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EVALUATION OF CHILDREN AS SOURCES OF BIOAEROSOLS IN A CLIMATE CHAMBER STUDY

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Emissions of viable particles from a group of children were measured under controlled conditions in a climate chamber that simulated indoor environmental exposure in day-care institutions with tight building envelopes and outdoor air supply by natural infiltration only. Bioaerosol sampling was simultaneously applied with slit samplers and sediment plates. A total of 142 strains was identified. Most of these were from sediment plates (95%) as the colonies on the slit sampler were more crowded and too confluent for separation. On sediment plates, coryneform bacteria dominated (27-85%), followed in frequency by micrococci (4-50%), *Staphylococcus epidermidis* and *saprophyticus* (12-43%), *Bacillus spp.*, most frequently *B. megaterium* (12-33%), and *Acinetobacter spp.* (11-14%). From the slit sampler plates, staphylococci dominated (67%), followed by coryneform species and micrococci (17%). Within the first hour after the group left the chamber, the number of colony forming units (CFU) suspended in the air decreased, corresponding to an equivalent dilution ventilation rate of 2.0 ACH (air changes per hour) for bacteria and 1.7 ACH for mold spores due to the catching of particles on surfaces and to die away of viable microorganisms. Accordingly, microbial surface contamination revealed an increase at the same time.

INTRODUCTION

Low natural ventilation rates in the range of 0.1 to 0.3 air changes per hour (ACH) have been enforced in children's day-care institutions built according to Danish building regulations and construction practices between 1972 and 1982 as part of energy conservation measures. We previously studied indoor air quality in such tight building envelopes with no mechanical ventilation to evaluate basic physical and chemical changes in the indoor environment due to human occupancy. When the prescribed number of children were in the rooms, based on a 5 m^3 room volume per child guidance, carbon dioxide was found to increase to 4000-5000 mL/m^3 within a few hours of occupancy if windows and doors were kept closed (Lundqvist et al. 1983).

From such buildings, severe occupant complaints of discomfort, acute illness symptoms, and concern about the possible health impact on the children were reported frequently in the public media. Consequently, since 1982, mechanical ventilation requirements, combined with heat recovery systems for new institutions, have been set in the Danish Building Code.

This has improved conditions in new buildings. Existing institutions without mechanical ventilation are now renovated and supplied with such installations. In addition, in several cases wall-to-wall carpets have been replaced with hard floor materials such as linoleum, to improve indoor climate (Gravesen et al. 1986).

We studied the effect on the indoor climate and indoor air quality in an existing institution of an after-installed mechanical ventilation system delivering an air change rate of 2.5 per hour (Lundqvist et al. 1986). With the identical number of persons present, the same activities, and the same outdoor climatic conditions, significantly lower carbon dioxide concentrations, lower absolute air humidity, and lower floating dust content were found each day in the mechanically ventilated group room in compari-

son with a corresponding squad room with natural ventilation, and an air change rate of approximately 0.5 per hour. These findings were in agreement with calculations based on dilution ventilation equations.

However, the differences in bacteria and microfungi counts, measured as colony forming units (CFU), showed variations to such an extent that significant differences between the two environments on each day could not be demonstrated. So far, the results indicated that the ventilation rates should be higher than 2.5 ACH to reduce the levels of airborne microorganisms in day-care institutions. The same findings occurred in a follow-up study of the effects of replacing the floor carpeting with cork linoleum in one group room in the same institution (Aalykke et al. 1987).

The aim of the present study was to measure the emission rates of viable particles from a group of children under controlled conditions in a climate chamber with no surface or air contamination before entrance to the room. In numerous recent field studies, most emphasis has been given to the indoor allergens in air and house dust, including species and numbers of fungi (Reed and Swanson 1986). Notwithstanding the well-documented importance of these components, we felt that corresponding interest in the bacterial flora, in particular regarding species distribution, has been missing. Therefore, this study focused most on the composition of the bacterial flora.

MATERIAL AND METHODS

The study was carried out in a climate chamber with a floor size of 2.90 x 4.15 m and a ceiling height of 2.70 m at the Institute of Environmental and Occupational Medicine (formerly the Institute of Hygiene) University of Aarhus, Denmark. All stainless steel surfaces in the chamber were cleaned with distilled water before entry into the chamber and the room was flushed with clean air filtered in an HEPA filter. After entry, room ventilation was reduced to equal an air change rate of 0.15 ACH, comparable to conditions in real-life, tight building envelopes with natural infiltration as the only fresh air supply.

