BACTERIAL AEROSOLS IN INDOOR AIR

by

Aino Nevalainen

ACADEMIC DISSERTATION

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ABSTRACT

Levels of indoor air bacteria in homes and the basic factors affecting them were investigated. The factors studied were occupancy, ventilation system, season and the type of dwelling. Samples were taken from new suburban townhouses, from farmhouses and from homes with a suspected microbial problem. Effects of air RH and temperature and the number of people present were also studied, as well as the diurnal and spatial variation in bacteria levels. The genera of airborne bacteria and their particle size distributions were characterized.

Bacteria samples were taken with six-stage impactors on TGY agar and the plates were incubated in room temperature for 3-5 days. Together about 2200 strains were isolated for further characterization of the bacteria.

Bacterial levels in homes varied $<10\text{-}10^4$ cfu/m^3. Indoor levels were always higher than outdoor levels. Outdoor levels were $\leq 10^2$ cfu/m^3 in winter when the ground was frozen and covered with snow. In spring to fall the levels were between $10^1\text{-}10^3$ cfu/m^3. In new homes, levels of airborne bacteria increased as long as two years after occupancy, after which they stabilized to the final level. This accumulation was independent on the ventilation system.

For removing airborne bacteria, mechanical supply and exhaust ventilation was a more effective system than natural ventilation or mechanical exhaust only. The relative humidity or temperature of air did not affect levels of airborne bacteria in either indoor or outdoor air. The number of people present during sampling affected significantly the levels of bacteria in indoor air, but the number of persons in the family did not. The proposal for an uppermost normal level of indoor air bacteria in homes is 4 500 cfu/m^3, when sampled with 6-stage impactor, using TGY agar and incubation at room temperature. The proposal does not apply to farmhouses.

The *Micrococcus/Staphylococcus* group was dominating in indoor air, but was a minor group in outdoor air, where *Pseudomonas* was the dominating genus. The differences between indoor and outdoor bacteria can also be seen in particle size distributions.

Actinomycetes were frequently found in homes with moisture problems but seldom in homes where no complaint had been made. Their occurrence evidently indicates a moisture problem in the house, and remedial actions are recommended. In farmhouses, however, actinomycetes belong to the normal flora because of the specific sources in the farming environment.

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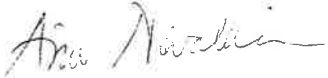

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1 INTRODUCTION

1.1 Bacterial aerosols

Airborne bacteria are ubiquitous; they are found in both indoor and outdoor air, even in the middle of oceans and in the upper layers of the atmosphere. Outdoors, bacteria are made airborne by dry processes such as mechanical disturbance of soils in farming, forestry and construction, and by wind. An example of a dry process indoors is the desquamation of human skin, which is the main source of indoor air bacteria. Another mechanism by which airborne bacteria are produced is droplet formation from bacteria-containing liquids, which after evaporation results in dry particles that remain airborne. These kinds of bacterial aerosols are formed by rain, in wastewater treatment and in wet-type cooling towers. Indoors bacterial aerosols are also formed by sneezing and coughing, which are the main sources of airborne infectious agents.

1.2 Airborne agents of disease

Transmission of diseases through air was suggested long before the existence of the agents (bacteria, viruses and other microbes) was shown. However, the medical importance of airborne infection has still been questioned in this century, as indicated in the review by Riley (1980).

There are two forms in which infections are transmitted through air. The first applies to droplets larger than 0.1 mm, which decend a short distance from the source. This form of personal contact infection is how most respiratory infections are transmitted from person to person (Riley 1982). The second form, which consists of dried droplet nuclei small enough to be suspended in air for long times and over great distances, is called airborne infection and is especially important in buildings. Field evidence concerning this route of infection has been introduced by Riley *et al.*(1962) for tuberculosis, by Riley *et al.*(1978) for measles and by Fraser *et al.*(1977) for legionellosis.

Furthermore, illnesses other than infections have also been associated with bacterial aerosols or their residues. Among these are chronic bronchitis, allergic alveolitis and organic dust toxic syndrome, usually caused by occupational exposure to high levels of bioaerosols. The causative agents are not well defined, but actinomycete spores and endotoxins of Gram-negative bacteria are often associated with these diseases (Lacey and Crook 1988).

1.3 Airborne bacteria and indoor air hygiene

Airborne particulate matter of biological origin is called a bioaerosol. In addition to bacteria, bioaerosols include fungal spores, pollen, algae, skin platelets and their residues.

The extensive literature on airborne allergens deals with both occupational environments and dwellings. Cases of asthma, hypersensitivity pneumonitis and other such diseases are mainly associated with fungi or actinomycetes (Lacey and Crook 1988). Airborne bacteria are often mentioned with this connection, but there is in fact very little data available.

Most of the research on bacteria in indoor air has been carried out in hospitals in an attempt to develop methods for the prevention of infections. Therefore the control and removal of airborne bacteria from indoor air are fairly well known (Rhame 1986). Applications of this control are used in operating rooms, in the food and pharmaceutical industry and in hi-tech clean room technology (Krüger 1987).

On the other hand, very little is known yet about airborne bacterial flora, the special characteristics of airborne infecters or the relationships between bacteria and other particles of air.

1.4 Importance of particle size

Regardless of whether a particle is inorganic, organic or of biological origin, its aerodynamic size determines its essential characteristics such as dispersion, removal and site of deposition in the human respiratory tract.

There are several ways of defining particle diameter. The most useful of these, aerodynamic diameter (d_A), is defined as the diameter of a unit density sphere (density = 1 g/cm^3) which has the same aerodynamic properties as the particle in question. It means that particles of any density or shape will have the same aerodynamic diameter if their settling velocity is the same (Reist 1984; see Figure 1).

The main factors which control the behavior of aerosol particles are diffusion, caused by random Brownian motion, and gravitational settling (Figure 1). In the respiratory tract, removal by impaction, *i.e.* the difference in inertia of gas and heavier particles, also becomes a very prominent way of settling. For small particles ($d_A < 2 \mu m$) the dominating force is diffusion, and the dimension of their movement is m^2/s. This follows Fick's law of diffusion (1):

(1) $$J = -D\frac{dc}{dx}, \text{ where}$$

J = flux of particles crossing a unit area per unit time ($1/m^2$ s)
D = diffusion coefficient (m^2/s)
dc/dx = concentration gradient (kg/m^4)

As the particle diameter increases, diffusion becomes negligible; the determining force is the gravity, and the particle motion follows Stokes' law (2). The settling velocity of a particle increases with increasing diameter and mass:

(2) $$F = 3 \pi \eta v d, \text{ where}$$

F = the force on the particle (kgm/s^2)
η = dynamic viscosity of the medium (kg/ms)
v = relative velocity between the air and the particle (m/s)

(3) $$v = \frac{\rho d_p^2 g}{18 \eta}$$

d_p = diameter of the sphere, ρ = density of the particle (kg/m^3)

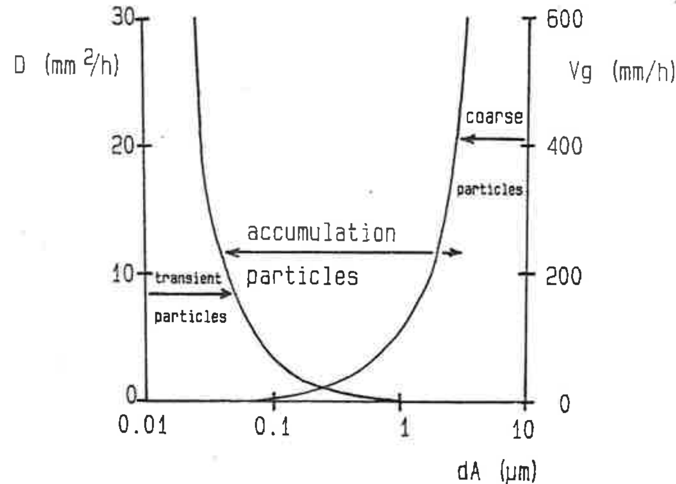


Figure 1. Factors that dominate aerosol particles vs. particle size. Transient particles are formed by homogenous nucleation, and they rapidly grow by condensation and agglomeration to stable accumulation particles. Coarse particles are formed by mechanical action and their residence times in air are short due to sedimentation. D = diffusion V_g = sedimentation due to gravity

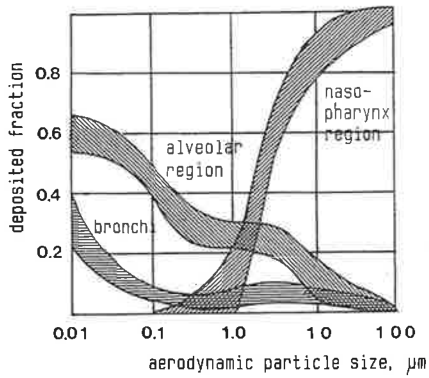


Figure 2. Deposition of particles in different regions of the respiratory tract (Task Group on Lung Dynamics 1966).

The health effects of aerosol particles are also related to their size. How deep a particle is inhaled into the respiratory system depends on the aerodynamic diameter of the particle.

Large particles are deposited in the upper parts of the respiratory tract and most are swallowed. Large particles may affect health if they contain allergens. A fraction of particles smaller than 2 μm reach the bronchi but are subsequently removed by the ciliary action of mucous membranes. Some particles <0.5 μm reach the alveolar stage and about 50% of them are exhaled (Figure 2). Most of the effects on pulmonary health are caused by particles small enough to reach this region behind the ciliar activity. The fraction of particles deposited in the respiratory tract also depends on the size of the airways, or the age of the exposed individual, and the pattern of breathing, *i.e.* nose or mouth breathing, breathing frequency and tidal volume. During slow deep breathing, deep-lung deposition increases (Glenn and Craft 1986).

For purposes of regulation and practical hygiene, concepts of respirable and non-respirable particle fractions are used. However, particle behavior in the alveolar region does not allow a sharp division of particles into two fractions. Thus there are several definitions of these concepts (Reist 1984): the British Medical Research Council (BMRC) defined the respirable fraction as that which penetrates to the alveolar stage (1952). In the 1961 standard of the U.S. Atomic Energy Commission (AEC) respirable particles are defined as those that penetrate to the nonciliated portions of the lung. The American Conference of Governmental Industrial Hygienists

(ACGIH) has given an almost identical definition. These definitions are based on sampling efficiency curves for aerosol samplers (Glenn and Craft 1986).

1.5 Survival of airborne bacteria

Survival of bacteria in air has been studied experimentally since the 1930's, but the determinants are still rather obscure. Air is an extreme environment for bacteria where their survival is limited by environmental stress. Only bacteria resistant to dryness, UV radiation and chemical contaminants can remain viable. In spite of this, it has been suggested that bacteria can divide at least once while airborne (Dimmick *et al.* 1979). Airborne division has been shown experimentally with *Serratia marcescens*, but there has been no later confirmation of this observation nor is there any information about whether other bacteria can divide while airborne. On the other hand, the concept of microbial viability has been reestablished in modern microbial ecology. Traditionally, the criterion of viability has been the ability to divide. With indirect measurements of specific activities such as activity of various enzymes, photosynthesis and respiration it has been shown that bacteria may be viable even when they cannot be seen to divide (Roszak and Colwell 1987).

The mechanisms of death for airborne bacteria are DNA or RNA damage (Strange and Cox 1976), or loss of cellular potassium (Anderson *et al.* 1968). It has been suggested that the death mechanism is dependent on the relative humidity of air (Benbough 1967, Donaldson 1978).

1.5.1 Temperature and humidity

Present data on the effects of air temperature and relative humidity on bacterial survival does not allow systematic, detailed analysis. In laboratory experiments there are many factors that influence simultaneously, such as the strain of the organism, the culture medium, the method of aerosolization, the atmosphere into which the organisms are aerosolized and the method of sampling. Thus it is difficult to separate out the effect of one factor (Donaldson 1978, Cox 1987).

Information about the effects of temperature and humidity on bacterial aerosol survival has been produced with single strains of bacteria, such as *Serratia marcescens* (Kethley *et al.* 1957), *Francisella tularensis* (Cox and Goldberg 1972) and *Escherichia coli* (Cox 1987). This information comes mainly from experiments in which bacterial suspensions have been aerosolized. There are very few field results about the effects of temperature and relative humidity on bacterial aerosol survival.

Generally speaking, low temperatures permit microbial survival. Microbial strains are maintained in the refrigerator and stored at deep-freeze temperatures (-70°C, or in liquid N_2 at -190°C). Several reviewers (Hatch and Wolochow 1969, Strange and Cox 1976) claim that low temperatures also favour bacterial aerosol survival and that increasing temperatures increase death rates, but they do not define these temperature ranges. In the study of Kethley *et al.* (1957) this trend was shown to be true in the temperature range -40°C... +30°C, where the death rate of *Serratia marcescens* was increased at relative humidity levels of 20...80%.

The literature on effects of relative humidity on airborne bacterial survival is extensive but no final answers have been reached. Obviously, injuries to and death of airborne bacteria are due to several causes, depending both on the particular organism and on many simultaneous environmental factors.

In air, bacterial cells are susceptible to dehydration. Dehydration as such does not necessarily kill bacterial cells, as is known from the general practice of freeze-drying them; but their metabolic activities are reduced (Hatch and Dimmick 1966). On the other hand, rehydration may be lethal, due to osmotic shock (Hatch and Dimmick 1966).

The death rate of airborne bacteria has been reported either to increase or to decrease with increasing relative humidity (RH) (Hayakawa and Poon 1965). Hatch and Dimmick (1966) reported that abrupt shifts, both increasing and decreasing RH, were lethal to airborne bacteria. Bacterial genera differ in their ability to withstand different humidities. *Escherichia coli* survives best at high humidities up to 90%, while RH levels under 40% are favourable to *Serratia marcescens* and staphylococci (Hatch and Wolochow 1969). For pneumococci and hemolytic streptococci the mid-range of humidity is the most lethal (Hatch and Wolochow 1969). *Legionella pneumophila* survived best at 65% RH, was less stable at 90% and least stable at 30% (Hambleton *et al.* 1983, Dennis *et al.* 1988).

In bacterial aerosol survival there are many additional factors involved such as the protecting effect of inorganic salts or sugars added to the aerosolized suspension (Poon 1968, Strange and Cox 1976), or the medium in which bacteria have been cultured prior to use in an experiment. Increased death rate after shifts in humidity were observed in organisms cultured and aerosolized from a rich medium but not from a minimal nutrient medium (Hatch and Wolochow 1969).

Surprisingly, there is a total lack of reported experimental work done with strains originally isolated from air. In the airborne state many factors are lethal and damaging to bacteria, and yet there are always viable cells present (see 1.1). In addition to spores for which air is a natural route of dispersion, vegetative cells are also found in air. It is not known what features make some strains so resistant to various stresses.

Why, for example, does *Legionella* survive (Dennis *et al.* 1988) and retain its virulence for long periods of time and after transport over long distances from the generation site?

Studying those strains that are known to survive while airborne could lead to better understanding of the factors that regulate airborne survival. More field research is also needed to evaluate whether environmental factors have any impact on airborne bacteria in general.

1.5.2 Effect of light

The effect of light on bacterial cells has been studied mainly in the laboratory with terrestrial and aquatic organisms. Both UV- and visible light produce harmful effects on DNA and membranes (Whitelam and Codd 1986, Larson and Berenbaum 1988). It is not clear, however, whether these observations can be applied to airborne microbes (Gregory 1973, Whitelam and Codd 1986, Cox 1987).

Intracellular or extracellular pigments, usually carotenoids, protect microbes against lethal photodynamic reactions (Minnikin and O'Donnell 1984, Whitelam and Codd 1986). Phototrophic organisms are also capable of altering the concentration of pigment; this may be a means of shielding the cell from light. Other shielding systems are the enzymes superoxide dismutase, catalase and peroxidase. Moreover, antioxidants, such as ascorbate, glutathione, α-tocopherol and histidine, may provide defense against photo-oxidative damage (Whitelam and Codd 1986).

Goff (1973) reported higher counts of airborne bacteria when air was sampled in the dark than when sampling was done in daylight. Likewise, Fedorak and Westlake (1978) found that shielding the otherwise transparent sampling device from light resulted in 3- to 8-fold increases in viable counts recovered near a wastewater treatment plant.

As such, the lethal effect of light on microbes is an old and established finding and it has hygienic importance. Ultraviolet light is routinely used for disinfection in the food and pharmaceutical industry, hospitals and microbiological laboratories (Rhame 1986). It has been found to be most effective as an overnight surface decontaminant, when high light intensities (20-50 $\mu W/cm^2$) can be used (Chatigny and Clinger 1969).

