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ORIGINAL ARTICLE

Determination and comparison of microbial loads in atmospheres of two hospitals in Izmir, Turkey

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Abstract

Introduction and Objectives. Nosocomial infections, also known as hospital-acquired infections, has become one of the most important health problems in health care units worldwide. The presented study aims to determine the average amount of microorganism loads and to show that the atmospheres of the two hospitals can be a potential source regarding nosocomial infections. The effect of surface and floor disinfection processes in the two hospitals and the antibiotic susceptibility of the bacterial isolates were also evaluated.

Materials and Methods. Microorganisms were isolated from air samples collected from different areas (patient wards, corridors, operating theatres and postoperative units) of the two hospitals in Izmir. Sampling was conducted between December 2006 – March 2007.

Results. During the 3-month sampling period, the average number of live microorganisms in the air samples collected from second-class environments in the hospital 1 and the hospital 2 was found to be 224.44 and 536.66 cfu/m^3, respectively. The average number of microorganisms in hospital 2 collected before the disinfection process was higher than those after the disinfection process. However, because of the closure of the air-conditioning system and the hepa filters after the disinfection process, this was reversed in hospital 1.
In total, 54 and 42 isolates were obtained from hospital 1 and hospital 2, respectively. 49 isolates from hospital 1 and 35 isolates from hospital 2 were identified as *Staphylacoccus* sp. The remaining isolates were identified as *Aerococcus* sp. and *Enterococcus* sp. *Pseudomonas* sp. was not determined in the air samples of the two hospitals.

Conclusions. It was detected that the microbial loads in the atmospheres of the two hospitals studied varied greatly depending on the number of people in the environment. As the results indicate, the total number of microorganisms in the atmospheres of operating theatres in both hospitals does not pose a threat according to the Air Microbe Index.

Key words

Nosocomial infections, *Aerococcus* sp., *Enterococcus* sp., *Staphylococcus* sp.

INTRODUCTION

Nosocomial infections, also known as hospital-acquired infections (HAIs), are defined as infections which are not in the incubation period during the admission of a patient to hospital, but develop 48-72 hours of admission, or sometimes do not appear during hospitalization but after the patient has been discharged [1, 2, 3]. The prevalence of microorganisms causing HAIs varies from hospital to hospital, from department to department, and even from time to time in the same hospital environment [4].

Nosocomial infections occur worldwide and affect both developed and underdeveloped countries. Infections acquired in health care settings are among the major causes of death and increased morbidity among hospitalized patients. They are a significant burden both for the patient and for public health. A prevalence survey conducted under the auspices of World Health Organization (WHO) in 55 hospitals of 14 countries representing 4 WHO Regions (Europe, Eastern Mediterranean, South-East Asia and Western Pacific) showed that an average of 8.7% of hospital patients had nosocomial infections [1, 4]. Annual HAI prevalence studies revealed that among 100 admissions, Greece had 9.1%, Spain – 7%, Norway – 5.1%, and Slovenia – 4.6% [4, 5, 6]. It was no surprise that the highest prevalence of HAI occurred in intensive care units and acute care surgical and orthopedic settings. Old age, multiple morbidities, disease severity and decreased immunity increase patient susceptibility. Poor infection control measures are an overall risk factor, as are certain invasive procedures including central venous or urinary catheter placements. Antimicrobial misuse is associated with drug-resistant HAI [4].

In recent years, among the gram-positive cocci, *Staphylococcus aureus* in particular has been isolated as an effective agent of several nosocomial infections, such as bacteremia, pneumonia, and surgical wound infections [7]. Although coagulase negative *Staphylococci* are naturally present on human skin and mucous membrane structures, they can also frequently be isolated from clinical samples and can cause serious pathogenesis in infections resulting from contaminated medical instruments, and in immunosuppressed individuals [8, 9, 10]. In Turkey, Gram-negative microorganisms are also another cause of nosocomial infections [11]. This has been reported to be due to the selection of resistant Gram-negative pathogens during treatment with broad spectrum antibiotics [12].