Two groups of six children each, ages 4 to 6 years, entered the chamber, which was measured to have a germ content approximated to zero before entrance. As the net volume of the chamber was 32.5 m^3 and the floor area 12 m^2, the occupancy was equal to 2 m^2 per child, corresponding to the prescribed area in group rooms of day-care institutions. The total plain surface area of the chamber—including floor, ceiling and walls—was 64 m^2, which gives an area to volume ratio close to 2.0.

The duration of occupancy in the chamber was one hour, and within this period in the chamber, no door openings took place in order to keep all particulate matter emitted from the occupants available for collection and measurement. Carbon dioxide, water vapor, and temperature were allowed to rise to simulate conditions in real-life indoor environments. Occupancy was repeated in the afternoon after a break at noon, at which time the climate chamber was aired with 15 ACH filtered air, and the floor cleaned. Measurements were continued after the occupants left the chamber in order to follow the elimination rates of air and surface contamination in the empty room in comparison to the ventilation rate maintained at 0.15 ACH. Then, after one hour of quiet conditions, the floor surface was swept with a nozzle of compressed air for a short moment in order to resuspend settled particles into the room air.

In the chamber, the children wore their own clothes, but no outdoor shoes were worn in order to avoid the possibility of taking soil materials from outdoors. The stay in the chamber was spent with varying activity levels from quiet to moving around. The children were only exposed to their own presence in the chamber, which was not thought to influence their assessed status of normal health. Colony forming units (CFU) of bacteria and mold spores were collected from the air on two slit samplers, producing a dish for counting every 10 minutes, which alternated from bacteria to mold spore plates. Settling plates (14 cm diameter) were placed in four positions on two horizontal levels: 50 cm below the ceiling and on the floor near the walls. These were collected after one hour exposure to settling particles. Samples from the walls were taken by contact plates (Rodac). The sampling volumes were considered to be of no influence to the contaminant concentrations. Bacteria were grown on Plate-Count-Agar at 30°C, for 48 hours; fungi spores were grown on Czapek Agar at 30°C for 48 hours. For the contact plates (Rodac), the medium was also Plate-Count-Agar.

There is no general agreement about the ideal way of sampling viable bacteria and fungi from the atmosphere. In this study slit samplers (model BIAP) were chosen for air sampling, although their sampling principle might have some systematic errors (temporary overcrowding of parts of the Petri dish due to nearby activity). Therefore calculations of both mean and median values for the entire sampling periods were made for comparisons.

RESULTS

Initially, it was clearly demonstrated that human occupancy is an immediate source of viable microorganisms to the room air. Secondly, these can colonize on room surfaces at the same time.

The graphs in Fig. 1 give the consecutive values for bacteria and mold spore colonies obtained from the two slit samplers in the afternoon experiment in which resuspension of particles was managed in the second hour after emptying the chamber. In spite of the limited number of observations, the dynamic conditions and the trends in the air contamination levels are clearly seen in the figures. The number of airborne bacteria increased to a level of 5000 CFU/m^3 after one hour of occupancy in the unvented climate chamber (initially clean and with no sources of particles in the room itself). The corresponding level of mold spores was 1000-1500 CFU/m^3. However, the amount of viable organisms in the bioaerosol was also influenced by the die away of organisms and by surface deposition.

This could be confirmed by measurements in the empty chamber after the group left the chamber. Within the first hour after exit, the concentration of airborne bacteria decreased, corresponding to an elimination rate by dilution ventilation of 2.0 ACH and 1.7 ACH for mold spores with a variation range of ±0.2 ACH between the experiments conducted. Resuspension of possibly settled particles by forced air movements in the chamber in the second hour of the post-occupation period did not increase viable airborne particle counts.

Sediment plates showed no significant differences between numbers of settled viable particles at floor level and at the upper level 50 cm below the ceiling (Friedmann Two-way Analysis of Variance, $p = 0.157$). This indicates that most airborne particles were small enough to be carried around by the air currents in the chamber in the occupied period (Table 1). After the occupants left the chamber, the numbers of CFU on sediment plates returned to background level within an hour; no increase due to the resuspension manoeuvre was seen. Contact plates from the stainless steel wall surfaces showed a growing number of bacterial colonies during the day, opposite to the immediate decrease in airborne viable organisms after disappearance of emission sources (Fig. 2). This finding was unexpected and not supplemented by other measurements, such as investigating for the presence of an invisible moisture layer on the walls.