1.6 Sources of bacterial aerosols

1.6.1 Indoor sources

In indoor air, the main source of bacterial aerosols is usually human beings. Desquamation of skin, movement, talking, sneezing and coughing produce bacterial aerosols (Lidwell 1967). Bacterial aerosols may also be created by disturbing previously settled dust and by handling textiles, food, plants, waste or other material contaminated by bacteria. In addition to this "normal" dispersal, there may be other sources which result in the release of excess amounts of bacteria or spores into indoor air. Among these are contaminated humidifiers or microbial growth due to water damage in the building.

1.6.1.1 Human skin

The area of the integument of an adult is 1.75 m^2, the surface layer of which consists of some 10^8 scales, each about 30x30x3-5 μm in size (Noble 1975). This whole cell layer is lost and replaced about every 4 days. This means an emission of more than 10^7 scales per person per day (Noble 1975). Certain individuals, whether healthy or with a skin disease, may be "dispersers", who shed unusually large numbers of bacteria into their surroundings (Noble *et al.* 1976).

Physical activity increases this dispersal. Walking has been shown to produce about 10^4 scales per minute (Sciple *et al.* 1967). In hospital studies it has been shown that showering may also increase the dispersal of bacteria, at least temporarily (Cleton *et al.* 1968). Clothing prevents bacterial emission, but really effective prevention is only approached by totally enclosing the body in protective garments (May and Pomeroy 1973).

Bacteria on the skin form microcolonies of 10^2-10^5 viable cells. These colonies may be far apart from each other and it is estimated that only 10% of the skin scales carry bacteria (Noble 1975).

Skin bacteria that normally colonize either on the surface or in follicles are known as "residents", as opposed to "transients", which are occasional contaminants and do not replicate on the skin. A third group of bacteria, called temporary residents, do not belong to the normal skin flora but may temporarily colonize the skin surface (Roth and James 1988).

The normal resident flora is known to be composed of relatively few genera (Leyden *et al.* 1983). The main groups are Gram-positive cocci of the genera *Micrococcus* and *Staphylococcus*. Kloos *et al.* (1974) reported that of 115 individuals studied 96%

carried *Micrococcus* on their skin. About 10 species of the genus *Staphylococcus* are also frequently isolated from skin, and staphylococci are the dominating group on the feet (Marshall *et al.* 1987) and on the subungual regions of the hands (McGinley *et al.* 1988). Gram-positive rods such as *Corynebacterium* and *Brevibacterium* are also common, but the only significant Gram-negative resident is the genus *Acinetobacter* (Roberts and Highet 1986).

The skin is a dry mechanical barrier from which contaminating microbes are continually removed by desquamation (Roberts and Highet 1976). The integumentary system contains a microbial ecosystem with many interactions, especially antibiosis. An example of this was an experiment where even deliberate inoculation with *Pseudomonas* sp. did not produce lesions on the skin (Leyden *et al.* 1980).

1.6.1.2 Mouth, throat and nasal cavities

Jennison (1942) showed that talking, coughing and sneezing produce droplets that carry bacteria. In a study by Duguid (1945), one sneeze generated 10^6 droplets less than 100 μm in diameter, while speaking a hundred words produced 250 droplets of equal size. Droplets small enough to evaporate before reaching the ground remain airborne, carrying the bacteria and other non-aqueous material of the original droplets (see 1.2).

Typical bacteria found in the nose and nasopharynx are aerobic coryneforms, *Staphylococcus epidermidis* and *Staphylococcus aureus* (Youmans *et al.* 1975). In the mouth, streptococci are the most numerous group of bacteria. They form about half of the viable counts in the saliva and on the dorsum of the tongue and they are also common in dental plaque (Hardie 1980). Other bacteria that occur generally, although in low numbers are staphylococci, lactobacilli, *Actinomyces*, *Bacteroides*, *Fusobacterium*, spirochetes and anaerobic vibrios (Hardie 1980).

1.6.1.3 Humidifiers

Air humidifiers are potential sources of airborne bacteria. A number of cases of disease, especially occupational diseases, are related to thermotolerant bacteria (Kohler *et al.* 1976) or bacterial endotoxins (Rylander and Haglind 1984) generated and released by humidifiers. So-called humidifier fever is a flu-like illness that occurs typically in the evening of the first working day after a break, subsiding in 24-48 h and not recurring despite continuing exposure (Finnegan *et al.* 1987). Cases of humidifier disease have been reported from hospitals (Smith *et al.* 1977), printing plants (Pickering *et al.* 1976, Rylander and Haglind 1984) and even from homes (Harris *et al.* 1984). Cases of a similar syndrome, caused by tap water with a high concentration of endotoxin, have also been reported (Nordman 1984).

Stagnant water in cooling systems or humidifying devices provides a good environment for replication of bacteria and other microbes. Burge *et al.* (1980) analyzed the water of 110 domestic humidifiers and found thermophilic bacteria and mesophilic fungi in most of them, and occasionally thermophilic actinomycetes. There are several possible ways in which these contaminants may be aerosolized (Keleti and Shapiro 1987).

All humidifiers, other than the evaporative type, are potential aerosol generators. However, excess bacteria have usually not been shown in air, although circulating or standing water has been heavily contaminated (Marko 1983). Most well-documented cases of humidifier fever have been caused by spray-type devices.

Another up-to-date source of bacteria is whirlpool spas, which are warm water pools with a built-in aerosol-generating system. The generated droplets have been shown to be of respirable size and possible carriers of microbes, e.g. *Legionella* (Baron and Willeke 1986).

1.6.1.4 Other indoor sources of bacteria

No systematic analysis has been published on the sources of indoor air bacteria. Bacteria found on different surfaces may become airborne. Normal domestic flora has been studied by Finch *et al.* (1978), who surveyed bacteria on different surfaces of 21 homes, and by Scott *et al.* (1982), who investigated the surface bacteria in 200 homes. The most common isolates on kitchen surfaces were *Bacillus* sp. and coagulase negative members of the family *Micrococcaceae*. Enterobacteria were common on kitchen sinks, draining boards and dishcloths. In bathrooms, most surfaces were contaminated with micrococci and *Bacillus* sp., while *Escherichia coli* was isolated only sporadically. No *E.coli* was found in the sinks of two new, unoccupied houses, but within one week of occupation a large number of *E.coli* were isolated (Finch *et al.* 1978). Thus the effect of occupancy on colonization of the sink surface was rapid. A summary of the two surveys of domestic bacteria is shown in Table 1.

Table 1. Bacterial flora on domestic surfaces

Bacterial group	Isolation frequency	Surface sampled	Ref.
Enterobacteriaceae			
E.coli	+ + +	dishcloth,sink,drain board	1
	+ +		2
Klebsiella	+ +	"	1
	+		2
Citrobacter	+ +	"	1
	+		2
Enterobacter	+ +	"	1,2
Pseudomonas sp.	+ +	dry and wet surfaces	2
Ps. aeruginosa	+ +	drain board, window sill	1
	+	"	2
Micrococcaceae	+ + +	dry and wet surfaces	1
Staphylococcus aureus	+ +	towels	1
	+	dry and wet surfaces	2
Bacillus sp.	+ + +	dry and wet surfaces	1
B.cereus	+	dry and wet surfaces	2

symbols: + + + a major genus, isolated in 40% of the samples
+ + found frequently, in 10-40% of the samples
+ found regularly, in <10% of the samples
References: (1) Finch *et al.* (1978), (2) Scott *et al.* (1982)

In Finch's study no air samples were taken. Scott *et al.* (1982) mention air sampling on blood agar plates but report merely that the airborne colonies detected were mainly micrococci and Gram-positive bacilli. It can be assumed, however, that bacteria from the surfaces are released into the air by aerosol formation from splashing water or mechanical disturbance. Gerba *et al.* (1975) showed that the toilet bowl is a continuous source of aerosolized coliforms which are shed overall in the bathroom. Detailed information about the interaction between surface and airborne flora is not available.

Ventilation ducts through which air is delivered to and collected from different parts of the building are dry and clean if they are functioning ideally (Jantunen 1987). If, however, water is condensed or leaks into the duct, microbial growth will occur. Aerosolization of spores or other particles from this growth occurs easily with the air stream. Although this may be common, there are few reports where the duct system alone has been reported to be the source of microbes in indoor air. In problematic cases there is usually a humidifying or cooling system associated with the duct system (Shaffer and McDade 1962, Weiss and Soleymani 1971, Ager and Tickner 1983).

Renovation work inside a building may also be a source of bioaerosols. Several cases of hypersensitivity pneumonitis due to *Bacillus subtilis* and *B.licheniformis* have been reported from renovation in a bathroom (Johnson *et al.* 1980).

1.6.2 Sources of outdoor bacterial aerosols

Outdoors, there are natural sources of airborne bacteria. These bacteria may also be transported into indoor air, but the amount and mechanism of transport is poorly known.

Soil is undoubtedly the main source of airborne bacteria. Soil includes large amounts of microbes: fungi and bacteria, including actinomycetes. It is estimated that 1 g of soil includes 10^9 bacterial cells (Atlas and Bartha 1987). Hence any movement of soil that releases dust also generates bacterial aerosol.

Plant leaves are prone to colonization by bacteria. Leaves are dry habitats with scarce supplies of nutrients for most microbes, but they do have their own ecosystems, the composition of which depends on the roughness and amount of hair on the leaf surface (Campbell 1985).

Airborne transmission is important for phytopathogenic (pathogenic to plants) bacteria such as *Erwinia*, *Pseudomonas* and *Xanthomonas* (Harrison 1980). Theoretical calculations of the distances traveled by *Erwinia carotovora* showed that 10^9 cells/m^2 were deposited 10 km downwind from the source (Harrison 1980).

Agriculture, forestry, biotechnical industry and waste treatment are heavy emitters of bacterial aerosols. Harrison and Hattis (1985) estimated that the microbial aerosol release from different activities in a greenhouse was 10^7-10^{11} microbes/day. Kenline and Scarpino (1972) calculated that bacterial emission from an aerated wastewater basin is about 440 bacteria/m^2/s, which is about $3x10^9$ bacteria/day from a 75 m^2 basin. According to Bovallius *et al.* (1980), viable bacteria from a wastewater treatment plant were transported a distance of 3 km distance downwind.

1.7 Sampling methods of airborne bacteria

Airborne bacteria are sampled by methods based on cultivation of colonies or on direct counts of stained cells examined under a microscope. Different types of particle samplers can be applied for bacteria sampling: impactors, filters, liquid impingers and methods based on sedimentation. Because of both the qualitative and quantitative heterogenity of the sampled material, no single method is adequate for all purposes. Nor is any method suitable for detecting total counts, bacterial flora and

particle size distribution. Consequently, the sampling method must be chosen according to the purpose of the analysis.

1.7.1 Impactors

The principle upon which impactors are based is the aerodynamic characteristics of particles, *i.e.* their inertia (Reist 1984). The air stream in the sampler is forced to change its direction, and particles with sufficient inertia or aerodynamic size (see 1.4) will impact on the collection surface. Several types of impaction samplers are available.

Since Andersen (1958) published a description of a six-stage cascade impactor for viable particle sampling, this method has become an almost worldwide standard (Gorman *et al.* 1979, Morey *et al.* 1986).

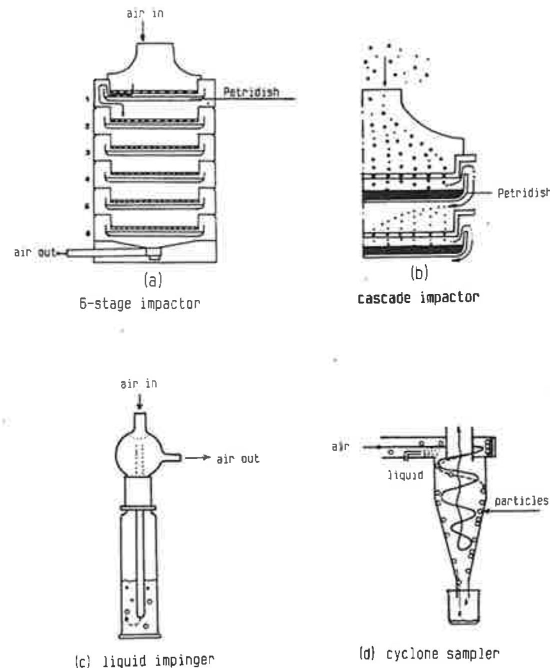


Figure 3. Principles of airborne bacteria sampling devices.

This sampler is capable of sampling particles of $<1...>8$ μm of aerodynamic size. The particles are collected on Petri dishes filled with culture medium (Figure 3). After incubation the visible colonies are counted and the results are expressed as colony-forming units per unit volume. In heavily contaminated environments ($>10^6$ cfu/m^3) the usefulness of the six-stage impactor is limited because very short sampling times, a few tens of seconds, must be used (see 1.7.3).

Modifications of the original sampler have been introduced by May (1964) and by Lidwell and Noble (1965), who improved the yield of large (>5 μm) particles by adding one more stage to extend the range of the sampler. Although well-founded, these ideas have not been applied further to field research, probably because the large particles are not considered important from the standpoint of health (see 1.4).

Jones *et al.* (1985) showed that for fungal spores the sixth stage alone gave a good estimation of the yield of the six-stage sampler. This N6 method was later also considered suitable for airborne bacteria and was recommended by the ACGIH Committee on Bioaerosols for routine surveys of viable microorganisms in office environments (Morey *et al.* 1986).

A two-stage cascade impactor of either disposable plastics (Curtis *et al.* 1978) or stainless steel (Gillespie *et al.* 1981) divides the aerosol into respirable and non-respirable fractions. Different patterns of particle size distribution are achieved with 6-, 7- and 8-stage impactors (Andersen 1958, May 1964, Curtis *et al.* 1978). Macher and Hansson (1987) introduced a cascade impactor suitable for personal sampling. In this sampler gelatin is used as the collection surface. Several other types of impactors have been developed for various situations (Andersen and Andersen 1962).

1.7.2 Liquid impingers

Impingers are usually all-glass samplers with water or other fluid as the collecting medium. The collection mechanisms are impaction, sedimentation and the diffusion occurring in the bubbles (Figure 3). Analyses of the catch are made by cultivating the sampling liquid on suitable media. There are both single-stage and multi-stage impingers (May 1966) which allow size fractioning of particles. The all-glass impinger was proposed as a standard method for sampling airborne microorganisms (Brachman *et al.* 1964), but according to the later literature the proposal has not been well-accepted.

1.7.3 Filter sampling

Sampling methods based on cultivation underestimate counts of species that do not grow or grow slowly on the sampling medium. This can be overcome by using cellulose or gelatin filter sampling followed by direct counting of bacterial cells under a microscope.

The CAMNEA method developed by Palmgren *et al.* (1986) is based on filter sampling and on analyses by both direct counting of stained cells and cultivation of viable cells. This method gives total counts that are 10-10^3 times higher than those sampled with the six-stage impactor in heavily contaminated environments (Palmgren *et al.* 1986, Laukkanen *et al.* 1987).

1.7.4 Other methods

A slit sampler (Bourdillon *et al.* 1941) is a one-stage impactor where the agar plate rotates, thus allowing long sampling periods (up to 1 hour).

In a cyclone sampler (Errington and Powell 1969) air is introduced radially into the upper part of a cylinder so that it makes several revolutions inside the cylinder before exiting axially along the cylinder centerline (Figure 3)(Reist 1984). The bacteria-carrying particles are rinsed down into the collection fluid. Another type of cyclone sampler is the RCS centrifugal sampler in which the bacteria are impacted on agar strips (Reiss 1981).

Sedimentation sampling by exposing open Petri dishes to the air also provides an estimation of airborne bacteria. This method overestimates the rapidly falling larger particles but is extremely simple and therefore cheap.

1.7.5 Comparison of sampling methods

No systematic evaluation has been made which compares the yields of all different samplers. Comparisons of the collection efficiencies of different sampling methods are listed in Table 2. There it can be seen that comparisons between different methods have been carried out both in the laboratory and in various field conditions, from classrooms to animal facilities and waste treatment plants. No straightforward conclusion can be made on the basis of these data. In agricultural and waste treatment environments where air is heavily contaminated, the Andersen six-stage impactor was less effective than the Hi-Vol sampler, liquid impinger, cyclone sampler or membrane filter; but it was better than the slit sampler or two-stage

impactor. In some studies the results have been contradictory (Lundholm 1982, Leong *et al.* 1987).

Table 2. Comparisons of the collection efficiency of different samplers. Differing efficiency is expressed by: better > worse.

