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Microbial investigation of the air in an apartment building

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SUMMARY

The microbial and viral flora in the ventilating ducts of an apartment building was evaluated. Several types of sampler (slit sampler, Andersen sampler, large volume air sampler) were used to evaluate the hourly, weekly and seasonal variation of this flora. The mean bacterial concentration was 17·2 c.f.u./m^3 with a maximum level at 07.30 h (41·3 c.f.u./m^3) and a minimal concentration in the early afternoon (8 c.f.u./m^3). The bacterial concentration observed correlated with the relative humidity in the air-ducts although there were no seasonal differences. The bacteria were mainly gram-positive cocci (73·5%) represented by a large number of *Micrococcaceae* (47·1%); gram-positive bacilli accounted for 14·2% of the isolates, gram-negative bacilli 12·0% and gram-negative cocci 0·3%. The majority of the bacteria-carrying particles were in the respirable range with 80·4% of them being less than 5 μm. The methods used did not result in the isolation of viruses during the winter sampling period.

INTRODUCTION

The daily time budget of industrial cities indicates that about 90% of our time is spent indoors in residences, offices or schools, and more than half of that time in our own dwelling (Moschandreas, 1981). According to Hinkle & Murray (1981), bacteria and viruses causing respiratory diseases are among the common causes of illness and represent the fifth cause of mortality in U.S.A. These illnesses are acquired mainly in the indoor environment. Four mechanisms of transmission of respiratory diseases have been recognized: contact, dust, respiratory droplets and droplet nuclei. Airborne infections are caused by droplet nuclei and occasionally dust, whereas the respiratory droplets are implicated in contact infections and there is some epidemiological evidence showing the role of the airborne route in the transmission of respiratory diseases. For example, it is largely admitted that influenza virus, measles and tuberculosis are airborne infections (Couch, 1981; Langmuir, 1961).

Reduced infiltration or ventilation, or recirculation of the air in buildings are

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proposed to save energy consumption (Hollowell, Berk & Traynor, 1979). These measures can affect the quality of indoor air by increasing its microbial content. Riley (1979) demonstrated the role of the ventilating system in an outbreak of measles in an elementary school and Houk (1980) showed that the recirculation of air in a naval vessel had been implicated in an epidemic of tuberculosis.

Quantitative studies on the microbial content of intramural spaces have mainly been performed in public spaces such as hospitals (Bourdillon & Colebrook, 1946; Greene *et al.* 1962), schools (Williams, Lidwell & Hirch, 1956), factories (Favero *et al.* 1966; Hirch, 1951); and a few were done in residential houses (Finch, Prince & Hanksworth, 1978; Scott, Bloomfield & Barlow, 1982). The present study was aimed at the evaluation of the microbial flora of the interior air of an apartment building to determine if it was a threat to public health.

MATERIALS AND METHODS

Apartment building

The apartment building chosen was located in the city of Laval, Quebec, Canada. It has 24 floors and a total of 282 apartments of 1–5 rooms. There were about 700 people and 60 pets. The socioeconomic status of the residents was middle to high-class, many of them being professionals and bureaucrats.

Each apartment has two ventilating apertures, one located in the kitchen over the cooking area and one in the bathroom. The collected air is directed towards one of the four independent collection ducts and evacuated outside without recirculation. In each collection duct, the air is exhausted at 400 m^3/min.

During October 1981 to June 1982, the bacterial and viral contents of two of the four collection ducts was studied. The samples were taken in the ducts just before the air was exhausted at the upper most level of the building.