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HAIs are becoming increasingly more important in Turkey. They prolong the hospitalization period, increase treatment costs and boost mortality and morbidity rates [13]. Therefore, the aim of the presented study was to determine the average amount of microorganism loads in the atmospheres of two hospitals, and to show that the atmospheres of these hospitals can be a potential source of nosocomial infections caused by *S. aureus*, coagulase (-) *Staphylococci* (CNS), *Enterococci* and *Pseudomonas*. The effect of surface and floor disinfection processes in the two hospitals and the antibiotic susceptibility of the bacterial isolates were also evaluated.

MATERIALS AND METHODS

Collecting air samples. Microorganisms were isolated from air samples collected from different parts of two hospitals in Izmir. Both hospitals are equipped with active air-conditioning (ventilation) systems, laminar airflow units, hepa filters and UV lamps. Sampling was conducted between December 2006 – March 2007. During the study, three air samples were collected from the patient wards and corridors once a month for three months, and six air samples from operating theaters, corridors and postoperative units twice a month, a day before and after the weekly-performed general disinfection, also for three months.

Air sampling was performed with MAS-100 Eco (Microbial Air Monitoring Systems, Merck) according to the standards set by its producer. In order to determine the total amount of live microorganisms in the hospital air, Count Agar (Difco 0479-17 PCA) was used, whereas *S. aureus* and other *Staphylococcus* species were detected with Baird-Parker Agar (LAM-M LAB 85) and *Staphylococcus-Streptococcus* Selective Medium (Oxoid CM 331 Columbia Blood Agar + antibiotic inhibitor =CNA Agar). For the isolation of *Enterococcus*, Kanamycin Esculine Azide Agar (Merck 1.05222 KEAA) was used. *Pseudomonas* Agar P (Difco) and Bacto Cetrimide Agar (Difco 0854-17-8) were used to detect *Pseudomonas aeruginosa*.

After the air samples used for sampling were incubated in petri dishes for 24 h, the growing colonies were counted. The number of live microorganisms in the aspirated air sufficient to form a colony was calculated with the following formula:

$$\frac{\text{Colony count in the petri dish (cfu)} \times 1000\ (\text{l})}{\text{Aspirated air (l)}} = \text{cfu/m}^3$$

Identification of the isolates. Identification of organisms isolated from the distinguishing and selective mediums, depending on their colonial characteristics at genus and species level, were performed with conventional methods described in Bergey's Manual of Systematics [14, 15] and Procaryotes [16], as well as with identification panels such as Api Staph (Biomerieux, France) and Api 20 Strep (Biomerieux, France). For identification, *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 29998 and *Micrococcus luteus* ATCC 9341 were used as control strains.

Determination of antibiotic resistance in isolates. Antibiotic sensitivity tests for the bacteria isolated and identified in both hospitals were performed with the disc diffusion method on Muller Hinton Agar medium according to the National Committee for Clinical Laboratory Standards (NCCLS) [17].

Statistical analyses. First of all, descriptive statistics on the data were calculated and the graphics were drawn. Then, one-way analysis of variance (ANOVA) was used to determine whether there was a difference between the species regarding their inhibition zone diameters. SPSS was used for the analysis and $p<0.05$ was considered significant.

RESULTS AND DISCUSSION

Nosocomial infections are an important health problem in Turkey and many other countries [18]. Nosocomial infections with the highest morbidity and mortality mostly occur in intensive care units. The fact that antibiotic-resistant strains are especially encountered in intensive care units is one of the most important factors which make treatment difficult and increase the costs [4, 5].