Efficiency of methods	Testing environment	Reference
And.2-stage < 8-stage	swine houses, classrooms	1
And.2-stage > May 3-stage	wastewater spray irrigation	2
And.2-stage < 6-stage	wastewater/sludge treatment	3
And.6-stage < Hi-Vol	waste handling	4
And.6-stage > slit sampler	cotton mill	5
And.6-stage < MSLI	waste handling	6
And.6-stage < cyclone	cowshed	7
And.6-stage < cyclone	outdoors	7
And.6-stage (=) RCS	occupational environments	8
And.6-stage (=) Marple personal 8-stage	laboratory	9
And.6-stage (=) drum sampler	laboratory	10
And.6-stage vs. midget impinger: variable	occupational environments	5, 8
And.6-stage < AGI	cowshed	7
And.6-stage > AGI	laboratory	5
And.6-stage < membrane filter	animal quarter	7
And.6-stage < membrane filter	waste handling	6
And.6-stage < membrane filter	landfill	11
Gelatin filter (=) cellulose ester filter	pharmaceutical industry	12
Gel.filter > cellulose acetate filter	laboratory	13
Personal impinger (=) AGI	laboratory (*Bacillus subtilis*)	14
Reuter centrif. > slit sampler	laboratory	15

abbreviations:
And. = Andersen, see text; "n-stage" refers to cascade impactors with n stages
Hi-Vol = high flow rate (20...30 l/s) air sampler
MSLI = multi-stage liquid impinger
AGI = all-glass impinger

References: (1) Curtis *et al.*(1978), (2) Zimmerman *et al.*(1987), (3) Gillespie *et al.*(1981), (4) Gorman *et al.*(1979), (5) Lundholm (1982), (6) Crook *et al.*(1988), (7) Henningson *et al.*(1981), (8) Leong *et al.*(1987), (9) Macher *et al.*(1987), (10) Andersen *et al.*(1962), (11) Laukkanen *et al.*(1987), (12) Hecker *et al.*(1983), (13) Koller *et al.* (1974), (14) Macher *et al.*(1984), (15) Placencia *et al.*(1982)

1.7.6 Determination of total viable counts

In hygienic environmental monitoring, total bacteria counts are determined mainly when water or food samples are investigated. The methods originally developed for this purpose are based on the idea that only organisms that grow at the temperature of the mammalian body, +37°C, are of hygienic importance. Thus, for decades standard methods based on this idea were generally accepted for routine bacteria monitoring of water and foodstuffs (Greenberg *et al.* 1981). However, bacterial growth at lower temperatures may seriously affect the quality of foodstuffs even if the strain is not pathogenic. On the other hand, bacterial toxins may cause disease and these toxins may be produced at temperatures other than +37°C.

Reasoner and Geldreich (1985) showed that an incubation temperature of +35°C did in fact yield much lower colony counts from water samples than did +28 or even +20°C. Consequently, it is evident that, in addition to the standard plate count of bacteria incubated at +37°C or +35°C, the "heterotrophic plate count" at a lower temperature should also be determined.

Traditionally, in studies of airborne bacteria the same culture media have been used as for microbes of clinical importance and for those living in water. However, extreme conditions like air, seawater and very cold or warm environments select their own microbial populations (Atlas and Bartha 1987), which surely differ from those in mammalian organisms. The ecological limitations of these populations have been studied in soil and water microbiology (Reasoner and Geldreich 1985, Olsen and Bakken 1987), and the same principles may hold true for the microflora of the air.

Reasoner and Geldreich (1985) showed that growth medium containing only a fraction of the nutrients of those in standard plate count medium resulted in significantly higher bacterial counts from drinking water. Excessive nutrients, rather than lack of them, may be the reason for the low colony numbers on rich media. Apparently the oligotrophic bacteria, which grow at very low nutrient concentrations (1-15 mg C/liter), fail to grow on rich media (Olsen and Bakken 1987).

Apparently there is no way to determine the literally total count of bacteria by cultivation with any medium and temperature combination; any choice of these conditions is selective. Thus the concept of total counts is limited and should always be used with a precise description of the conditions of sampling and cultivation.

1.8 Identification of bacterial genera

Prokaryotes are traditionally classified and identified according to their morphological, biochemical and physiological characteristics. In modern bacterio-

logy serological, genetic and chemotaxonomic methods are also utilized. Identification of a bacterial strain begins with observation of its colony characteristics: pigmentation, appearance, swarming and size. Gram-staining, morphology and motility are basic features. Division of bacteria according to the above-mentioned principles is presented in Table 3.

Table 3. Division of bacteria into major taxonomic groups (Bergey's Manual of Systematic Bacteriology 1984)

Aerobic/microaerophilic, Gram negative bacteria

Aerobic, Gram negative rods and cocci
e.g. Family *Pseudomonadaceae*
Family *Legionellaceae*
Family *Neisseriaceae, incl.:*
Genera *Moraxella, Acinetobacter*
Genus *Flavobacterium*
Genus *Francisella*

Facultatively anaerobic Gram negative rods
e.g. Family *Enterobacteriaceae*, incl.:
Genera *Escherichia, Klebsiella, Citrobacter, Enterobacter, Serratia*

Anaerobic Gram negative rods
Sulfate- or sulfur reducing bacteria
Anaerobic Gram negative cocci
Rickettsias and Chlamydias
Mycoplasmas
Gram positive cocci
e.g. Family *Micrococcaceae*, incl.:
Genera *Micrococcus, Staphylococcus*
Genus *Streptococcus*
Endospore-forming Gram positive rods and cocci
e.g. Family *Bacillaceae*, incl.:
Genus *Bacillus*
Nonsporing Gram positive rods
Mycobacteria

Biochemical and physiological characteristics such as presence of certain enzymes, utilization of different nutrients, temperature range of growth, relation to free O_2 and type of metabolism are equally necessary for identification. Computerized systems are commonly used to help in comparing the features of an unknown strain with those of classified bacteria. Principles of identification and characterization of prokaryotes have been presented e.g. by Staley and Krieg (1984) and Trüper and Krämer (1986).

1.9 Quantitative and qualitative aspects of airborne bacteria

1.9.1 Bacteria levels in indoor and outdoor air

Few data are available on monitoring of bacteriological air quality; those available include such special environments as submarines (Morris and Fallon 1973) and spacecrafts (Favero and Puleo 1980).

For previous measurements various sampling methods, different culture media and incubation times have been used; therefore the results are poorly comparable. As a rough summary, however, the levels of airborne bacteria are within 10-10^4 cfu/m^3 in homes and schools and in long-range traffic vehicles where special attention has been paid to ventilation. In all of these, the main sources of airborne bacteria are people. Reported levels of airborne bacteria are presented in Table 4.

Occupational environments with heavy contamination by airborne bacteria are cowsheds and mills in agriculture (Dutkiewicz *et al.* 1978, Kotimaa *et al.* 1984), the wastewater industry (Lundholm 1982, Nevalainen *et al.* 1985, Boutin *et al.* 1986) and even landfill areas (Laukkanen *et al.* 1987). In these environments, the levels of airborne bacteria are several orders of magnitude higher than in "normal" indoor air, 10^3-10^8 cfu/m^3, and the main sources are organic materials that produce dust or droplets when handled (Table 4).

In Sweden bacteria levels in outdoor air were reported to range from 0-10^2 cfu/m^3 on the coast to 10^2-10^3 cfu/m^3 in cities (Bovallius *et al.*1978a). Similar levels were found by Wright *et al.* (1969) in Minnesota and Mancinelli *et al.* (1978) in Colorado, in the U.S.A. Similar levels of bacteria were found even at an altitude of 170 m (Wright *et al.* 1969).

Long-range transport of viable bacteria has also been reported. During a monitoring program in Sweden an exceptionally high bacterial count was recorded. Trajectory calculations showed that the bacterial aerosol originated from a dust storm near the Black Sea (Bovallius *et al.* 1978b).

Table 4. Levels of airborne bacteria in different indoor environments, occupational spaces and outdoors.

Sampling site	Sampling method, medium and incubation temperature	Bacteria level cfu/m^3	Ref.
PRIMARY SCHOOL,			
classrooms	slit sampler/ serum agar, 37°C	$8x10^2$-$8x10^3$	(1)
cloakrooms	"	$5x10^3$	
CLERICAL OFFICES	"	10^3	
HOMES,			
dwelling room	"	10^2	
dining room	slit sampler/ broth agar, 37°C	10^1-10^2	(2)
HOMES	6-stage impactor/TGY-agar, 20°C	10^2-10^3	(3),(4)
SUBMARINES	slit sampler/ blood agar, 37°C	10^2	(5)
SUBWAY STATIONS	slit sampler/ TS-agar, 37°C	10^2-10^3	(6)
OUTDOOR AIR,			
urban	slit sampler/serum agar, 37°C	10^2	(1)
"	2-stage impactor/ TS-agar, 35°C	0-10^3	(7)
"	6-stage impactor/ TGY agar, 20°C		(3)
"	6-stage impactor/ TGE agar, 24°C	10^2-10^3	(8)
"	6-stage impactor/ TSA agar, 35°C	10^2-10^3	(9)
coast	"	<10-10^2	(8)
HOSPITALS			
operation theatre	slit sampler/ blood agar, 37°C+25°C	10^2	(10)
patient rooms	membrane filter/ blood agar, 37°C	<10-10^2	(11)
AGRICULTURE			
swine confinement building	6-stage impactor/ placenta agar, 35°C	$3x10^5$	(12)
poultry confinement building	"	$4x10^5$	"

References: (1) Williams *et al.*1956, (2) Lidwell 1948, (3) Pellikka *et al.*1986, (4) Raunemaa and Ruokolainen (1986), (5) Morris and Fallon 1973, (6) Yoshizawa *et al.*1983, (7) Jones and Cookson 1983, (8) Bovallius *et al.*1978a, (9) Lee *et al.*1973, (10) Tjade and Gabor 1980, (11) Kiosz *et al.*1984, (12) Clark *et al.*1983

1.9.2 Genera of airborne bacteria

The difficulties in analyzing information on airborne bacterial flora are even greater than those mentioned above because of the variability in both sampling methods and analysis procedures. Consequently, the reported data presented in Table 5 are expressed by symbols instead of numbers. In summary, micrococci and staphylococci are reported to be the most dominant group in indoor air and are also common in outdoor air. The frequency of all other groups of bacteria differs greatly in different reports.

Table 5. Occurrence of bacterial genera in different environments. hosp=hospital air, subw=subway station air, submar=submarine air.

group of bacteria	indoor air(1)	outdoor air (2)	hosp (3)	subw (4)	submar (5)	grain mill(6)
micrococci	+ + +	+ + +	+ + +	+ + +	+ + +	+
staphylococci	+ +	+ +			+ +	+ + +
aerococci	+	+ +			+	
streptococci	+	+		+ +	+	+
Gram positive rods			+			+
corynebacteria	+ +	+ +		+ +	+ +	+
sporeforming rods	+ +	+ +		+	+	+
actinomycetes				+		+
Gram negative rods	+	+ +	+	+ +	+ +	+ + +

Symbols: + + + a dominating group, >40% of isolates
+ + found frequently, 10-40% of isolates
+ found regularly, <10% of isolates

References:
(1) Williams *et al.*1956, Lidwell 1974, Ueda and Kuwabara 1980, Tyndall *et al.*1987
(2) Wright *et al.*1969, Mancinelli and Shulls 1978, Tyndall *et al.*1987
(3) Davies and Noble 1962
(4) Szám *et al.*1980, Yoshizawa *et al.*1983
(5) Morris and Fallon 1973
(6) Dutkiewicz *et al.*1978

1.10 Hygienic importance of bacteria in indoor air

Airborne infections have been shown to occur in association with many bacteria (see 1.2). Aerosols of e.g. *Legionella*, thermophilic actinomycetes and possibly mycobacteria may be dispersed inside buildings (LaForce 1984). Likely reservoirs of these agents are cooling towers, contaminated filters, air ducts, and certain types of humidifying units in the heating, ventilation and air conditioning systems (Ager and Tickner 1983). Cases of these types of infections and other building-associated illnesses have been documented (LaForce 1984). In recommendations for indoor hygiene, measurements of biological pollutants are encouraged in order to detect possible agents of these diseases (WHO Working Group on Indoor Air Quality 1988). However, there is an almost total lack of background information about the normal ranges of bioaerosol levels in homes, public buildings and other such environments where people spend most of their time. This applies especially to airborne bacteria. As indicated before (see Table 4), the scant information available on indoor bioaerosols applies mainly to heavily contaminated occupational environments. These data have little value for evaluating whether the air in a public building or in a private home is contaminated with disease-causing bacterial aerosol. This was the starting point of the present study.

1.10.1 The importance of airborne bacterial counts

Measurement of total counts of airborne bacteria is a way of expressing the impact of the people present in a confined space, or to show the burden of organic material present in an occupational environment. So far, however, the information available on this issue is mainly qualitative. No dose-response curves for this kind of exposure are available, nor have hygienic standards been set for airborne bacteria.

The earliest proposals for bacteria standards were based on the idea that airborne saprophytic bacteria, such as *Streptococcus salivarius*, which is found in the saliva, are indicators of the the presence and spread of airborne pathogens (Gordon 1904, Wells 1934, Bourdillon *et al.* 1948). Cross-infection with measles is an example often referred to (Reid *et al.* 1956).

Most airborne bacteria are not pathogens. Only recently has the more general value of airborne levels of bacteria as a hygienic indicator been introduced (Morey *et al.* 1986). This is based on the idea that the efficiency of ventilation and therefore the quality of indoor air could be monitored with this parameter. Furthermore, by measuring total bacteria or bacterial groups rather than a single species, other possible sources than humans can also be detected.

In some studies a total microbial count of about $1 x 10^3$ cfu/m^3 has been considered as the action level (Morey *et al.* 1984). The scientific basis for this limit has not been made thoroughly clear, however, and such suggestions are probably based on practical experience. There is an urgent need to create databases large enough to offer reference data on bacteria levels as well as data on cases of disease associated with specific sources of bacteria in indoor air. As mentioned previously, results obtained by measurement of airborne bacteria are strongly linked to the sampling method, and there is a great need to standardize the methods of both sampling and analysis for hygienic monitoring, as has been done for water and food samples.

A recommendation protocol for hygienic measurements in offices has been put forward by the American Conference of Governmetal Industrial Hygienists, ACGIH (Morey *et al.* 1986). According to this protocol, the sum of the total counts of fungi, bacteria (+35°C) and thermophilic actinomycetes (+55°C) should not exceed 10^4 cfu/m^3. If the amounts of airborne *Bacillus* spp. and Gram-negative rods in an initial screen do exceed 500 cfu/m^3, however, a building-associated source is presumed. As a second screen, an amount exceeding 500 cfu/m^3 of either *Staphylococcus*, *Streptococcus*, *Corynebacterium*, *Acinetobacter*, *Pseudomonas* or *Micrococcus* is regarded as an indication of the need for remedial action. For thermophilic actinomycetes, levels above 500 cfu/m^3 are also considered high (Morey *et al.* 1986). For special purposes, stricter requirements are given. For instance, the British recommendation for operating theatres is <1 cfu/m^3 *Staphylococcus aureus* or *Clostridium welchii*, or total bacteria counts <180 cfu/m^3 (White *et al.* 1983, Arrowsmith 1985).

Most airborne bacteria are surely harmless, but occasionally there are pathogens or opportunistic pathogens present and transported by air. The flora of air is very poorly characterized, however, and no systematic source analyses are available. It is not known whether the flora is essentially different in different environments. Nor is it known whether air flora is composed mainly of typical airborne genera or whether the sources define the composition. Hence, in order to detect etiological agents of diseases and to estimate their dose for health risk assessment, more precise information should be obtained about the total flora of the air.

1.11 Aims of the study

The purpose of this study was to investigate the role of bacterial aerosols in indoor air.

The detailed aims of the study were:

1. To determine the levels and particle size distribution of indoor airborne bacteria in homes
2. To study the effect of ventilation system and season on airborne bacteria in homes
3. To make a source analysis of bacteria in indoor air
   (a) by studying the effect of occupancy,
   (b) by determining the bacteria genera normally found in indoor air and
   (c) by comparing them with the possible sources of bacteria
4. To characterize the bacterial flora of normal domestic suburban and rural indoor air sampled with a six-stage impactor
5. To characterize the bacterial flora of the indoor air in homes with microbial problems and to compare it with the flora in normal homes
6. To test whether microbial problems due to water damage in a house can be demonstrated by analyzing the bacterial flora of the air
7. To compare the levels of airborne pseudomonads in indoor and outdoor air
8. To compare the levels of airborne micrococci and staphylococci in indoor and outdoor air, and
9. To propose a hygiene guideline for levels of indoor air bacteria.