Air sampling

Slit sampler. The slit sampler used was a slit-to-agar biological air sampler model STA-203 (New Brunswick Scientific Co.). This sampler is an impactor sampler (Bourdillon, Lidwell & Thomas, 1941). The air is drawn at 50 l/min by a vacuum pump throughout a fixed-slit orifice and air particles impacted directly on a rotating nutrient collecting medium located just below the narrow slit. Viable bacterial cells in the particles find immediately a suitable medium for growth. After an incubation period, the bacteria growing into visible colonies can be counted and evaluated. The slit-sampler was connected by 1·2 m of flexible tubing (of 0·5 cm diameter) to two of the air ducts. A stainless steel probe was inserted in each duct through a small opening and connected to the flexible tubing. To determine the optimal sampling time, air was sampled continuously for 24 h using the slit-sampler and half-hour changes of agar plates.

The samples were collected each Monday from 07.00 to 09.00 h. Before use, the sampling box of the slit sampler and the tubes were sterilized with 70% ethanol. The nutrient collecting medium was chocolate agar (150 × 15 mm) an enriched medium for the growth of common and fastidious bacteria. After sampling, the plates were incubated aerobically for 48 h at 37 °C and the colonies were counted and identified by standard methods. The fermentative gram-negative bacilli were

analysed by the API-20E system (API Laboratory Products Ltd.) and the non-fermentative gram-negative bacilli were identified by the method of King (1972). All media used in our experiments were obtained from Institut Armand-Frappier (Laval) and from the laboratories of the Ministry of Social Affairs (Ste-Anne-de-Bellevue).

Andersen sampler. The Andersen sampler collects air at 28·3 l/min through six stages with decreasing diameter holes in each succeeding plate. As the jet velocity increases with the decreasing diameters of the holes, smaller particles are recovered at each stage. Below each plate, an agar surface containing the nutrient agar receives the airborne particles separated according to their aerodynamic dimensions. The petri plates are then removed, incubated and the colonies counted by the 'positive hole' method (Andersen, 1958).

In our study, the Andersen sampler (model 0101) was connected to the vacuum pump supplied with the instrument. Like the slit sampler, the Andersen sampler was also connected to two similar flexible tubes each directed to a collection duct. Before each use, the instrument was sterilized by ethylene oxide. Three samples (15 min each) were taken weekly, one run with sheep blood agar and two with chocolate agar. The plates were incubated aerobically for 48 h at 37 °C and colony counts were calculated by the positive hole method.

Large-volume air sampler (LVAS). The LVAS used in these experiments to evaluate the viral content of the air, was a simple liquid scrubber (cyclone scrubber) similar to the one described by White *et al.* (1975). It operates at a flow rate of about 950 l/min. Airborne particles entering the apparatus are collected by impingement into a film of liquid and directed towards a collection vessel. The instrument, except the motor, was pre-sterilized by ethylene oxide before each use. The collection fluid was composed of MEM-medium (with Earle's salts) mixed with 0·5 % bovine serum albumin, 2 mM of L-glutamine and 50 μg/ml of gentamicin. Six 10-min samples were obtained weekly.

The fluids obtained were reconcentrated using Amicon Centriflo membrane filter cones (type CF 50A, Amicon Canada Ltd.) preconditioned with MEM-Earle's 0·1 % bovine serum albumin by centrifugation at 2000 rev/min for 30 min. Aliquots (0·33 ml) of the concentrate were inoculated into 12 tissue culture flasks (25 cm^2) of each cell line. Five cell lines were used: MDCK (ATCC CCL 34), Vero (ATCC CCL 81), HEp-2 (ATCC CCL 23), IMHP (human diploid lung fibroblast, Institut Armand-Frappier), and primary kidney cell culture of African Green Monkey Kidney, (Flow Laboratories). Cultures were incubated at 33 and 37 °C and observed for the presence of cytopathic effect. After two blind passages, a standard hemadsorption test using guinea pig red blood cells was also performed.