The number of live microorganisms in the air samples collected from the second-class environments such as patient wards and service corridors in hospital 1 and hospital 2 for three months is shown in Table 1. Although neonatal care units are considered as first-class environments according to DIN 1946/4 standards, premature units and operating theatres in hospital 1 were evaluated separately. There was no unidirectional flow system in the neonatal care unit in the hospital 1. The number of live microorganisms in the atmosphere of the premature unit is shown in Table 2. The number of live microorganisms in the air samples collected from the first-class environments, such as operating theaters, corridors and postoperative units, before and after the disinfection process is shown in Table 3.

While the number of microorganisms collected in hospital 2 before the disinfection process was higher than those after the disinfection process, this was reversed in hospital 1. When

Table 1. Number of live microorganisms in air samples collected from second-class environments – patient wards and service corridors in hospital 1 and hospital 2

Samples	Hospital 1				Hospital 2			
	Avg (cfu/m³) N=6	SD	Min. (cfu/m³)	Max. (cfu/m³)	Avg (cfu/m³) N=6	SD	Min. (cfu/m³)	Max. (cfu/m³)
1st month sample	223.33	145.28	40	410	516.66	377.07	130	1220
2nd month sample	323.33	203.43	40	410	390	476.10	30	1320
3rd month sample	126.66	77.37	40	230	703.33	377.71	290	1150
Avg of 1st, 2nd, and 3rd month	224.44	164.21	40	720	536.66	409.86	30	1320

SD – standard deviation; Avg – average

Table 2. Number of live microorganisms in the premature unit of hospital 1

Premature unit	Avg(cfu/m³) N=3	SD	Min. (cfu/m³)	Max. (cfu/m³)
Avg of 1st, 2nd, and 3rd month	38.33	1.178	35	40

SD – standard deviation; Avg – average

Table 3. Number of live microorganisms in first-class environments in hospital 1 and hospital 2 before and after disinfection processes

Samples	Hospital 1								Hospital 2							
	Before Disinfection				After Disinfection				Before Disinfection				After Disinfection			
	Avg (cfu/m^3) N=5	SD	Min. (cfu/m^3)	Max. (cfu/m^3)	Avg (cfu/m^3) N=5	SD	Min. (cfu/m^3)	Max. (cfu/m^3)	Avg (cfu/m^3) N=5	SD	Min. (cfu/m^3)	Max. (cfu/m^3)	Avg (cfu/m^3) N=5	SD	Min. (cfu/m^3)	Max. (cfu/m^3)
1^{ts} month sample	13	7.58	5	25	67	53.80	0	135	24	15.16	10	45	11	6.51	0	15
2^{tnd}month sample	7	7.58	0	15	3	2.73	0	5	23	29.70	0	75	26	31.50	0	75
3rd month sample	16	24.59	5	60	17	14.40	0	35	20	19.03	0	50	17	14.83	0	40
Avg of 1st, 2nd and 3rd month	12	14.85	0	60	29	41.19	0	135	22.33	20.60	0	75	18	19.98	0	75

SD – standard deviation; Avg – average

the cause of the increase was investigated, it was found that the air-conditioning system and the hepa filters which were switched on before the disinfection process, were turned off during the weekend, and thus the number of airborne live microorganisms increased fivefold after the disinfection process. The number of airborne live microorganisms in the operating units of hospital 1 – 0-135 cfu/m^3 during the 1st month, dropped to 0-55 cfu/m^3 during the 2nd and 3rd months, because the hepa filters were constantly switched on during those months.

Counts made during surgery showed that the environment where the surgical team was stationed had the highest particle concentration. Although it is impossible to completely eradicate particles and microorganisms in operating theatres, it is possible to reduce the number of particles and microorganisms in these places. This can only be accomplished by pumping air free of particles and/or microorganisms into the environment, and by removing the air laden with particles and microorganisms from the environment. This indicates the importance of air-conditioning (ventilation) in operating theatres [19].