A total of 142 strains was identified; most of these were from sediment plates (95%), as the colonies on the slit sampler frequently were too confluent for separation. On sediment plates coryneform species dominated (27-85%); followed in frequency by micrococci (4-50%); *Staphylococcus epidermidis* and *saprophyticus* (12-43%); *Bacillus spp.* most frequently *B. megaterium* (12-33%); and *Acinetobacte spp.* (11-14%). From the slit sampler plates, staphylococci dominated (67%), followed by coryneform and

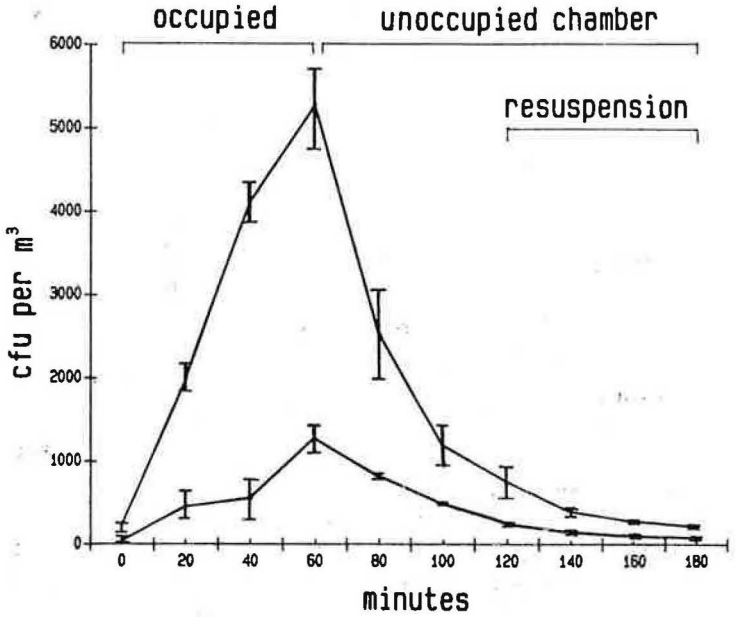

Fig. 1. Highest and lowest number of airborne bacteria (upper curve) and mold spores (lower curve) per m^3 as measured each 20 minutes in the climate chamber by two slit samplers. First hour: chamber occupied; Second hour: chamber unoccupied, elimination by natural die away of viable organisms; Third hour: same as second hour, but forced air movements in the chamber for resuspension of settled particles.

Table 1. Number of colony forming units (CFU) of bacteria settled on Ø 14 cm Petri dishes within one hour in four positions both on floor and 50 cm below ceiling in the six successive periods of the day: I) Empty chamber before entrance of occupants; II) Occupied chamber, first period; III) Empty chamber after occupants exit; IV) Occupied chamber, second period; V) Empty chamber after occupants exit; VI) as V), but with a resuspension manoeuver performed.

PERIOD	FLOOR	CEILING
I	16 18 20 37	22 15 18 37
II	235 209 263 275	227 216 250 245
III	28 20 26 28	27 28 21 20
IV	278 208 236 256	198 188 239 182
V	30 41 34 33	55 48 48 34
VI	9 9 5 7	11 12 16 3

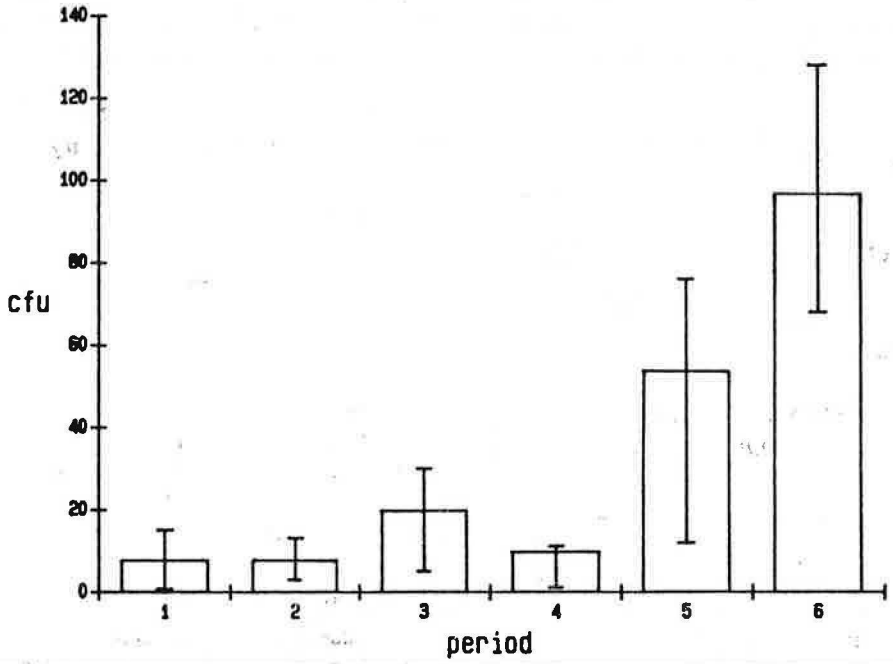

Fig. 2. Number of colony forming units (CFU) of bacteria on Rodac Plates showing surface contamination on the stainless steel walls in the climate chamber taken by contact in the successive six periods of the day: I) Empty chamber before entrance of occupants; II) Occupied chamber, first period; III) Empty chamber after occupants exit; IV) Occupied chamber, second period; V) Empty chamber after occupants exit; VI) as V), but with a resuspension manoeuver performed.

micrococci (17%). Twelve colonies were later examined from contact plates (Rodac-plates). From these plates were only isolated *Bacillus spp.* from the floor, from the ceiling an walls only coryneforms were isolated.