RESULTS

Time distribution

Bacterial counts obtained in half-hour increments during 24 h revealed a mean count of 17·2 colony forming units (c.f.u.) per m^3 of air. Maximum counts were recorded in the morning at 07.30 and 10.30 (41·3 and 37·3 c.f.u./m^3) (Fig. 1). Minimum counts were observed in early afternoon and in the evening. Samples were taken at regular intervals during the period of this study and bacterial counts

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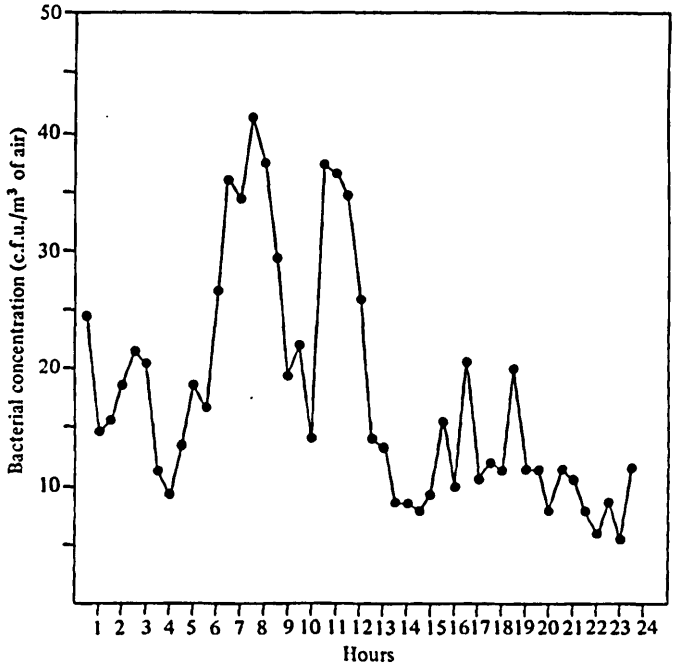

Fig. 1. Hourly variation of airborne bacterial level.

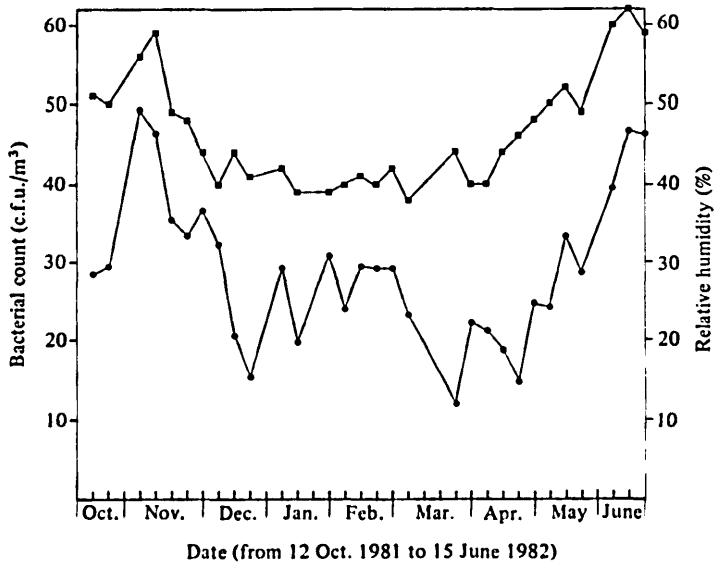

Fig. 2. Weekly distribution of airborne bacterial count and relative humidity of the air during the sampling period. (The correlation coefficient of Pearson test = 0·72.)

correlated with the relative humidity of the indoor air (Fig. 2). A positive correlation between the bacterial counts and the relative humidity was found (correlation coefficient of 0·72 by a Pearson test). Results reported as a seasonal average (autumn, winter and spring), do not significantly differ. We found a difference of less than 7 c.f.u./m³ of air between the seasons.