The surface and floor disinfection process in surgery units, another objective of the presented study, plays an important role in the reduction of dust particles likely to disperse into the air. The disinfection processes conducted in both hospitals are similar: after each surgery, residues are collected. The floors in hospital 1 are cleaned with a solution prepared with hypochlorous acid and in hospital 2 with a solution prepared with chloral tablets. Operating tables and lamps are routinely cleaned with several disinfectants every week. In hospital 2, disinfection of the air is accomplished only with the air-conditioning system. On the other hand, in hospital 1, the air is also disinfected. However, it was seen that the disinfection of the air was insufficient during the weekends of the first month because the hepa filters were switched off. In both hospitals, the effectiveness of the disinfection process and its effect on the quality of the air also depend on the number of patients and healthy people around on weekdays and at the weekends [20, 21]. During the presented study, the number of people in the surgery units varied between 0–3. In hospital 1, the number of microorganisms in one of the operating theatres was 60 cfu/m^3 just after surgery, whereas it was 5 cfu/m^3 in another theatre which was empty before and after the sampling process. During the sampling processes, conditions such as temperature, humidity, pressure and operating filters were the same in both theaters. The only difference was the presence or absence of people. The main source of bacteria in operating theaters is the skin flora of the people present, and the number of airborne bacteria increases as the number of people in the theater increases. The factors such as the number of patients and visitors in patient wards during sampling process and airing time also affected the density of microorganisms in the atmosphere. The average number of live microorganisms during the 3-month sampling period was 224.44 (± 164.21) cfu/m^3 and 536.66 (± 409.86) cfu/m^3 in the patient wards of hospital 1 and hospital 2, respectively. Since hospital 1 is a State hospital and serves only civil servants, the number of patients and visitors was lower than in hospital 2, which might be the factor affecting the microorganism loads in patient wards. The fact that hospital 2 is a private hospital serving anyone, more patients are operated on there, and thus the number of patients and visitors is higher than in hospital 1, and might be the cause of the higher number of microorganisms in samples collected. It is remarkable that there were three or more people present at the points where the highest microorganism counts were reached during the sampling and PCA counting processes. Maximum microorganism counts in patient wards and in other rooms should not exceed 300-400 cfu/m^3 according to Air Microbial Index [22].

Airborne microflora in hospital rooms has been the subject of numerous studies as a potential cause of hospital infections. Most of the studies were performed in intensive care units, surgical units, haematological wards, maternity wards and other departments where the risk of infections is greatest [23, 24]. The levels of microorganisms found in most rooms were 10^1-10^3 cfu/m^3 [23, 25, 26, 27], except for rooms of high cleanness, such as operating theatres or transplant units, where the levels were 10^{-1}-10^1 cfu/m^3 [23, 24].

In total, 54 and 42 isolates were obtained from both hospital 1 and hospital 2, respectively. 49 isolates from hospital 1 and 35 isolates from hospital 2 were identified as *Staphylacoccus* sp. Of the 84 *Staphylococcus* isolates, 36 strains (42.86%) were identified as *S. aureus,* 7 of which were coagulase (+), and 29 coagulase (-). Of the other *Staphylacoccus* isolates, 19 strains were identified as *S. xylosus*, 13 as *S. haemolyticus*, 7 as *S. hominis*, 3 as *S. lentus*, 2 as *S. lugdunensis*. The remaining 4 isolates were identified as *S. chromogenes*, *S. epidermidis*, *S. saprophyticus* and *S. warneri*.

During the sampling process, 12 strains with positive esculin hydrolysis were isolated from 99 KEAA petri dishes. 3 isolates which were esculin (+) but did not show characteristics of *Enterococcus* through morphological and biochemical tests were identified as *Aerococcus viridans* 1 with the Api-Staph test. Of the enterococci isolates, 2 (22.22%) were identified as *E. faecalis* and 7 (77.78%) as *E. faecium*. *Pseudomonas* sp. was not determined in the air samples of the two hospitals.

In antibiotic sensitivity tests performed with the disc diffusion method, of the 36 *S. aureus* strains, 11 (30.56%)

were resistant to oxacillin and 5 (13.89%) were moderately sensitive. Also, 11 strains of *S. aureus* were identified as "methicillin resistant *Staphylococcus aureus* (MRSA)", and 5 strains were moderately sensitive to methicillin.