2 MATERIAL AND METHODS

2.1 The homes studied

Three kinds of homes were included in this study:

Group A: new suburban apartment homes, in houses with three different ventilation systems (n = 18),
Group B: farmhouses (n = 9), and
Group C: suburban homes with known moisture problem (n = 27).

The homes of the Group A were suburban townhouses completed in the fall of 1985 and located on an esker area in Kuopio, which is in eastern Finland. These townhouses were two-story concrete element buildings built on a slab. These houses were built for research purposes in order to study the effects of different ventilation systems on indoor air quality in homes. Thus they were identical in construction except for the ventilation system, which was either natural ventilation based on gravity (house A1), mechanical exhaust ventilation (house A2) or mechanical exhaust and supply ventilation (house A3). In these houses a wide research program on indoor air was carried out during 1985-1988 (Savolainen *et al.* 1988). The bacteria studies were a part of this program.

The ventilation was dimensioned to 0.9-1.0 1/h in house A2 and to 1.8-2.0 1/h in house A3. The apartments, 18 in all, were 2-3 room homes of 40.5 or 56.5 m^2 in area with 2-5 inhabitants each. The occupants were mainly young families with small children.

The farmhouses (Group B) were located in the farming area surrounding Kuopio. Of the nine farms three were dairy farms, two poultry farms, two pigfarms and two combined poultry and pig farms. The occupants of the farmhouses were families of two to seven persons. The farm-houses were chosen from a group of farms where an extensive research program on occupational health hazards was carried out by the Kuopio Regional Institute of Occupational Health (Pönni 1987). The characteristics of the homes are presented in Table 6.

Table 6. The farmhouses studied

House no	main production	size of the house (m^2)	persons in the family	ventil. system	built in (renov)	mater
B1	dairy farm	120	4	natural	1975	wood
B2	dairy farm	150	6	mech –>	1980	brick
B3	dairy farm	90	3	natural	1947	wood
B4	poultry farm	140	2	natural	1977	brick
B5	poultry & pig farm	200	5	natural	1956	wood
B6	poultry farm	200	4	natural	1947(-80)	wood
B7	poultry &cattle-fattening farm	104	6	natural	1950	wood
B8	dairy farm	250	7	natural	1880	wood
B9	pig farm	400	3	natural	1950(-83)	wood

mech –> mechanical exhaust ventilation

The homes of the Group C were chosen from a nationwide survey on microbial problems in homes. The homes included in the survey, 142 in all, had been reported to the health authorities because of indoor air problems. Of these homes, 27 were selected for an indoor air survey. The selection criteria for air sampling was either a known moisture problem in the house or microbial problem-associated health complaints of the tenants reported by a physician. Among the selected homes, 17 were townhouse apartments, six were single-family houses, and four were apartment homes in multistory apartment buildings. The area of the homes varied from 60 to 180 m^2 and the ventilation system was mainly either natural with or without kitchen exhaust fan or mechanical exhaust ventilation. In two cases there was a mechanical supply and exhaust ventilation with air recirculation. The characteristics of the homes of Group C are presented in Appendix 1.

Kuopio, which is an industrial and educational center, is also the administrative capital of Savo province and has a population of about 80 000 inhabitants. The climate in this part of Finland is subarctic with 5 months of plentiful snow cover. The mean temperature is -10°C in January-February and +13°C... +16°C in June-August (Atlas of Finland 1988).

2.2 Sampling strategy

About one week in advance, the occupants of the selected homes were informed by phone of the sampling to come. Samples were taken in the daytime during weekday working hours, and the occupants were either at home, for example, housewives with their children, or absent. The occupants were encouraged to continue their normal daily routines and to avoid extra cleaning or other such activities prior to sampling. There were always two persons carrying out the sampling.

Samples were taken indoors in living rooms, bedrooms or kitchens. In one series of sampling in the fall 1987, parallel samples were taken in the kitchen, bedroom, living room, bathroom and vestibule of the same apartment of Group A.

In the homes of the group A, samples were taken in six periods:

1. Fall 1985, when the houses were completed but not yet occupied,
2. Winter 1986, when the apartments had been occupied for about 4 months,
3. Spring 1986, after about 8 months of occupancy,
4. Fall 1986, after a year of occupancy,
5. Fall 1987, after two years of occupancy, and
6. Fall 1988, after three years of occupancy.

Each sampling period lasted two weeks. In the first period each apartment was sampled four times a day and in the later periods twice a day, in the morning and in the afternoon. The samples were taken in the room where the temperature and humidity sensors had been installed. Depending on the occupants, this room was used either as a bedroom or a living room.

A 24-hour period was sampled at one-hour intervals during the day (0700-2200 h) and two hour intervals in the night (2200-0700 h) in the same apartment.

Outdoor samples were taken outside the houses in the morning and afternoon of each sampling day. The sampling site was in the yard, within 10 m of the sampled house. The sampling height was 1.5 m. In rainy weather the sampling apparatus was placed under a shelter.

Before the first sampling in the unoccupied homes, the ventilation had been adjusted to the recommended level (see 2.1) and the windows had been closed one day prior to sampling. After the apartments were occupied, the occupants could adjust the ventilation and indoor temperature as they liked.

The automatic monitoring system for indoor temperature, relative humidity, the flow of exhaust air and details of the ventilation systems have been described elsewhere (Savolainen *et al.* 1988).

The farmhouses (Group B) were sampled once late in the fall of 1985. In each farmhouse 5-14 samples were taken in the kitchen and in the living room (Table 7). An outdoor sample was taken in the yard of each farm.

Table 7. Timing of the farmhouse sampling in the fall of 1985

House no	Number of samples	Time of the day
B1	6	05.30-09.30
	6	13.00-17.30
B2	6	07.00-09.30
	8	11.00-16.30
B3	5	06.30-08.30
	3	19.00-21.00
B4	7	07.30-12.30
B5	5	14.30-17.30
B6	6	11.45-14.30
B7	6	15.15-18.00
B8	5	06.15-11.15
	6	13.15-17.15
B9	10	07.30-12.00

The homes with moisture- or mold problem (Group C), cited here as problem homes, were sampled once in April-May 1987. In each home samples were taken in at least two rooms, near a damage location and in another reference room. Outdoor air samples were also taken at each location.

In all apartments sampled, the temperature, relative humidity and number of people present were recorded.

2.3 Bacterial aerosol sampling

Bacterial samples from the air were taken with 6-stage impactors (Andersen 10-800) on Petri plates containing tryptone-glucose-yeast extract (TGY) agar, which is a suitable growth medium for most heterotrophic bacteria. Cycloheximide (0.5g/l) was used in the medium as a fungicide. The sampling times were 10-20 min indoors and 20-30 min outdoors and the sampling height was 1.5 m. The sampling volume flow was 28 l/min. The samplers were calibrated prior to each sampling period and the level of adequate suction was secured with a rotameter connected to the pump of the sampler.

The Petri plates were incubated in the dark at +21-+23°C for 3-5 days. The colonies were counted and the counts corrected according to the method of Andersen (1958). The counts of dry, actinomycete-like colonies were differentiated from other colonies of bacteria.

Of the total 450 air samples, 16 were chosen for further laboratory analyses in order to characterize the bacterial flora. The samples were selected to represent indoor air before and after occupation, during different seasons, different types of homes (Groups A, B and C), different rooms of a home and outdoor air. The samples chosen for this purpose are presented in Table 8.

Table 8. Air samples chosen for characterization of bacterial flora (sampled with 6-stage impactor)

	Indoor			Outdoor
Group A	A1 natural	A2 mech – >	A3 mech < – >	
fall 1985				
before occupancy	x	x		x
winter 1986	x	x	x	
spring 1986	x	x	x	
fall 1986		xxx		
farmhouses	B1	B2	B4	
	x	x	x	
problem homes	C20	C21	C22	C24
	x	x	x	x

x represents one sample, which includes six plates with 0-400 colonies each
xxx three separate samples

Altogether, about 2200 colonies were isolated for further characterization. From the plates with fewer than 100 colonies, all the colonies were isolated. If the number of colonies exceeded 100, only those on half a plate were isolated, and when there were >200 colonies, only one-fourth were isolated.

The colonies were cultured first on TGY plates to secure pure growth. During characterization, the strains were kept in semisolid agar tubes at +4°C. These stock cultures were renewed 2-3 times a year.

2.4 Characterization of the bacteria

All strains were Gram stained, and oxidase and catalase tests were made according to the methods of Stanier *et al.* (1966) and Kovacs (1956).

Gram-positive rods were tested for sporulation. The growth on agar at different NaCl concentrations (5%, 6.5%, 10%, 15% and 20%) was determined for Gram-positive cocci. Biochemical test kits (API Systems, S.A.) were used for bacterial identification as follows:

kit type	type of studied strains
API 20 NE	Gram-negative rods and cocci
API Staph	Gram-positive cocci
API 20B	Gram-positive rods

Some of the strains were further characterized by their motility, growth at different temperatures (+4°C, +37°C and +55°C), ability to hemolyse blood and utilization of caseine.

2.4.1 Identification of the Pseudomonas group

Bacteria belonging to the genus *Pseudomonas*, family *Pseudomonadaceae*, are Gram-negative, nonspore-forming rods that are catalase positive. Most of them are oxidase positive (Bergey's Manual of Systematic Bacteriology 1984). The strains fulfilling these criteria were tested further to confirm the identification. Growth at temperatures of +4°C, +37°C and +55°C was registered. The oxidative/ fermentative carbohydrate metabolism of the strains was tested with the method of Hugh and Leifson (1953). The identification was completed with API 20NE-kits. The

Gram-negative rods that were catalase positive, oxidase positive, showed oxidative carbohydrate metabolism in Hugh-Leifson test and gave the identification of *Pseudomonas* in the API 20NE kit by at least "good" (>90% probability) were registered as *Pseudomonas* sp.

2.4.2 Identification of the Micrococcus/Staphylococcus group

The genera *Micrococcus* and *Staphylococcus* belong to the family *Micrococcaceae*. These two genera are typically Gram-positive or Gram-variable cocci that occur in pairs, tetrads or clusters. They are able to grow in a medium with 10-20% salt concentration. These genera are differentiated from each other by the facultative aerobic growth of *Staphylococcus* compared to the obligate aerobic growth of *Micrococcus*.

Gram-positive cocci that occurred in pairs, tetrads or clusters were tested for their growth in nutrient medium containing 5%, 6.5%, 10%, 15% and 20% NaCl. Their aerobic/anaerobic growth was tested and they were identified with API Staph kits. Strains for which the genus name was given by at least "very good" (99% probability) were registered as *Micrococcus* or *Staphylococcus*.

The flow chart of the characterization process of the bacterial isolates is presented in Figure 4.

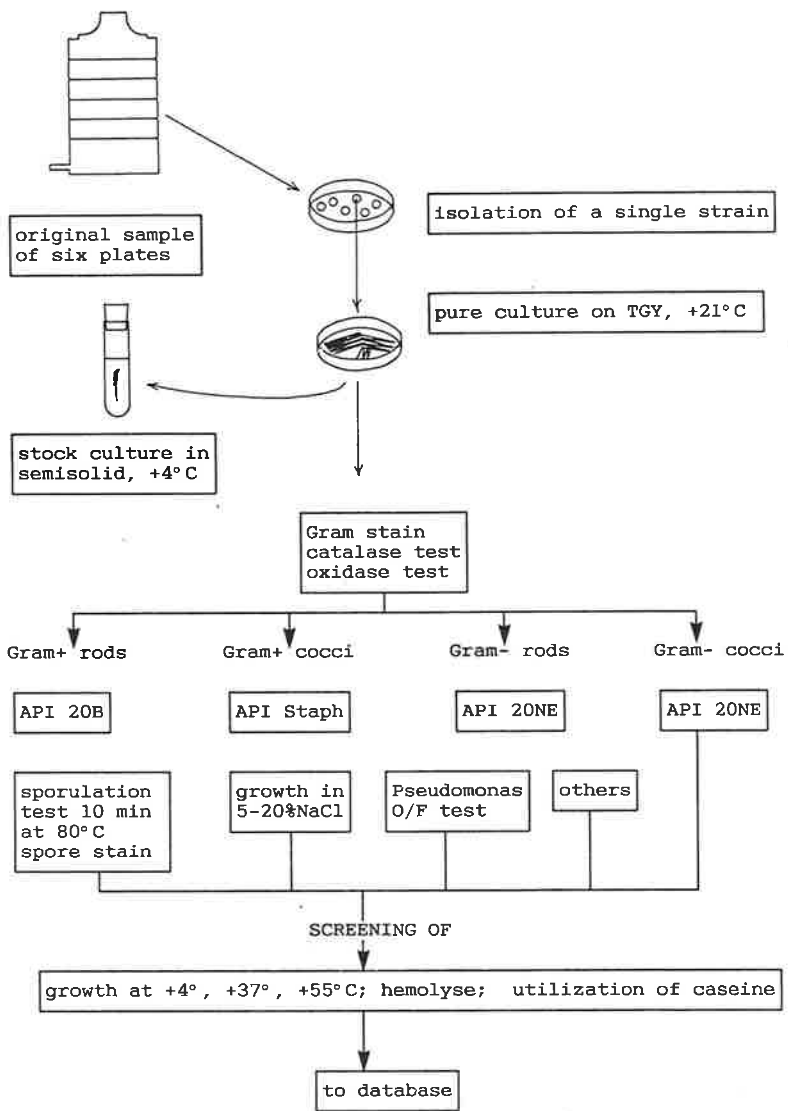


Figure 4. Flow chart of the process of characterizing the isolated bacteria.

2.5 Data processing and statistical analyses

The distributions of the bacteria levels were close to lognormal distribution. Hence the logarithms of the counts were used in statistical calculations. Non-parametric tests for independent data were used to test the statistical significance of the differences in bacteria levels. The statistical tests used are presented in Table 9.

The data from the strain characterization was processed with a modified dBaseIII + and statistical analyses with SPSS PC+ software using a microcomputer.

Table 9. Statistical tests used

purpose of the test	test used
normality of bacteria level distributions	Kolmogorov-Smirnov test
effect of season and occupancy and room use on bacteria levels	Kruskal-Wallis one-way analysis of variance Mann-Whitney U-test
differences between ventilation systems	Kruskal-Wallis one-way analysis of variance Mann-Whitney U-test
effect of room use and number of people present	Mann-Whitney U-test

3 RESULTS AND DISCUSSION

3.1 Discussion of methods

A six-stage impactor with a non-selective bacteria medium was used for sampling airborne bacteria. All the plates were incubated at room temperature, +21...+23°C. There are, however, no really "non-selective" cultivation methods for bacteria because different strains have vast differences in growth requirements (Roszak and Colwell 1987). Selection of one sampling method and one incubation type results in a limited spectrum of the variability of bacteria.

This study was designed to explore the building- and occupant-related aspects of airborne bacteria rather than screening the whole flora. Consequently, a well-described sampling method, which is generally used as a reference method (see 1.7.1), was chosen. The same applies to the medium. The airborne flora has been poorly characterized, and hence there is so far no specified choice of a plate-count medium for airborne bacteria. The standard plate-count medium for water samples (Standard Methods 1981) was used because most aerobic and facultative anaerobic heterotrophic bacteria grow on it. On most plates, the number of colonies was less than 400, which verifies that the sampling times were properly chosen (Andersen 1958).

The individual strains characterized in this work were bacteria that are able to form colonies in the sampling conditions used. Bacteria species caught by different sampling methods or with different media were not compared in this work.

The bacterial strains isolated for characterization were selected to represent indoor air before and after the occupancy of apartments, during different seasons, in different rooms, in suburban and farmhouses, both "normal" homes and those with suspected microbial problems, and in outdoor air. From the samples that were chosen for this part of the work, all the colonies were isolated without selecting them in any way.

3.2 Levels of airborne bacteria

3.2.1 Homes

The geometric means (GM), geometric standard deviations (GSD) and ranges of the bacteria levels in indoor of homes and in outdoor air are presented in Tables 10 and 11. Levels in new suburban townhouses (Group A) are presented in Table 10.

Table 10. Levels of airborne bacteria (GM, GSD and range) in suburban townhouses (group A including houses A1, A2 and A3) and in outdoor air during the three first years of occupancy. n indicates the number of samples.