The distribution of species was largely independent of the circumstances, although periods of activity showed a tendency to more diversity in species. The strains isolated were subjected to 33 tests of morphology, colonial morphology, physiology, and biochemistry in accordance with recommended tests (Seiler 1983). Three reference strains (NCIB 113's, 11092 11491) of *Arthobacter spp.* and NCIB 11437 of *Brevibacterium* were examined by the same methods. According to these examinations, 86% were *Arthobacter* and 14% were *Brevibacterium.*

DISCUSSION

Bioaerosols that are respirable in size and shape are well-known to play a role in disease transmission and acute respiratory diseases, such as hypersensitivity pneumonitis and allergic reactions (types I and IV). An inherent problem in conducting airborne microorganism studies is that the emission of microorganisms and aeroallergens may not be expected to be the same in various groups of individuals or in the same group from day to day during the seasons of the year. The outbreak of infectious diseases may also be expected to cause temporary but dramatic changes. This should limit the conclusions to be drawn from this study.

However, the occurrence of staphylococci, bacillus, and micrococci is in accordance with those counts reported from indoor atmospheric studies by most authors. What is specific for this examination in the initially clean climate chamber is the frequent occurrence of coryneform bacteria.

The coryneform bacteria, of which several species form part of the normal flora of the human respiratory tract and other mucous membranes, represent a very inhomogeneous group comprising 22 taxa. *Corynebacterium, Arthrobacterium, Brevibacterium, Microbacterium,* and *Propionibacterium* are the most frequent potential pathogens (*C. diphtheriae* are not present). The explanation for these species' predominant representation may be the closed environment of the climate chamber. Furthermore, coryneform bacteria have good survival ability under dry conditions. They have small dimensions (0.5-1 μm in diameter and 3-5 μm in length), which can keep them volatile in contrast to the micrococci.

The qualitative distribution and quantitative amounts of the mold spores will vary within the season. In this study, with no external ventilation and indoor sources, we suppose that the mold spores were carried into the chamber by the clothes.

Sediment plates are generally considered useful for collecting the variety of species present, but they are questionable as a concentration measurement tool. Microbial surface contamination on the walls showed an unexpected growth, which remains to be explored and explained. The study confirmed that the human emission of microorganisms in an enclosed, occupied space is a complex variable in itself, and produces nonlinear relationships and non-normal population distributions (Burger and Solomon 1987). Measurements of the steady state concentrations under various conditions showed that the continuous loss caused by die away has to be considered in calculating emission rates of viable airborne microorganisms from humans in occupied rooms.

Ventilation rates higher than 2-3 ACH are needed to eliminate from the room air an emitted bioaerosol to avoid deposition and subsequent growth on surfaces in the room. This is in accordance with general practice in laminar airflow, ventilated, clean rooms. Once brought into a room or into a building by dust, shoes, and clothes, microorganisms can stay alive for a long time and cause health disorders among susceptible individuals.

Yet, prevention has to be enforced against pathogens such as *Legionella pneumophila*; *Aspergillus sp.*; thermophilic actinomycetes; and, if present, *Mycobacterium tuberculosis* (LaForce 1986). Precautions against virus and vector-borne infections will normally be taken separately when any epidemics are in outbreak.

The American Industrial Hygiene Association (AIHA) has prepared a manual dealing with indoor environmental quality and suggested that levels of microorganisms exceeding 500 CFU/m^3 warrant a detailed environmental survey in each case (Gammage and Kerbel 1987). However, this action level should be more specific and related to the non-occupied, mechanically ventilated, and naturally ventilated room.

This conclusion is based on the results from this climate chamber study and from the previously quoted field measurements where values ranging from 1000 to 10 000 CFU/m^3 were found. The high and fluctuating emission rates of viable microorganisms from the occupants must be considered separately, as these sources may easily raise levels in excess of 500 CFU/m^3.

Conditions in rooms with furniture and with large variations in textured surface areas may act differently from the stainless steel chamber environment, and should be expected to accumulate and disperse

microorganisms depending on both humidity and binding forces. The concept of every room surface as a temporary or permanent sink for airborne pollutants and contaminants should generally be further explored in addition to ventilation, clean-up, and emission control strategies.

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