Of the other CNS strains, 28 (58.33%) were resistant to oxacillin (MRKNS), and the rest were sensitive. Resistance rates to methicillin were 73.68% in *S. xylosus*, 0% in *S. epidermidis*, 66.66% in *S. lentus*, 50% in *S. lugdunensis*, 0% in *S. warner*, 100% in *S. saprophyticus,* 100% in *S. cromogenes*, and 57.14% in *S. hominis*.

When the sensitivity rates of *S. aureus* to other antibiotics were considered, it was seen that 100% of coagulase (+) *S. aureus* strains were resistant to ampicillin, while 82.75% of coagulase (-) *S. aureus* strains were resistant to ampicillin. These rates showed that ampicillin was the antibiotic to which the *S. aureus* strains were most resistant. *S. aureus* strains and other CNS strains were most sensitive to vancomycin (0% resistance).

When the resistance rates of MRSA strains were considered, it was observed that they developed multi-resistance in addition to oxacillin resistance. 8 MRSA strains (72.72%) were resistant to 4 or more antibiotics. Resistance rates of MRSA strains to antibiotics were as follows: 0% to vancomycin, 54.5% to ciprofloxacin, 18.1% to chloramphenicol, 36.6% to amoxicillin clavulonic acid and 36.6% to gentamicin. (Tab. 4, Fig. 1a).

S. aureus needs special mention, especially methicillin (MRSA). The proportion of *S. aureus* isolates among intensive care unit patients that are resistant to MRSA as well as oxacillin or nafcillin, is on the rise at approximately 60% [4, 28].

Resistance rates of *Enterococcus* species to antibiotics found in the presented study were as follows: none of the 7 *E. faecium* and *2 E. faecalis* strains was resistant to vancomycin. However, *Enterococcus* strains were all resistant (100%) to ciprofloxacin. 5 of the *E. faecium* strains (71.4%) were resistant to ampicillin, whereas 2 of them (28.5%) were sensitive. One of the *E. faecalis* strains (50%) was resistant to chloramphenicol, while the other (50%) was moderately sensitive. Of the *E. faecium* strains, only one (14.28%) was resistant to chloramphenicol. Table 5 gives descriptive statistics on the antibiotic sensitivity zone diameters (mm) of *Enterococcus* and *Aerococcus* strains. The highest mean value (25±3.05) belongs to 'va' antibiotic (Fig. 1b).

Enterococci currently account for 12% of nosocomial infections and 8% of all nosocomial bacteremia [29]. The incidence of enterococcal bacteremia due to *E. faecium* is increasing. In a previous study, it was found that mortality was significantly higher among patients infected with *E. faecium* than among those infected with *E. faecalis*. This was true particularly among patients with monomicrobial or nosocomial bacteremia, those who had previously received antibiotic treatment, and those who had cancer [30].

Table 4. Descriptive statistics for antibiotic sensitivity zone diameters (mm) of *Staphylococcus* strains

	N	Minimum	Maximum	Mean	Std. Deviation
c	84	.00	32.00	21.2976	6.9625
gen	84	.00	38.00	21.5833	8.3409
cc	84	.00	44.00	24.6429	9.7963
amc	84	10.00	45.00	26.2857	7.9858
sxt	84	.00	32.00	12.5357	12.2075
am	84	.00	44.00	17.7619	11.8318
ox	84	.00	38.00	13.8929	8.5616
cxm	84	.00	32.00	16.3929	8.9846
cip	84	.00	36.00	17.5952	10.0566
va	84	18.00	26.00	21.7738	1.4425
ipm	84	.00	44.00	17.4762	14.5175
nb	84	.00	32.00	19.5000	9.1842
Valid N (listwise)	84				

am – ampicillin; amc – amoxycillin/clavulonic acid; c – chloramphenicol; cc – clindamycin; cip – ciprofloxacin; cxm – cefuroxime sodium; gen – gentamycin; ipm – imipenem; nb – novobiocin; ox – oxacilline; sxt – trimetoprim sulfametaxosol; va – vancomycin.