	Bacteria levels cfu/m^3			
Group of samples	n	GM	GSD	Range
(A) total 1986-88				
indoors	183	550	1.32	0-11 900
outdoors	89	110	1.38	2-2 200
(A) winter 1986				
indoors	40	140	1.45	0-2 400
outdoors	17	16	1.07	0-70
(A) spring 1986				
indoors	36	680	0.91	100-5 900
outdoors	18	145	1.69	2-3 100
(A) fall 1986				
indoors	36	520	0.90	30-2 700
outdoors	18	120	1.03	20-850
(A) fall 1987				
indoors	36	1 200	0.80	200-11 900
outdoors	18	150	0.75	30-560
(A) fall 1988				
indoors	35	1 100	1.12	20-11 800
outdoors	18	130	1.14	30-2 200

GM = geometric mean
GSD = geometric standard deviation

Bacteria levels varied according to season and the range of these bacteria levels was quite wide, from 0 to 10^4 cfu/m^3.

Bacteria levels in the other two groups of homes, farmhouses (Group B) and problem homes (Group C), are presented in Table 11.

Table 11. Airborne bacteria levels in farmhouses (Group B), in suburban homes with suspected microbial problem (Group C) and in outdoor air. n indicates the number of samples.

		Bacteria levels cfu/m^3		
Sampling site	n	GM	GSD	Range
(B) FARMHOUSES				
indoors	77	800	0.92	130-4 900
outdoors	3	100	1.30	30-400
(C) PROBLEM HOMES[1]				
indoors	46	990	1.13	60-11 700
outdoors	11	110	1.82	10-480

[1] This group includes townhouses, single-family houses and apartments
GM = geometric mean
GSD = geometric standard deviation

The geometric mean of the bacteria levels was lower (statistical significance in parentheses) in new suburban townhouses (see Table 10, Group A, total) than in either farmhouses, group B ($p<0.001$) or in the problem homes, Group C ($p<0.0005$). The levels in the new suburban townhouses rose during the study period, however, and after two years of occupancy they were higher than in farmhouses or in problem homes. The range of the bacterial levels in the new suburban townhouses was the same as in the problem homes, which was greater than in farmhouses.

There is very little reference data about the levels of bacteria in indoor air. The results of three previous surveys made in Finland are shown in Table 12.

Table 12. Bacteria levels in indoor air reported in previous studies. n indicates the number of sampled sites.

		Bacteria levels cfu/m^3		
Sampling site	n	GM	Range	Ref.
homes	59	730	200-4 600	(1)
homes	16	640	200-4 000	(2)
offices	31	60	20-300	(3)
day-care centers	17	1 300	200-8 500	(1)

References: (1) Pellikka *et al.* 1986, (2) Raunemaa and Ruokolainen 1986, (3) Pellikka *et al.* 1985

The sampling method used in this study was the same as that used in three previous studies; therefore the data are comparable. The range of the bacteria levels in this study is wider than in the previous studies. The bacteria levels in homes seem to be an order of magnitude higher than in offices and lower than in day-care centers.

In farm environments there are natural sources of airborne bacteria such as soils, cattle, animal feed, hay and straw. These bacteria may be carried inside the home on clothes and shoes and also in the air, as has been shown to occur with fungal spores (Pasanen *et al.* 1988). In spite of these specific sources, however, the bacteria levels in farmhouses were not especially high (Table 11).

The bacteria levels observed in the homes with suspected microbial problems, 60-11 700 cfu/m^3 (GM 990 cfu/m^3), were higher than in the other spring samples but were of the same order of magnitude as those in the group A homes in the fall of 1987 and 1988. Thus the level of total bacteria in indoor air does not necessarily indicate a moisture problem in the home or the microbial growth associated with it.

Some of the highest bacteria levels (>5 000 cfu/m^3) in the problem homes were likely to be caused by insufficient ventilation or overcrowding. The area/occupant ratio of these homes was quite low, 19.4m^2/occupant, compared to the national average of 29.9 in the year 1987 (Official Statistics in Finland 1988a). In the home with the highest bacteria level this ratio was 13.8. No statistically significant correlation between number of people present during sampling and bacteria levels was detected in this group of homes, although the number of children per area and their activity explained the high bacteria levels in daycare centers in a previous study (Pellikka *et al.* 1986).

3.2.2 Bacteria in outdoor air

Outdoors, the levels varied from 0 to 3 100 cfu/m^3, which is the same range as reported by Wright *et al.* (1969), Bovallius *et al.* (1978a) and Mancinelli *et al.* (1978). The outdoor levels of bacteria were always lower than the indoor levels in occupied homes ($p<0.00001$), which confirms that indoor sources are dominating contributors to the amounts of bacteria found indoors.

Bacteria in outdoor air may also contribute to the levels in indoor air, but this transport is poorly known.

3.3. Results of the three-year monitoring of bacteria

A three-year study was carried out in a group of three new suburban townhouses (Group A, houses A1, A2, A3), including six apartments each. The otherwise identical houses had different systems of ventilation. Bacteria monitoring was started before the occupants moved in and continued for three years after occupancy. In addition to the bacteria studies, other parameters of indoor air quality were also studied, including ventilation, radon, formaldehyde, fungal spores and total suspended particles. Results of the parameters other than bacteria have been reported elsewhere (Reponen *et al.* 1987, Kokotti *et al.* 1988, Reponen *et al.* 1988, Savolainen *et al.* 1988).

3.3.1 Bacteria levels during the first year of occupancy

The levels of indoor air bacteria during the first year of occupancy, as well as the corresponding outdoor levels, are presented in Figure 5. Samples were taken in the fall before the occupants moved in, in the winter, in the spring and again the next fall.

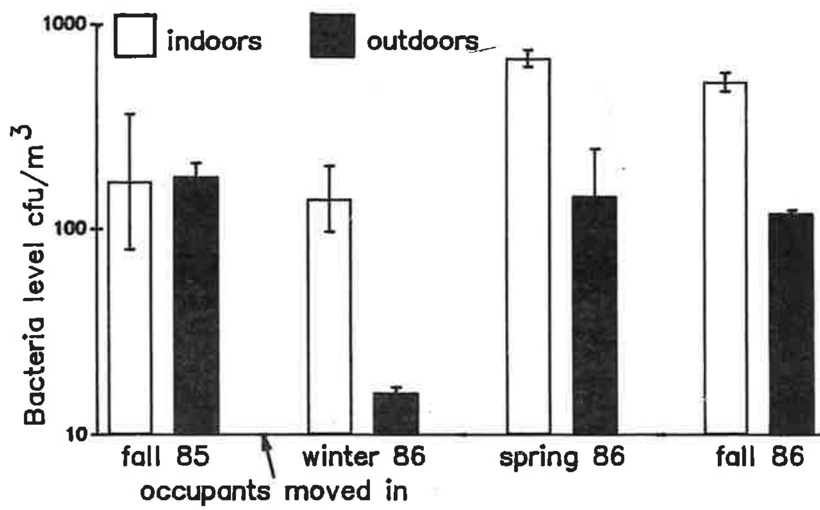


Figure 5. Bacteria levels in new suburban townhouse homes before the occupants moved in and during the first year of occupancy.

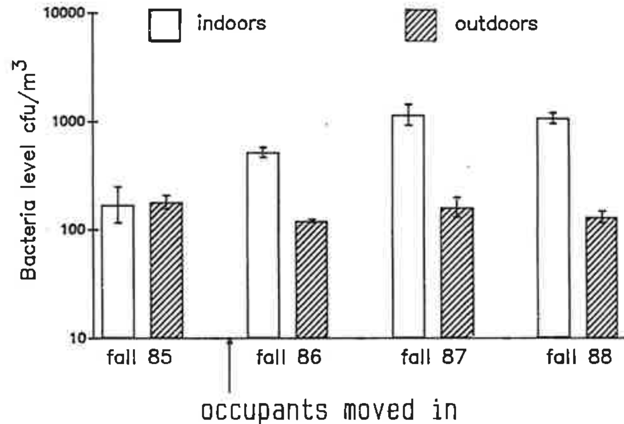


Figure 6. Bacteria levels in new suburban townhouse homes before the occupants moved in and after 1, 2 and 3 years of occupancy.

The wintertime levels of bacteria in the Group A homes were statistically significantly lower than in spring ($p<0.0001$) or in fall ($p<0.0005$). The spring and fall levels in the same year (1986) were of the same magnitude (Figure 5). The fairly low wintertime levels may be due to the fact that the apartments had been occupied only for four months before this sampling period. For practical reasons it was not possible to repeat these wintertime samplings and thus the seasonal variation could not be confirmed.

The fall levels of bacteria in the homes increased by an order of magnitude during one year of occupancy. This was due to the occupants and their activities, because there was no corresponding change in outdoor levels.

3.3.2 Bacteria levels in 1985-1988

The geometric mean (GM) of the bacteria levels in newly completed, unoccupied apartments was 170 cfu/m^3 and increased statistically significantly ($p<0.0005$) during the first year of occupancy, to GM 520 cfu/m^3. After two years of occupancy the levels had risen further to GM 1150 cfu/m^3, which again was statistically significantly ($p<0.0005$) higher than a year before (Figure 6). This increase occurred in all three houses with six apartments each, irrespective of ventilation system (see 3.3.3). After three years the bacteria levels had stabilized to a GM of 1070 cfu/m^3 (Figure 6, Table 13).

These results show that occupancy of an apartment brings along a population of airborne bacteria. Apparently it takes two years before the bacterial accumulation from the occupants and their activities becomes stabilized. The accumulation of bacteria in indoor air up to two years after occupancy of the apartments is a new finding.

The reasons for this accumulation of bacteria are not obvious, although it is known that humans are the most important source of indoor airborne bacteria (see 1.6.1). However, the number of persons living in these apartments did not increase during the study period. Therefore, increasing levels of bacteria during the first two years cannot be explained by an increasing number of occupants. On the other hand, occupancy is a prerequisite for build up of bacteria sources. In these homes the area/occupant ratio was low *i.e.* 20.0, while the national average was 28.9-29.9 during the years 1985-87 (Official Statistics of Finland 1988b). Since the occupants and their activities are a continuous source of bacteria, less space leads to higher concentration of bacteria-carrying particles.

There was no marked decrease in ventilation rates that would have explained the observed accumulation of bacteria (Figure 7). In the houses with mechanical

ventilation (A2 and A3) the ventilation rates decreased from the fall of 1986 to the fall of 1987. This may explain part of the increase in bacteria levels. However, in the house A1 the natural ventilation was increased during this period and yet the bacteria were also accumulated in this house (see 3.3.3).

An interesting observation was the decrease in formaldehyde concentrations that occurred simultaneously with the increase of bacteria levels in these homes (Reponen *et al.* 1988). Formaldehyde is an effective bactericide, but the possible association between formaldehyde and airborne bacteria must be confirmed in laboratory studies.

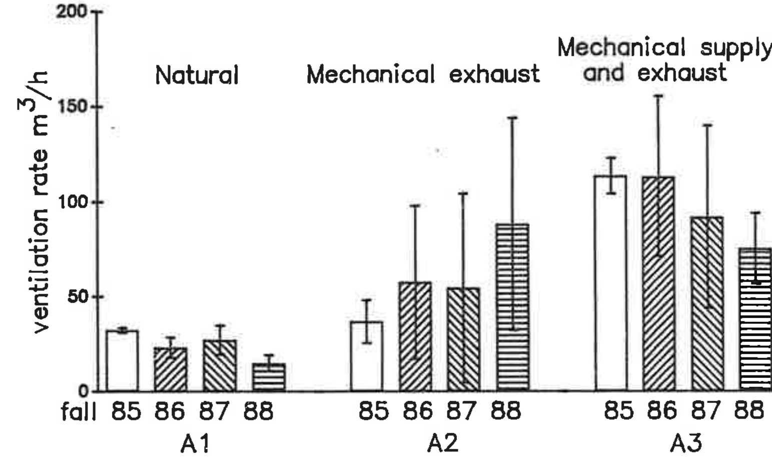


Figure 7. Ventilation rates in new suburban townhouses during the first three years of occupancy.

3.3.3 Effect of the ventilation system

The homes included in Group A were three houses with different systems of ventilation. The results from the sampling series before and after 1, 2 and 3 years of occupation are presented in Table 13. The differences in bacteria levels between the three ventilation systems are shown in Figure 8.

During the first sampling period before the occupants moved in, no significant differences in bacteria levels were detected between houses with different ventilation systems, and the same was true during the first year of occupancy. Only after two years of occupancy, in the fall of 1987, were statistically significant differences in bacteria levels detected between houses with different ventilation systems (Figure 8). In the homes with mechanical supply and exhaust ventilation (A3) the bacteria levels were lower than in those with mechanical exhaust only (A2, $p<0.05$) and in those with natural ventilation (A1, $p<0.005$). After three years of occupation, the statistical significance of differences between the ventilation systems disappeared.

Table 13. Bacteria levels in homes with different ventilation systems before occupation and after 1, 2 and 3 years of occupation. The yearly sampling period was in September.

	GM(GSD) of the bacteria levels (cfu/m³)			
Time of sampling	Total Group A	House A1 natural ventilation	House A2 mech –> ventilation	House A3 mech <–> ventilation
before occupation (Sept.1985)	170(0.47)	160(0.41)	210(0.35)	160(0.58)
after 1 year of occupation (Sept.1986)	520(0.90)	590(0.92)	720(0.70)	340(0.97)
after 2 years of occupation (Sept.1987)	1 150(0.80)	1 690(0.74)	1 470(0.66)	610(0.63)
after 3 years of occupation (Sept.1988)	1 070(1.12)	1 410(0.86)	1 000(1.41)	880(1.06)

mech –> mechanical exhaust ventilation
mech <–> mechanical exhaust and supply ventilation
GM geometric mean
GSD geometric standard deviation

Because the only difference between the three houses was the ventilation system, valid comparison could be made. Two-way mechanical ventilation seems to be most effective in removing bacteria-carrying particles from indoor air. This agrees with the results of Spiegelman and Friedman (1968).

The occupants could adjust the ventilation as they wished. As reported elsewhere (Savolainen *et al.* 1988), the occupants were frequently disturbed by the noise caused by the ventilation fan and turned it off during the night. Hence the mechanical ventilation did in fact resemble the so-called natural ventilation based on gravity. This obviously abolished some of the advantage of the more efficient air removal. If this had not occurred, the differences might have been even larger.

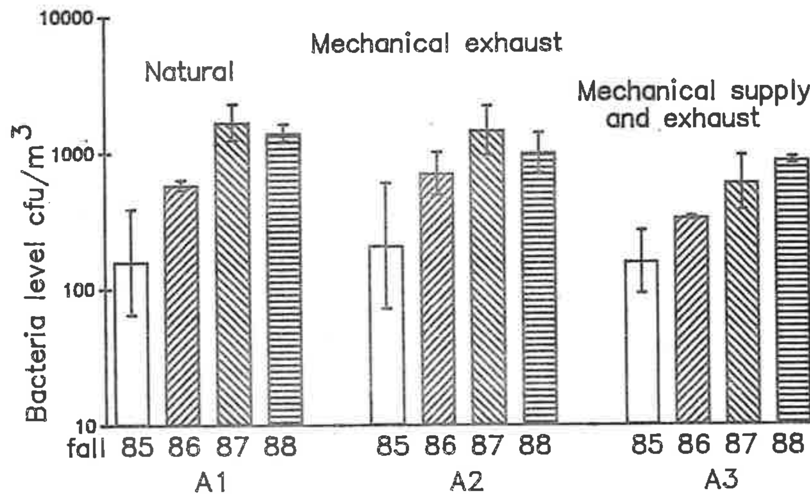


Figure 8. Bacteria levels during three years of monitoring in the three houses with different ventilation systems.

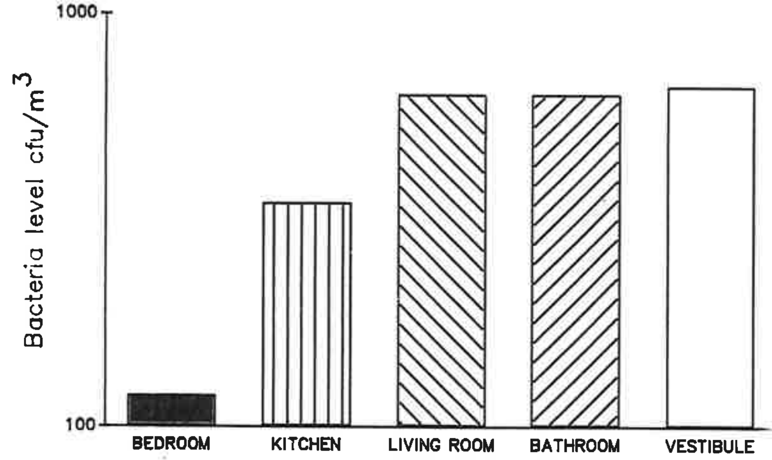


Figure 9. Bacteria levels in different rooms of an apartment. The samples were taken simultaneously.

