantages of the device [18,19].

We also consider that quick sampling and ability to determine rarely existing microorganisms in the air (*S. aureus* and *Enterococcus* spp.) are the advantages of air sampler, whereas sterilization and calibration, requirement increased cost, and the limited amount of air that can be used for sampling are the disadvantages of the device.

It is reported that the number of colonies insulated via passive method, which is performed by using settle plates, is directly proportional with the level of contamination in the air [36-38]. The advantages of this method was reported as low cost, easy application, ability to measure harmful part of airborne contamination, multi-sampling at the same time from different locations, significant outcomes concerning critical surfaces (such as operation site), comparable and usually valid outcomes, having no effect on ventilation of the environment, and microorganism growth under the natural conditions. The disadvantages of this method can be listed as unknown volume of the sampled air, requirement of large particles, insufficient for fungal spore evaluation, long sampling time, and it is not always accepted by the official guidelines [18,19]. In addition, it has been expressed that the IMA rule, which has been used in this present study, gives the opportunity to compare the results of different studies, which have been conducted at different places about microbial air contamination by different researchers, provides easy and generally acceptable parameters for guidelines, and is cheap and easily applicable. It is stated that IMA, which is tested in different environments, has always given true results in accordance with the real conditions, and that it has provided more reliable outcomes than volumetric measurements [18].

We think that inexpensive and practicable allowing multi sampling at the same time from different places based upon comparable and usually valid outcomes and the presence of microorganism growth under the natural conditions are the advantages of settle plate method when the unknown volume of the air sampling and longer times of sampling are deemed as the disadvantages.

We consider that acceptable value for air sampler determined by using ROC curves (258 cfu/m³ for bacteria and 126 cfu/m³ for fungi) is an important step to create an uncontaminated standard for the indoor air quality of autopsy room. Similar studies which are conducted at different centers will be helpful for establishment of a national standard thus the acceptable standards shall be established based upon the microbial count for minimum contamination of the room air.

Many organisms isolated in the study can be found in normal flora of the healthy people or in the respiratory air thus it is difficult to determine the source of these organisms. These microorganisms could be originated from both the staff and cadavers and more importantly they could not cause infections in immunocompetent people. It is difficult to comment the increase in bacterial colony counts of air as there is no standard for the amount of bacteria that can be exist in the air of autopsy room. Although the gram negative bacteria such as *E. coli*, *K. pneumoniae* and *P. vulgaris* had been rarely insulated in the study we think that they were originated from cadavers. These gram negative bacteria spread infections usually through other ways rather than the air however they can be a potential hazard for ambient air.

Our findings could support the importance of primary containment for protecting autopsy staff and secondary containment for protecting environment from infectious agents at autopsy room.

5. Conclusion

1- Even if the increased number of bacteria and fungi colonies during the time of autopsy is not found significant, it is obviously seen that it represents serious risk for the people with immunosuppressive or the people having an open scar.

2- We consider that the design of an autopsy room like ventilation-air supply,, disinfection of storage of contaminated, hepa filtration systems, biosafety cabins for tissues, structuring the autopsy table drainage, downdraft tables in accordance with internationally approved guides and protocols should be appropriately required. Moreover, the air flow ways and working areas should be designed in the way that they can separate the contaminated and uncontaminated areas from each other and these areas are required to be marked clearly, so that the staff members should certainly

obey the inter-area passage rules, and that risk assessment should be done prior to all autopsy sessions.

3- As it is also mentioned in the literature, using only safety equipments is not enough for protection from infections during autopsy; additionally we think that forensic medicine specialists, pathologists, autopsy technicians and laboratory staff are required to be trained on this issue.

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