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Table 1. *Percentage of bacteria-carrying particles in the ventilation ducts according to their aerodynamic size*

Andersen sampler (stage)	Particles size (μm)	Bacteria-carrying particles (%)
6	< 1·0	30·9
5	1·0–2·0	33·0
4	2·0–3·3	10·2
3	3·3–4·4	6·2
2	4·4–9·0	6·4
1	> 9·0	13·2

Table 2. *Bacterial species isolates from air ducts and their percentage frequency*

Gram-positive cocci	
Micrococcaceae: 47·1 %	
Micrococcus spp.	32·7
Staphylococcus (coagulase negative) spp.	14·1
Staphylococcus aureus	0·3
Streptococcaceae: 26·4 %	
Aerococcus spp.	12·6
Streptococcus (group D)	4·6
Streptococcus (group viridans)	2·0
Streptococcus spp. (other groups)	7·2
Gram-positive baccilli	
Bacillaceae: 7·2 %	
Bacillus cereus	1·2
Bacillus subtilis	0·8
Others	5·2
Corynebacteriaceae: 7·0 %	
Gram-negative bacilli	
Enterobacteriaceae: 0·3 %	
Non fermentative: 11·7 %	
Gram-negative bacilli	
Acinetobacter spp.	4·1
Pseudomonas spp.	2·9
Branhamella spp.	1·8
Moraxella spp.	0·9
Flavobacterium spp.	0·6
Others	2·0
Gram-negative cocci	
Neisseriaceae: 0·3 %	

Particle size

The particle size studied with the Andersen sampler revealed that 80 % of the viable particles were smaller than 5 μm and that the majority of them were less than 2 μm (Table 1).

Bacterial isolates

Every week for 15 weeks, the first 25 c.f.u. encountered using the slit sampler were identified. Table 2 summarizes the results obtained and Table 3 reports the relative concentration of each genus. The average total concentration was 21·8 c.f.u./m^3 of air. The gram-positive cocci were the most frequent (73·5 %)

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Table 3. *Concentration of various airborne bacteria in the ventilating ducts*

Airborne bacteria	C.f.u./m³ of air
Micrococcus spp.	7·28
Staphylococcus spp. (negative coagulase)	3·21
Staphylococcus aureus	0·08
Aerococcus viridans	2·37
Streptococcus (group D)	0·89
S. (group viridans)	0·55
S. spp. (other groups)	1·54
Bacillus cereus	0·26
B. subtilis	0·19
B. spp. (other groups)	1·17
Corynebacterium xerosis	0·25
C. ulcerans	0·12
C. haemolyticum	0·03
C. spp. (other groups)	1·10
Enterobacter agglomerans	0·06
Acinetobacter calcoaceticus	0·86
Pseudomonas vesicularis	0·22
P. spp. (groups CDC)	0·26
P. putrefasciens	0·07
P. picketti	0·06
P. paucimobilis	0·03
P. spp. (other groups)	0·06
Moraxella osloensis	0·34
Branhamella catharrhalis	0·31
Flavobacterium meningosepticum	0·13
Other non-fermentative gram-negative bacilli	0·31
Neisseria spp.	0·07
Total	21·82 c.f.u./m³

followed by gram-positive bacilli (14·2%), gram-negative bacilli (12·0%) and gram-negative cocci (0·3%). A detailed study of the gram-positive cocci showed that *Micrococcus* spp. (7·28 c.f.u./m³) were most frequently encountered. Similar concentrations of staphylococci (3·29 c.f.u./m³), streptococci (2·98 c.f.u./m³) and aerococci (2·37 c.f.u./m³) were observed. Few *Staphylococcus aureus* were isolated (0·08 c.f.u./m³) as compared to coagulase negative staphylococci (3·21 c.f.u./m³).

Among the gram-positive bacilli, there were similar concentrations of *Bacillus* spp. (1·62 c.f.u./m³) and *Corynebacterium* spp. (1·50 c.f.u./m³). In the bacillus group, *Bacillus cereus* and *Bacillus subtilis* were often observed. Very few enterobacteriaceae were isolated (0·3%) and they were exclusively represented by *Enterobacter agglomerans*. There was a concentration of 2·65 c.f.u./m³ of non-fermentative gram-negative bacilli represented principally by *Acinetobacter* and *Pseudomonas* spp.