3.3.4 Spatial and diurnal variation of bacteria in indoor air

In the homes of the Group A, the use of the rooms differed for different families so that the corresponding room could serve as either a living room or a bedroom. However, the humidity sensor was always placed in an identical location in similar apartments and the bacteria samples were taken in this room. The bacteria levels were higher ($p < 0.05$) when the space served as living room than when it was a bedroom.

In a series of five parallel samples, the bacteria levels were compared in different rooms of an apartment. The samples were taken in the fall of 1987. The results are presented in Figure 9. Unexpectedly enough, the bacteria levels were lowest in bedrooms. This does not support the presumption that making beds and other handling of textiles would be a major source of airborne bacteria. Other activities seem to be more important sources. The observed differences between rooms suggest that when one evaluates air hygiene, airborne bacteria should be sampled in more than one room of a house or an apartment.

The diurnal variation in bacteria levels in homes was studied in two new suburban townhouses and two farmhouses. The results of this monitoring are presented in Figures 10-12.

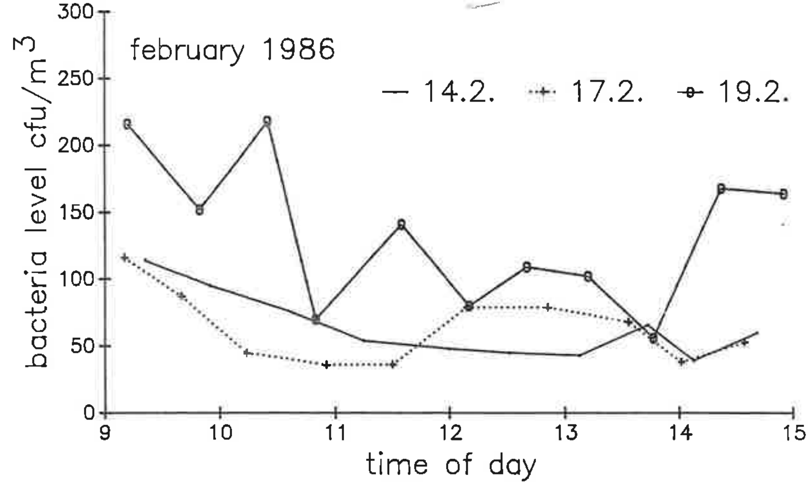


Figure 10. Bacteria levels in an apartment during the daytime when the occupants were away at work. The serial monitoring on consecutive days was part of a humidification experiment (see 3.4.3).

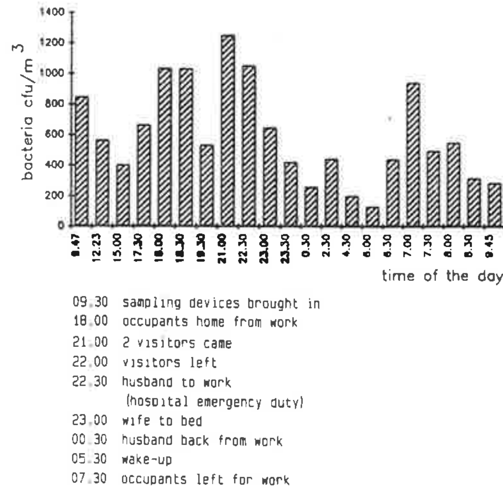


Figure 11. Diurnal variation in bacteria levels in an apartment during a 24-hour monitoring period.

As seen in Figures 10 and 11, the diurnal variation in bacteria levels is mainly due to different activities of the occupants. The bacteria levels decreased slowly after the occupants left for work, and stayed quite stabilized during the day (Figure 10). In the other apartment (Figure 11), bacteria were monitored for 24 hours. Here the peaks due to different activities can be seen clearly. Similar monitoring patterns have been presented by Lidwell (1948), who used a slit sampler with Hartley broth agar/24h at +37°C.

In two farmhouses bacteria were monitored during the daytime between 0530 h and 1730 h. The diurnal variation in bacteria levels is presented in Figure 12. Bacteria levels varied according to a similar pattern in both kitchen and living room. The largest variation in bacteria levels can be seen in farmhouse 2 (Figure 12). The indoor level of bacteria increased threefold in the morning and by an order of magnitude in the afternoon after the farmer came in from the cowshed. Evidently bacteria are transported by humans from the cowshed and other work facilities into the house, even if the working clothes are changed prior to coming indoors.

Due to large variations in bacteria levels in both space and time, a single sample does not indicate the air quality of the whole space. For meaningful evaluation of indoor air hygiene in a home, more than one sample should be taken. In order to reach valid conclusions, it is necessary to carefully record the number of people present and the activities of the occupants at the time of sampling.

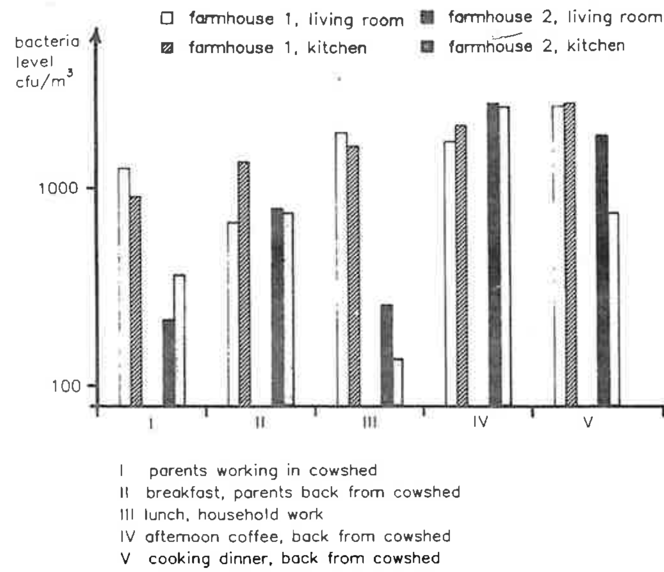


Figure 12. Bacteria levels in two farmhouses between 05.30 h and 17.30 h.

3.4 Other factors affecting bacteria levels in indoor air

The importance of the number of persons present during sampling was calculated using the total data for all groups of homes (A, B and C). The effect of temperature and humidity on outdoor levels of bacteria was calculated from the total indoor and outdoor data.

3.4.1 How number of people present affects bacteria levels

The number of people present during sampling was always recorded. The data were grouped according to the number of people present: either personnel only (≤ 2 persons) or occupants also present (>2 persons). The bacteria levels were statistically significantly ($p<0.0001$) higher when there were more people present. This was expected, as the impact of people on indoor levels of airborne bacteria is well known (Lidwell 1948). This effect was seen when the total data were combined, although it was not evident in the problem homes alone (see 3.2.1).

To study the effects of children on bacteria levels, the homes were divided in two groups according to whether there were children in the family or not. There was no statistically significant difference in the bacteria levels between the homes of the families with children and those occupied by adults only.

Although the number of persons present during sampling is a dominating factor in determining bacteria levels, an accumulation of bacteria also occurs (see 3.3.2) that is not dependent on the strength of the source. Other important factors are the type and practice of ventilation, and the cleaning habits and other characteristics of everyday life. Evidently these factors, mainly the habits of the occupants rather than the size of the family, determine the level of bacterial accumulation.

3.4.2 Effect of relative humidity and temperature on bacteria levels

Relative humidity had only a marginal effect on levels of airborne bacteria (Figures 13a-b and 14). As seen in Figure 13, both bacteria levels and relative humidities were higher in spring and fall than in winter. The reason for this, however, is not their mutual dependance, but rather climatic factors.

In a cold climate, the wintertime indoor humidity is very low, owing to the difference in temperature between indoor and outdoor air, which usually is more than 30°C. A water content of 1gH_2O/kg air, which means 60% relative humidity at -10°C, results in only 7% RH in indoor air at +20°C. When the temperature difference decreases, the humidity gradient also decreases. Thus the indoor humidities in a subarctic climate are always higher in the spring and fall than in winter. The reasons for the low bacteria levels in the winter samples have already been discussed (see 3.3.1).

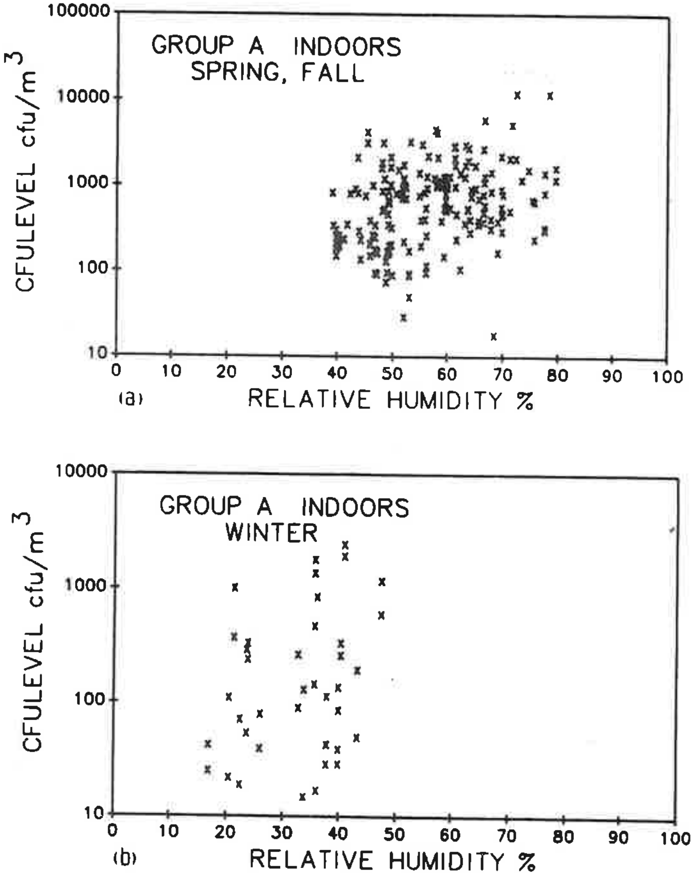


Figure 13. Effect of relative humidity on bacteria levels (a) in spring and fall and (b) in winter.

Relative humidity had no effect on outdoor levels of bacteria, either (Figure 14).

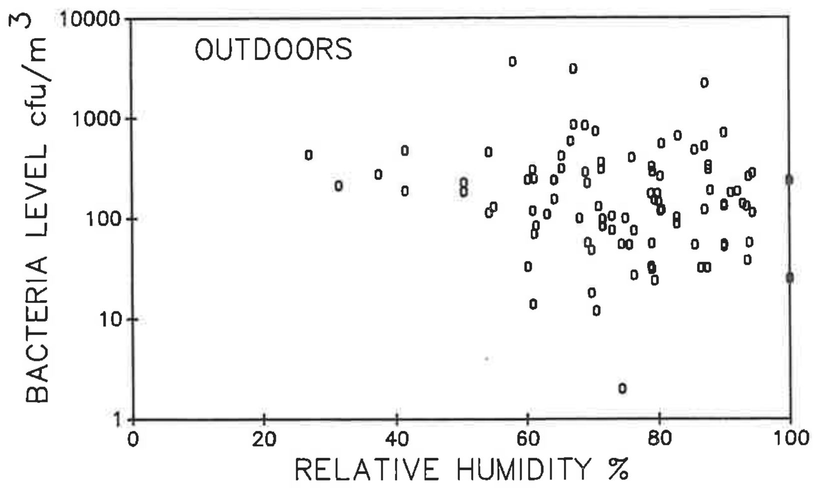


Figure 14. Effect of relative humidity of air on outdoor levels of bacteria.

These results do not support the conclusion of Sterling *et al.* (1985), who claimed that a relative humidity of 30...60% would minimize levels of airborne bacteria in indoor air. Their conclusion was based on data about a few single bacterial strains (*Escherichia coli*, *Aerobacter aerogenes*, *Mycoplasma* and *Serratia marcescens*) and does not necessarily apply to other bacteria (see 1.3.1). Total levels of bacteria, as monitored in this study, were not affected by the humidity range mentioned, and hence the conclusion of Sterling *et al.* (1985) must be regarded as premature.

The effect of humidification on airborne bacteria was studied in an experiment where an apartment was humidified for one week and the bacteria levels were monitored. The results are presented in Figure 10. The humidity in the apartment was raised from 40% to 70%, and a slight rise in the bacteria levels was observed. This increase may be due to better survival of bacteria in higher humidity. However, it was the effect of humidification rather than that of humidity *per se* that was studied in this experiment.

In an earlier experiment (Pellikka *et al.* 1985) bacteria were monitored in 5 humidified apartments. An increase in relative humidity from 18% to 62% did not significantly affect the level of bacteria in indoor air.

There are very few field results about the effect of humidity on levels of indoor air bacteria. The results of this study suggest that the effect as such is marginal. There are, however, indirect associations such as season and climatological factors. Evidently the effects of air humidity and the humidification of otherwise dry air must be separated. The effect of humidity or humidification is also dependent on the genus of bacteria (see 1.5.1).

When the results for level of bacteria are plotted against temperature, (Figures 15 and 16), air temperature does not explain either the high or the low levels of airborne bacteria. The temperature range indoors was +16...+31°C and thus the result does not apply to temperatures <10°C, in which bacterial survival is generally enhanced. Samples were taken outdoors at this temperature range, but no effect can be seen here either. Thus the air temperature did not affect levels of airborne bacteria in either the indoor or the outdoor data.

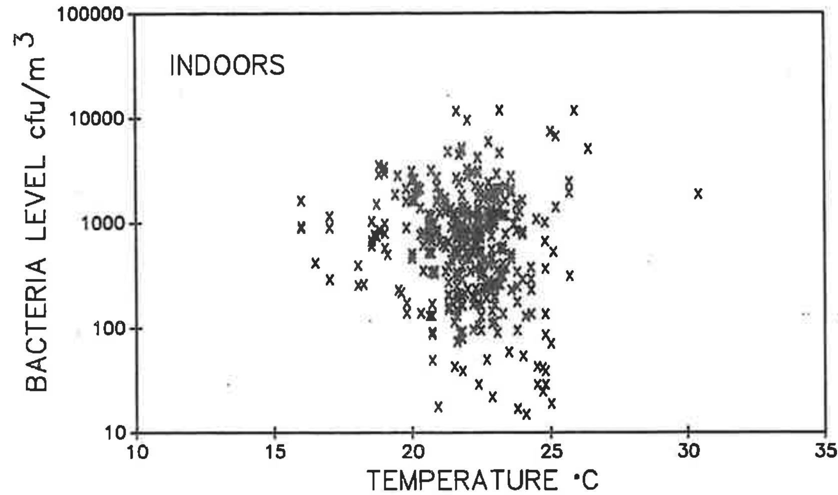


Figure 15. Effect of air temperature on levels of indoor air bacteria.

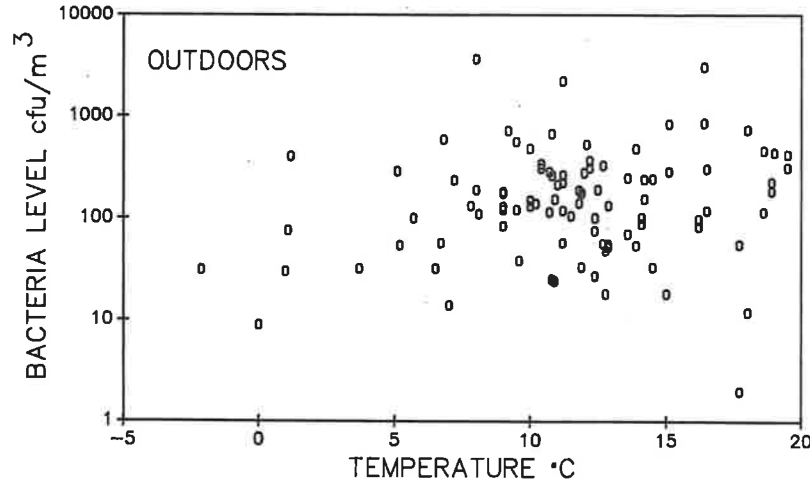


Figure 16. Effect of air temperature on levels of outdoor air bacteria.

3.5 Particle size distribution of airborne bacteria

The particle size distributions of the indoor and outdoor bacteria are presented in Figures 17-19.

In the suburban townhouse apartments, before occupancy (fall 1985) indoor bacteria were clearly of outdoor origin (Figure 17). There were no differences in particle size distributions between indoor and outdoor air or between the different ventilation systems.