Table 5. Descriptive statistics for antibiotic sensitivity zone diameters (mm) of *Enterococcus* and *Aerococcus* strains

	N	Minimum	Maximum	Mean	Std. Deviation
c	12	.00	36.00	21.1667	9.7406
gen	12	.00	14.00	5.9167	6.3024
cc	12	.00	42.00	17.5833	14.2858
amc	12	.00	32.00	18.6667	11.2439
sxt	12	.00	12.00	1.0000	3.4641
am	12	.00	30.00	12.8333	12.5831
ox	12	.00	.00	.0000	.0000
cxm	12	.00	11.00	1.6667	3.9158
cip	12	.00	16.00	5.9167	7.4034
va	12	20.00	30.00	25.0000	3.0451
Valid N (listwise)	12				

am – ampicillin; amc – amoxycillin/clavulonic acid; c – chloramphenicol; cc – clindamycin; cip – ciprofloxacin; cxm – cefuroxime sodium; gen – gentamycin; ox – oxacilline; sxt – trimetoprim sulfametaxosol; va – vancomycin.

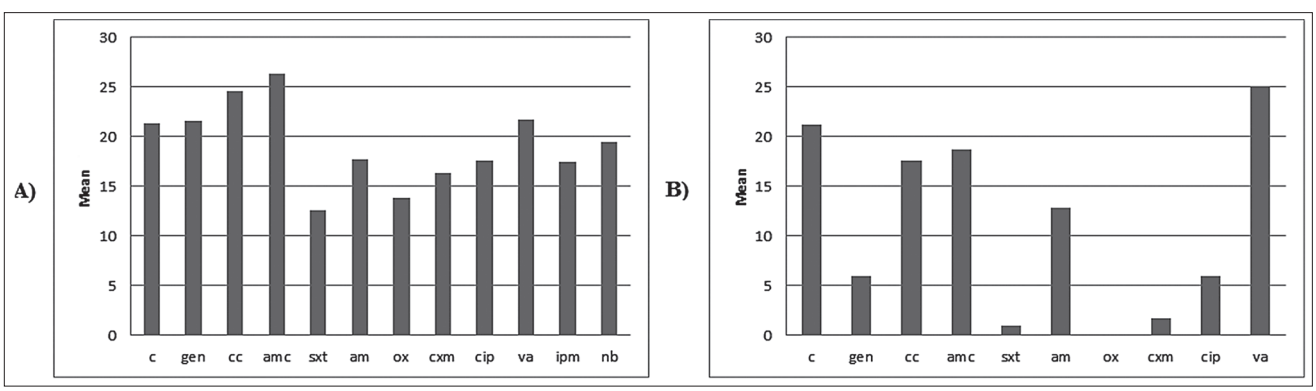

Figure 1. Bar graphs of antibiotic sensitivity zone diameters (mm) of the strains – A). Results for *Staphylococcus* strains – B). Results for *Enterococcus* and *Aerococcus* strains

CONCLUSIONS

It was detected that microbial loads in the atmospheres of the two hospitals studied varied greatly, depending on the number of people in the environment, and that the most important factor helping control the atmosphere in surgery units microbiologically were the air-conditioning systems. When the results obtained were evaluated according to the Air Microbial Index, it was seen that airborne microbial loads in the atmospheres of the operating theaters in both hospital did not pose a danger. Although it is impossible to eradicate microorganisms completely, by taking appropriate precautions and using air filters and air-conditioning systems in surgery units and intensive care units effectively, the number of microorganisms can be kept at a safe level. Only if the above-mentioned infection control regulations and actions are thoroughly applied, can nosocomial infections be prevented.

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