Virological analysis

In this study, 48 m³ of air were sampled each week during 20 weeks. Viruses were not detected in any of the samples.

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DISCUSSION

The generation of airborne micro-organisms of indoors spaces is mainly due to human activities: expiratory atomization (sneeze, cough, loud speaking; Duguid, 1946), household activities (bedding, dressing, sweeping; Riley & O'Grady, 1961) and, in some instances, by the building itself (air conditioning, toilets; Gerba, Wallis & Melnick, 1975). Except for some spore-forming bacteria, these micro-organisms are always attached to air borne particles. There are three categories of particles harbouring micro-organisms. (1) Droplets larger than 100 μm that emanate principally from the mouth and nose during expiratory atomization, or from the flushing of toilet bowls, are sufficiently large to sediment rapidly without desiccation by evaporation. These droplets stay airborne for only a very short period of time and remain in the immediate vicinity of their source; they are considered in contact infection. (2) Droplets smaller than 100 μm desiccate before sedimenting, forming residues of 10 μm and less, called droplet nuclei, that remain airborne for long periods until they are breathed or ventilated (Wells, 1955). (3) Dust which consists of assorted sizes of particles that exist on surfaces such as floors, walls, clothing or bedding is periodically resuspended in the air as a result of household activities.

In the present study, bacteria-carrying particles in the air of ventilating ducts were found mainly as droplet nuclei as more than 80 % of them was smaller than 5 μm. Noble, Lidwell & Kingston (1963) who have determined the size distribution of bacteria-carrying particles in the air of occupied rooms using a size-grading slit-sampler found in offices a mean median equivalent diameter of 7·7 μm at low ventilation flow. A mean median equivalent diameter of 10·0 μm was found at higher ventilation flow rate, the smaller particles being removed more rapidly when the ventilation rate was increased. We found a larger percentage of very small viable particles in the ventilating system than the values reported for occupied rooms. These results were expected because droplet nuclei generated in indoor environment stay airborne long enough to find their way to the aeration system whereas the large particles sediment rapidly in the room or are retained on the wall of the ducts. However, the methods used may have underestimated the larger bacteria-carrying particles because the Andersen sampler does not recover these particles as efficiently (May, 1964; Lidwell & Noble, 1965).

A higher concentration of bacteria was observed in the morning, other studies have reported a positive correlation between human activities and bacterial counts (Greene *et al.* 1962; Williams, Lidwell & Hirch, 1956). Duguid & Wallace (1948) had reported that even slight activities could produce 1000 bacteria-carrying particles per minute and Riley & O'Grady (1961) found that, under domestic activities, the bacterial content of air can rise to about 1000/m^3 (300/ft^3). The afternoon is normally a resting period and because fewer individuals are present in their apartment, a lower bacterial level was observed in our study.

The observed variations in the weekly bacterial counts may be due to changes in human activities from one week to another, but if we consider constant activity during the sampling period, they could be related to environmental conditions such as relative humidity. A positive correlation was demonstrated by Wells & Zapposodi (1948) between the bacterial counts and the relative humidity

of the air. Nevertheless, the effect of relative humidity on airborne bacteria is controversial. Some have stated that the death rate of the airborne bacteria is greatest at high relative humidity (De Ome, 1944) whereas others claim intermediate levels to be the most lethal (Dunklin & Puck, 1948). According to Webb (1959), the stability of airborne organisms varies with the types of bacteria encountered. In the present studies, the seasonal influence on the bacterial counts was minimal. This suggests that in indoor spaces, the airborne contamination is not a function of extramural climatological conditions and agrees with a study in a hospital in Minneapolis (Minnesota), where the investigators found a difference of only 60 c.f.u./m^3 (1·7/ft^3) between summer and winter months (Greene *et al.*, 1962).