In winter, spring and fall samples from 1986, the effect of occupancy can also be seen in particle size distributions of airborne bacteria. In the spring and fall samples the ventilation systems differed in their removal of bacteria-carrying particles $<5 \mu m$ in size. These differences were emphasized even more after two years of occupancy (see Figure 18).

The particle size distributions of four consecutive years are presented in Figure 18. In the time series 1985-1988 the impact of occupancy could be shown in the particle size distributions (Figure 18). The outdoor air distributions were different than those indoors and thus the contribution of the outdoor air to indoor bacteria was negligible.

For larger ($5 \mu m$) particles the distributions were almost similar in all three ventilation systems during the time period 1986-1988. This was expected, as ordinary ventilation does not remove this size fraction.

As indicated before, the size of a particle determines its behavior. If the ventilation coefficient is 0.5, the air of the space is changed once in two hours. For a 5 μm particle it takes about 30 minutes to settle from a height of 1.5 m, and for a 10 μm particle only 8 minutes. These particles remain airborne for only short times, and they are removed by gravity and resuspended again when disturbed mechanically. Ventilation is not a crucial factor in the removal of these particles.

The behavior of smaller particles is different; for example, a 1 μm particle does not settle down during 2 hours. It can be seen in Figure 18 that for smaller particles there are no systematic patterns. Evidently the intramural processes determine the particle size distributions.

During the first two years the mechanical systems of ventilation removed this fraction more efficiently than natural ventilation based on gravity did (Figure 18). The levels of small ($<5 \mu m$) particles in the house with mechanical supply and exhaust ventilation were slightly lower than in other houses. This difference later disappeared, however, as could also be seen in the bacteria levels (Figure 8).

In the farmhouses and homes with suspected microbial problems, the particle size distributions in indoor air are different from those in outdoor air (Figure 19). For large (5 μm) particles the patterns resemble those of the Group A homes. For smaller particles no systematic pattern can be detected in these homes.

The outdoor patterns of size distribution are variable, depending on the season. A large proportion of bacteria-carrying particles are smaller than 5 μm. This finding does not support the results of Lee *et al.* (1973) or Wright *et al.* (1969), who reported that most viable microorganisms in urban air were larger than 5 μm in diameter. These investigators used the same sampling method used in this study, but incubated the plates at $+35^{o}C$ instead of at room temperature, which was used here. Differences in incubation temperature may select different fractions of airborne flora. Furthermore, the outdoor samples in this study were taken in either suburban or farming areas, and no actual urban samples were included. The differences may therefore be due to different origin of the particles.

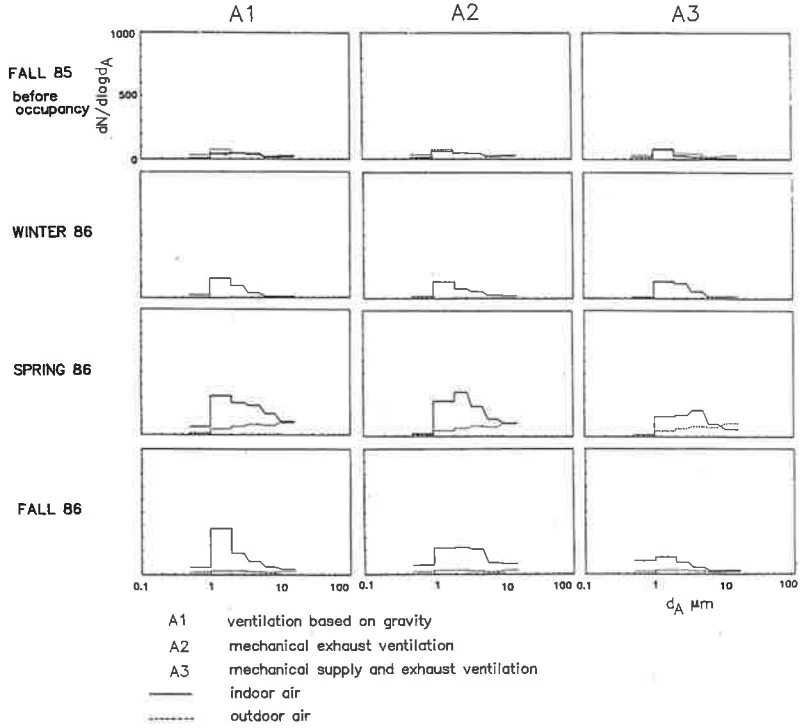


Figure 17. Particle size distributions of indoor airborne bacteria in the new suburban townhouse homes (Group A) before occupancy and in different seasons during the first year of occupancy. N = number of particles (cfu), D_A = aerodynamic particle diameter

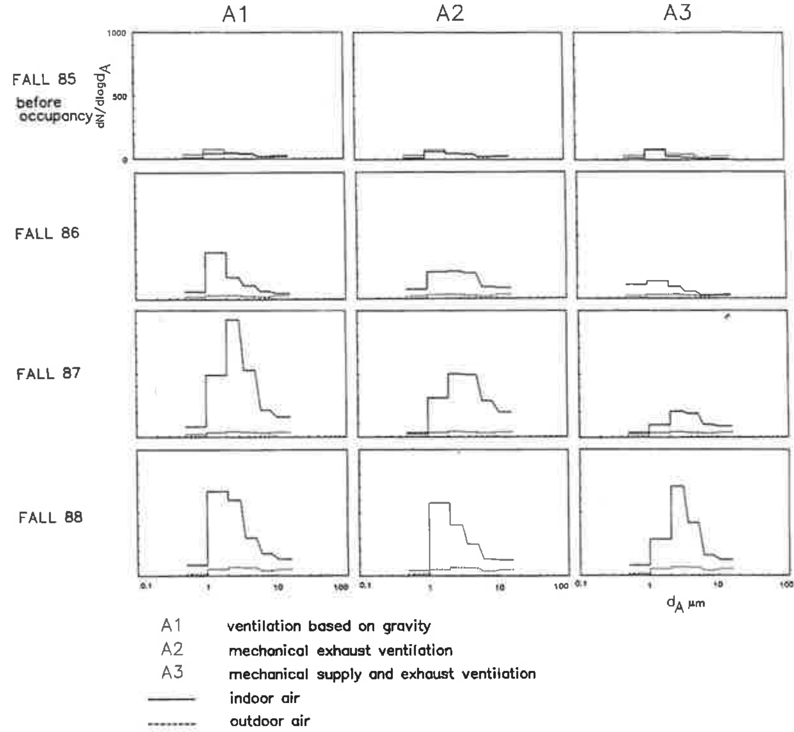


Figure 18. Particle size distributions of indoor and outdoor bacteria in townhouses before occupancy and during the first three first years of occupancy.
N = number of particles (cfu), D_A = aerodynamic particle diameter

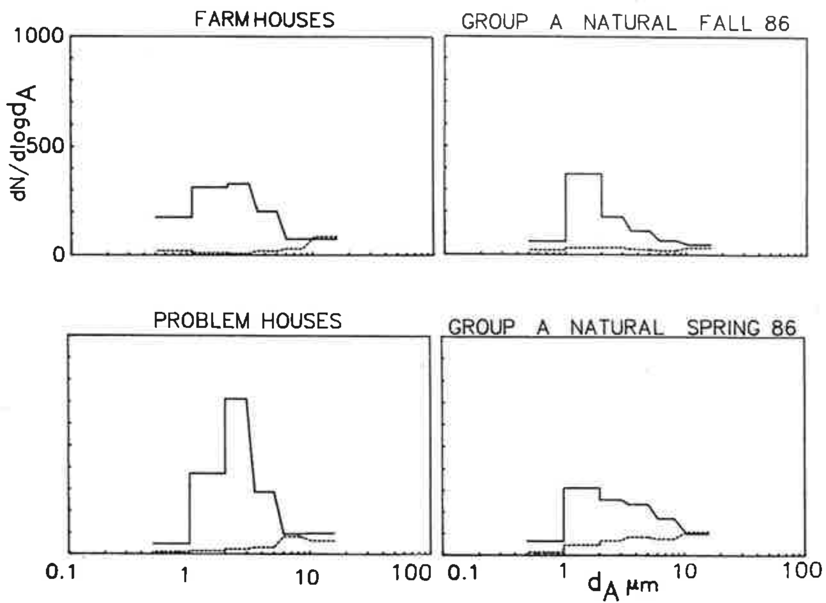


Figure 19. Particle size distributions of airborne bacteria in farmhouses and problem homes. For comparison, fall and spring 1986 distributions of the homes in Group A are also shown.

3.6 Proposal for hygiene guidelines

Data on bacteria levels in Group A from this study were combined with data from previous field studies (Pellikka *et al.* 1986, Raunemaa and Ruokolainen 1986). Data from the 1988 sampling of this study were excluded to avoid overrepresentation of homes that are smaller than average (see 3.2.2). The geometric mean (GM) and the upper limit under which 95% of results remain (GMx2GSD) are presented in Table 14. These results have been computed for indoor air data from homes and for corresponding outdoor data.

Table 14. Results from three field studies on indoor airborne bacteria (1), (2), (3).
n = number of observations

Sampling site	n	Bacteria level cfu/m^3 GM	GMx2GSD
indoor air, homes	162	570	4 500
outdoor air	139	60	1 600

GM = geometric mean
GSD = geometric standard deviation

References: (1) Pellikka *et al.*(1986), (2) Raunemaa and Ruokolainen (1986), (3) this study

For purposes of practical hygiene, there is a need for criteria for indoor air quality. Guidelines are needed for evaluating whether the bacteria levels are normal, whether there are abnormal sources of bacterial aerosols or whether ventilation is insufficient.

Present knowledge of the possible dose-effect relationships of bioaerosols is not sufficient to offer health criteria for any threshold limits. The problem can, however, be approached from another point of view. It can be presumed that the existing bacteria levels are "normal" in homes where the occupants have no complaints about indoor air quality or suspicions of building-associated health problems.

In biomedical sciences the upper limit of normality is commonly defined as the level below which about 95% of the results remain. Mathematically, this limit in a lognormal distribution is expressed as GMx2GSD (Reist 1984). Applied to data from three recent studies, the upmost normal limit for airborne bacteria is 4 500 cfu/m^3 (Table 14). Therefore, it is proposed that this be used as the uppermost normal level in homes in subarctic climate. If bacteria levels are higher than this, the reason for these high levels should be investigated and remedial actions should be initiated. Possible reasons for high bacteria levels are overcrowding, insufficient ventilation and specific intramural sources of bacteria.

This limit applies to suburban or urban homes. In farmhouses there are specific sources of bacteria (see 3.2.1), and high levels of airborne bacteria do not necessarily indicate an indoor air problem. It should also be reminded that the suggested limit applies to samples taken with the same method as described in this study.

3.7 The microbial flora of indoor and outdoor air

3.7.1 General features of the airborne flora

Colony appearance and morphology. A substantial proportion of the airborne colonies of bacteria were pigmented. Of about 1 300 colonies for which the color of the colony was systematically recorded, 50% were yellow, 8% were orange and 2% were red. These pigments are believed to protect cells from the damaging effect of light, as indicated previously (see 1.5.2). However, plenty of colonies in the air samples had no such pigment. Hence this protection is not a necessary prerequisite for bacterial cells to remain viable while airborne. On the other hand, the concentration of the pigment was not determined by chemical analysis and possible low concentrations of pigments were not detected.

The distribution of bacteria in indoor and outdoor air according to Gram staining and morphology is presented in Table 15.

Table 15. Distribution of bacteria in indoor and outdoor air according to Gram staining and morphology.

Gram staining and morphology	indoor air %	outdoor air %
Gram-positive cocci	60	3
Gram-negative cocci	3	0
Gram-positive rods	23	19
Gram-negative rods	14	78

In indoor air, Gram-positive cocci were a dominating group, but they made up only a small fraction of the bacteria in outdoor air. In outdoor air, Gram-negative rods were a major group, but were less common in indoor air. It can be seen from this distribution that in indoor air the bacterial flora differs from that found outdoors.

Catalase and oxidase reactions. Most strains (80%) were catalase positive and oxidase negative. Other combinations of these characteristics were sporadic. Frequency of the occurrence of these enzymes is presented in Table 16.

Table 16. Occurrence of catalase and oxidase enzymes in airborne strains of bacteria

	% of the strains
catalase positive, oxidase negative	80
catalase positive, oxidase positive	12
catalase negative, oxidase positive	2
catalase negative, oxidase negative	6

Catalase is the enzyme that decomposes hydrogen peroxide to oxygen and water, thus protecting the cell from the toxic effects of this compound. Oxidase is an enzyme included in the respiratory transport chain; its presence shows that there is cytochrome c in the transport chain (Stanier *et al.* 1979). The presence or absence of these enzymes is important for identification of aerobic bacteria.

Hemolysis and utilization of caseine. Among the isolated bacteria, 8% were hemolytic strains. These hemolytic strains consisted equally of α-hemolytic and β-hemolytic bacteria. Hemolysis is the ability to damage red blood cells, indicating a possible pathogen.

Caseine utilization was observed among 32% of the strains. Caseine is a milk protein used in some types of concrete-smoothing materials. In case of a moisture leakage to this material it offers an immediate growth medium for many bacteria that are generally found in indoor air. This is a strong contraindication for use of caseine as a constituent in building materials (see 3.4.7).

Occurrence of spore-forming bacteria. In indoor air, spore-forming strains were isolated only in unoccupied apartments (1.5% of the total amount of isolates), in the spring samples (2.4%) and in the farmhouse samples (3.5%). This was 7% of the Gram-positive rods. The numbers of spore-forming bacteria are presented in Table 17.

Table 17. Percentages of spore-forming bacteria in indoor and outdoor air. N = number of isolated strains, n = number of Gram+ rods

group of samples	N(n)	sporeformers % of total	sporeformers % of Gram+ rods
(A) unoccupied fall 1985	134(57)	1.5	3.5
outdoors fall 1985	130(22)	0	0
(A) occupied winter 1986	101(15)	1.0	6.7
(A) occupied spring 1986	452(90)	2.4	12.0
(B) farmhouses	598(140)	3.5	15.0
(C) problem houses	419(167)	0	0

The low percentage of spore-forming bacteria is somewhat unexpected because spores are naturally dispersed through air. Apparently the sampling medium did not favour the growth of this group of bacteria.

Temperature tolerance of airborne bacteria. All the strains grew at room temperature (+21°C... +23°C). Results of the growth tests at different temperatures are shown in Table 18.

Table 18. Growth of airborne isolates at different temperatures

+4°C	+37°C	+55°C
75%	98%	39%

As seen in Table 18, the temperature tolerance of airborne bacteria is wide. Most strains were able to grow at +4°C, almost all at +37°C and a majority of them even at +55°C. It can be assumed that the ability to grow at +37°C, the body temperature of humans, is a measure of how opportunistic they are. Their ability to grow at +55°C means that airborne bacteria are often thermophilic.

3.7.2 Identification of the bacterial genera

With the methods used, about 45% of the strains could be identified to genus. The usefulness of API test kits for identification of environmental strains is limited because the system is prepared mainly for clinically important bacteria. The API 20B kit prepared for environmental strains does not usually provide names for the strains studied. Further taxonomic information could be acquired with chemotaxonomical methods (Goodfellow and Minnikin 1985) and specific tests for different groups of bacteria (Skinner and Lovelock 1979).

The most common genus in indoor air was *Micrococcus* and the most common in outdoor air was *Pseudomonas*. Other genera identified from air samples (as well as their percentage of the total) are listed in Table 19.

Table 19. Bacterial genera identified from indoor and outdoor air and their percentage of the total number of isolated strains

Genus	frequency (%)
INDOOR AIR	
Micrococcus	30
Staphylococcus	10
Bacillus	2
Moraxella	2
Pseudomonas	1
Agrobacterium	<1
Acinetobacter	<1
Flavobacterium	<1
OUTDOOR AIR	
Pseudomonas	30
Flavobacterium	2
Acinetobacter	<1
Aeromonas	<1
Agrobacterium	<1
Micrococcus	<1
Staphylococcus	<1

The common occurrence of micrococci and staphylococci in indoor air has been reported previously (Lidwell 1974). Also in the present study, these groups of bacteria formed a major proportion of all indoor air samples.

Micrococci and staphylococci were not common among the outdoor isolates in this study, although Wright *et al.* (1969) and Mancinelli *et al.* (1978) reported large numbers of these groups.

Members of the genus *Pseudomonas* were a dominating group in outdoor air, but the occurrence of this genus in air has seldom been mentioned before. The sources of bacteria in outdoor air are variable (see 1.6.2) and the variations in the flora of outdoor air may also be large. As mentioned before, the composition and sources of outdoor airborne bacteria are poorly known.