Over the sampling period we found a mean concentration of 21·8 c.f.u./m^3 of air in the ventilating ducts. This agrees with the study of Pincus & Stern (1937) who found about 50 c.f.u./m^3 in the ventilating duct of a theatre, but is a low count when compared to the values reported for occupied rooms. Bourdillon, Lidwell & Lovelock (1948), cited by Hirch (1961) suggested a level of 1800 c.f.u./m^3 of air as a maximum level in any ordinary occupied spaces. However, only a fraction of particles carrying organisms are ventilated: only the droplet nuclei and some dust particles can reach the ducts and are exhausted.

The general distribution of the microbial types we have encountered suggests the importance of the gram-positive cocci. These are, with the corynebacteria, indigenous to human skin, hair and respiratory tract. The results obtained indicate that the major contamination source of the indoor air of the building was human activity as suggested by Greene *et al.* (1962), who stated that clean air contains predominantly gram-positive cocci. Few *enterobacteriaceae* (0·3 %) were found in the air of the ducts. This was to be expected because the *enterobacteriaceae* are mainly found in wet areas and that they are unstable in the airborne state (Scott, Bloomfield & Barlow, 1982). The non-fermentative gram-negative bacilli were in relatively high concentration (11·7 %) and, in a study reported by Greene *et al.* (1962), these bacteria were principally harboured by particles of 2 μm and less.

Viruses were not observed even though respiratory viruses such as influenza virus, measles virus, chickenpox virus, coxsackievirus A 21 and adenovirus types 4–7 have been shown to be transmitted by the airborne route (Couch, 1981). These viruses are more common in winter months when the relative humidity of the indoor air is low due to the heating (Knight, 1980). The main difficulties in isolating viruses from environmental sources are related to the low number of virus particles in contaminated air and the efficiency of isolation procedures. The efficiency of the LVAS was previously studied by Park (thesis submitted to the School of Medicine for the degree of Master of Science at the University of Ottawa, 1980) who evaluated the concentrating procedures and found that the overall efficiency of the method was about 50 %. Our results suggest, that since we were unable to isolate any virus in 1200 m^3 of air, that if they were present, it was at levels below our testing limits or because they were non-cultivable by our methods.

The daily average concentration of bacteria in the ventilating ducts was about 17·2 c.f.u./m^3 of air. Considering that the air is exhausted at about 400 m^3/min, the average concentration is estimated to be about 7000 c.f.u./min or approximately 10^7 viable particles in one day. With an electronic particle counter (Climett Ltd) an average of $1{\cdot}5 \times 10^6$ particles/min or $2{\cdot}2 \times 10^9$ particles/day was measured in the ducts. The percentage of particles harbouring micro-organisms is thus very

low, corresponding to about 0·45% of particles containing bacteria. This value is probably lower than the true value because the techniques used to sample the airborne bacteria are rarely 100% efficient. A theoretical study prepared for the Division of Building Research of the National Research Council (Canada) in 1980 estimated the viable particulate content of domestic ambient air. This was done to evaluate the feasibility of reducing energy consumption of an apartment building by the use of recirculated air. For an exhaust flow of 360 m^3/min, a level of $6{\cdot}6 \times 10^6$ viable particle count/min or $9{\cdot}5 \times 10^9$ bacteria-carrying particles in one day was estimated. It was assumed that the viable particulate count was about 0·16% of the total particulate count of the ventilating duct and concluded that the recirculation of exhausted air from toilets and kitchens can be safe and economical to the occupants of the appartment building provided some simple and elementary precautions are maintained such as filtering. From a microbial perspective, our study agrees with their conclusion. The microbial concentration is relatively low in the ducts of the building we investigated but the majority of the particle-harbouring micro-organisms are in the droplet nuclei state. Droplet nuclei are important in the transmission of airborne infection. According to Hatch (1961), the penetration and deposition of particles in the respiratory tract vary with the particle size. Particles lower than 5 μm can reach the lungs and cause infection in susceptible tissues if breathed. Thus it is very important to propose an efficient control system to limit the accumulation of bacterial cells if recirculation of air is performed.

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