3.7.3 Bacterial groups in different rooms of an apartment

There were differences in the flora between different rooms of an apartment. The bacterial groups in different rooms of an apartment are shown in Figure 20.

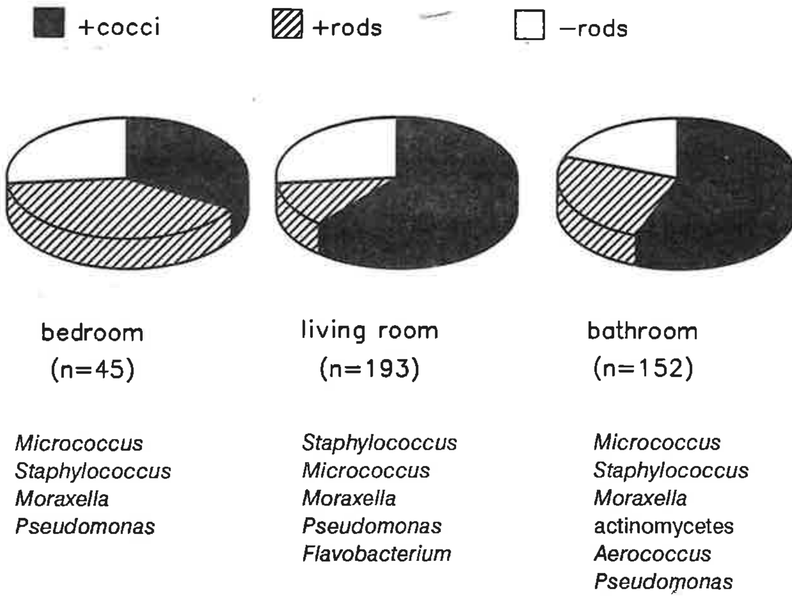


Figure 20. Airborne bacteria in different rooms of an apartment, according to morphological groups. The samples were taken simultaneously. The strains identified in each room are listed in order of frequency.

The largest differences were in the proportions of Gram-positive cocci and rods, while the proportion of Gram-negative rods was almost the same in different rooms.

3.7.4 Staphylococci and micrococci

The *Staphylococcus/Micrococcus* group was a dominating part of the bacteria in indoor air but occurred infrequently in outdoor air. In indoor air, the main source of these bacteria is humans. Micrococci and staphylococci are very common on human skin, as mentioned previously (see 1.6.1.1). However, they are also very common in soil, and thus their occurrence in outdoor air is to be expected. The outdoor isolates were only a minor part (n = 130) of this work, and it is possible that these cocci could be found in outdoor air samples taken at other times and locations.

The growth of staphylococci and micrococci in different concentrations of salt is presented in Figure 21.

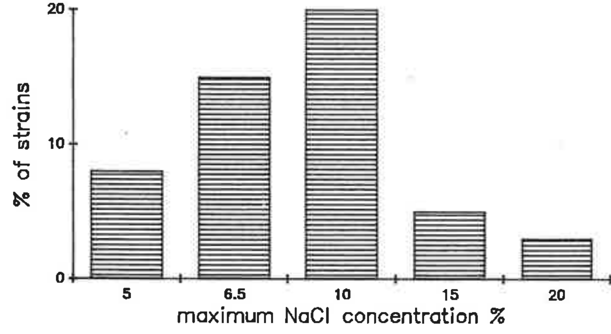


Figure 21. Growth of staphylococci and micrococci in different concentrations of salt (NaCl).

A considerable proportion of the isolated micrococci and staphylococci grew in high salt concentrations. About 50% of them grew in $\geq 5\%$ salt concentration and a third of them in $\geq 10\%$. Thirty-five strains were able to grow in salt concentrations as high as 20%. The ability to grow in high salt concentrations indicates good resistence to drying, which favours airborne survival.

3.7.5 Pseudomonas

In order to characterize the major groups of the bacterial flora in air, the genus *Pseudomonas* was chosen to represent a typical bacterial genus in nature. The occurrence of *Pseudomonas* was screened through all 2192 strains isolated.

The number of the *Pseudomonas* strains found in indoor and outdoor air is presented in Table 20.

Table 20. Occurrence of *Pseudomonas* sp. in indoor and outdoor air

sampling site and time	number of isolated colonies	number of *Pseudomonas* sp.(%)
INDOOR AIR		
(A) fall 1985 indoors, before occupation	134	7 (5.2)
(A) winter 1986 indoors	100	2 (2)
(A) spring 1986 indoors	451	1 (0.2)
(A) fall 1987 indoors	358	8 (2.2)
(B) farm-houses indoors	597	6 (1)
(C) problem homes indoors	418	6 (1.4)
OUTDOOR AIR		
fall 1985 outdoors	130	41 (32)

Pseudomonas strains are only a small fraction of the indoor airborne bacteria but are a major group in outdoor air. In samples from the unoccupied apartments (fall 1985) members of the genus *Pseudomonas* made up about 5% of the bacteria in indoor air but at the same time 32% of the bacteria in outdoor air.

In all the other indoor air samples, the *Pseudomonas* strains again made up less than 10% of the total bacteria (Table 20).

The genus *Pseudomonas* is an omnipresent bacterial group in soils and natural waters. Until now, little has been known about its occurrence in air. In this study the genus *Pseudomonas* was chosen to represent a typical bacterial genus found in nature, generally occurring in soils and waters. The results show that *Pseudomonas* is as common in outdoor air as it is elsewhere in nature. The results for outdoor air also show that *Pseudomonas* can be detected with the method used.

Indoors, the genus *Pseudomonas* is sometimes suspected to be associated with humidifier fever because of positive isolations in humidifier water (Covelli *et al.* 1973), but it has usually not been isolated directly from air. It is obvious that in cases of suspected health problems from contaminated humidifiers, detection of airborne *Pseudomonas* should be encouraged. It does not seem to be a major representative of the flora in indoor air, and thus its common occurrence in indoor air samples could indicate an intramural source.

Pseudomonas species. In outdoor air, the most common species was *Pseudomonas fluorescens*. In indoor air, other species were detected equally often. The identified species are presented in Table 21.

Table 21. *Pseudomonas* species identified in air

species	number of strains
Pseudomonas fluorescens	31
Pseudomonas putida	1
Pseudomonas luteola	4
Pseudomonas maltophilia	3
Pseudomonas paucimobilis	4
Pseudomonas oryzihabitans	1
Pseudomonas vesicularis	3
Pseudomonas stutzeri	1
Pseudomonas sp.	23

3.7.6 Actinomycetes

In the homes of the Group A, actinomycete colonies occurred sporadically. In the farmhouses they were found in large amounts, although their occurrence varied greatly. In the problem homes they were found regularly, *i.e.* in 70% of the homes, at levels of 2...60 cfu/m^3. The actinomycete levels in different homes are presented in Table 22.

Table 22. Actinomycete colonies in the indoor air of new suburban townhouses (Group A), problem homes (Group C) and farmhouses (Group B) (n= number of sampled homes)

sampling site	n	number of homes where actinomycetes found	actinomycetes cfu/m^3
suburban townhouse homes (A), spring	18	1	30
problem homes (C)	27	18	2-60
farmhouses (B)	9	9	5-1000

The problem homes were sampled in the spring and thus the results are comparable to the spring results of the Group A homes.

The frequency of actinomycete colonies in air samples from the problem homes was high compared to homes where there had been no complaint. Similar observations have also been reported also in Sweden (Hallenberg and Gilert 1983). The detected levels of actinomycetes, 2-60 cfu/m^3, were not high compared to levels in the farmhouses. In the farmhouses, however, there are specific sources of actinomycetes. The actinomycete levels in the animal shelters are high, due to the handling of hay and straw (Kotimaa *et al.* 1984). Thus the high levels in the indoor air of farmhouses can be explained by the transmission of spores from the animal shelters into the house, both on people and their clothing or in the air. The transmission of actinomycete spores by people is shown in Figure 23. The indoor levels of actinomycete spores in indoor air increased by two orders of magnitude after the farmer came in from the cowshed where he had handled moldy hay.

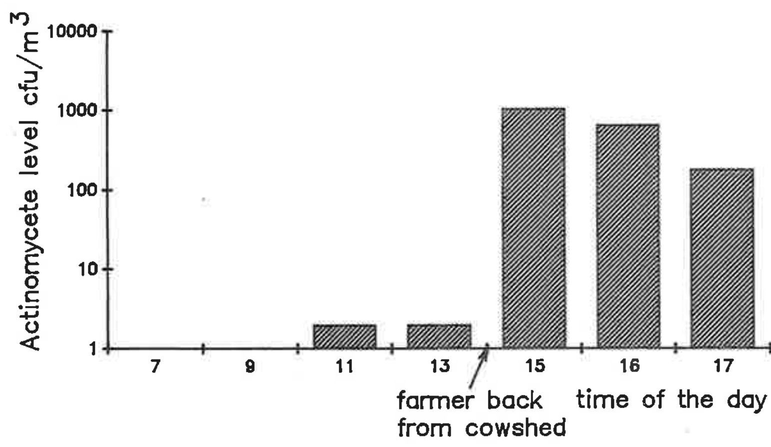


Figure 22. Levels of actinomycete spores in a farmhouse during the daytime. The farmer had handled moldy hay before returning to the house in the afternoon.

Actinomycete species in the problem homes. As a preliminary study, 20 of the actinomycete colonies from the problem homes were identified to species. The identifications were made by Dr. Ilona Buti from the Research Institute of Agricultural Chemistry, Hungarian Academy of Sciences, Budapest, Hungary. The results of the identification are presented in Table 23.

Table 23. Actinomycete species (n=20) isolated from the air of the problem homes

Species	n
Streptomyces lipmanii	4
Streptomyces flavogriseus	3
Streptomyces parvus	2
Streptomyces rishiriensis	1
Streptomyces tetanusemus	1
Streptomyces roseus	1
Streptomyces willmorei	1
Streptomyces nitrosporeus	1
Streptomyces sp.	5
Actinomyces ochroleucus	1

The role of these organisms in problematic indoor environments is of increasing interest. Moisture damage in a building may change the normal physical conditions, especially humidity, to enable microbial growth. This may be a suitable ecological niche for certain organisms. Many construction materials contain suitable nutrients for microbes; among these are wood, wallpapers, concrete, insulation materials and floor skim that contains caseine, an especially suitable nutrient for actinomycetes. A start in microbial growth of one type may lead to an ecological chain deteriorating the building.

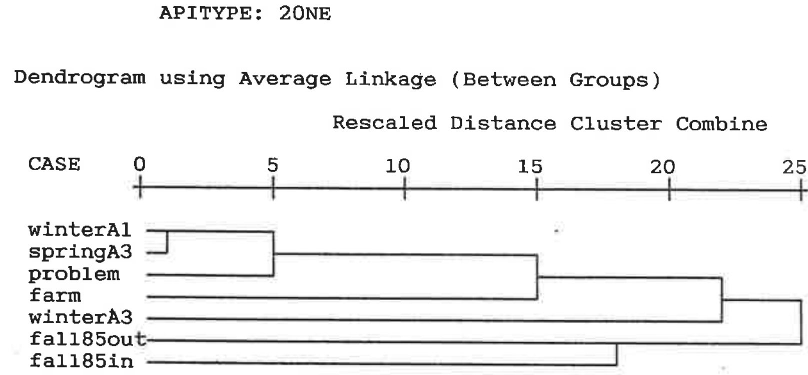
Another dimension of the occurrence of actinomycetes in moisture-damaged homes is their odor. These organisms have been shown to be the main cause of mud-like taste and odor in drinking water. The major components of the odor are geosmin and methyl-isoborneol (Gerber 1979). The possible role of actinomycete odors as an indoor air problem has only been suggested so far, but it will be one of the future challenges in research on indoor air and building hygiene.

3.7.7 Cluster analysis of the API profiles

Only 45% of the isolated strains could be identified to genus with the methods used. For the others, the probability of an accurate identification was not satisfactory. About 20 biochemical tests are made with each API test kit and their results give valid information about the characteristics of the strain, irrespective of whether a genus name can be proposed on the basis of the test results.

A cluster analysis (Everitt 1981) of the API profiles of the strains was made to test whether the bacterial flora is different in suburban townhouses (Group A), farmhouses and problem homes.

The profiles were divided into groups according to different sampling periods. These preformed groups were compared to each other by calculating the average of all two-profile similarity measures between the groups. A similarity matrix of the groups was used as input to a SPSS PC+ statistical package cluster procedure. The clustering method used in this procedure was average linkage between groups. As a result of the cluster analysis, dendrograms were made for API Staph and API 20NE profiles (Figure 23).



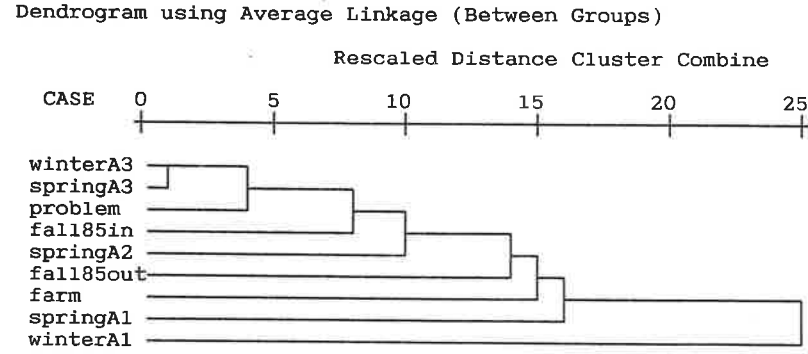


Figure 23. Dendrograms of the cluster analysis of API profiles between different sampling sites and periods. A1 = townhouse with natural ventilation based on gravity, A2 = townhouse with mechanical ventilation, A3 = townhouse with mechanical supply and exhaust ventilation

In the upper dendrogram of the API 20NE profiles, it can be seen that the fall 1985 (before occupancy) indoor and outdoor strains are of different origin than the other strains. The farm flora differs slightly from the winter and spring strains of the Group A and from the problem-home strains. However, no systematic differences could be seen between the groups.

In the other dendrogram of API Staph profiles, the winter strains of the houses A1 and A3 are close to the spring strains of the corresponding houses, but the spring strains of A1, A2 and A3 differ from each other.

In this preliminary analysis of the strains, no systematic differences could be seen between the airborne bacterial flora of the three groups of homes.

4 SUMMARY AND CONCLUSIONS

In this study levels of indoor air bacteria and the basic factors affecting them in homes were investigated. The factors studied were occupancy, ventilation system, season, and the type of dwelling. Samples were taken from new suburban townhouses, from farmhouses and from homes with a suspected microbial problem. Effects of air RH and temperature and the number of people present were also studied, as well as the diurnal and spatial variation in bacteria levels. The groups of airborne bacteria and their particle size distributions were characterized.

The results of the study contribute to our basic knowledge about bacteria in the indoor air of homes in subarctic climate and can be used as reference data in interpreting measurement data from hygienic monitoring and for detecting excess bacteria sources in buildings.

The conclusions of this study are:

1. Bacterial levels in homes have a wide range, $<10\text{-}10^4$ cfu/m^3. Indoor levels are always higher than outdoor levels.

2. Outdoor levels are low, $\leq 10^2$ cfu/m^3, in winter when the ground is frozen and covered with snow. In spring to fall the levels are more variable, between $<10\text{-}10^3$ cfu/m^3.

3. In new homes, levels of airborne bacteria increase due to the occupants and their activities. Bacteria are accumulated as long as two years after occupancy, after which they stabilize to the final level. This accumulation is independent of the ventilation system.

4. For removing airborne bacteria, mechanical supply and exhaust ventilation is a more effective system than natural ventilation or mechanical exhaust only. However, the premise on which this advantage is based is the adequate use of the system.

5. The relative humidity of air has only a marginal effect on levels of airborne bacteria. This applies to both indoor and outdoor air.

6. Air temperature does not affect levels of airborne bacteria in either indoor or outdoor air.

7. The number of people present during sampling significantly affects levels of bacteria in indoor air. The number of persons in the family does not alone determine the level; other factors, such as ventilation and cleaning habits, also contribute.
8. The proposal for an uppermost normal level of indoor air bacteria in homes is 4 500 cfu/m^3.

9. The *Micrococcus/Staphylococcus* group includes the dominating genera in indoor air, but is a minor group in outdoor air

10. *Pseudomonas* is the dominating genus in outdoor air, but makes up less than 10% of the bacteria in indoor air.

11. Actinomycetes are frequently found in homes with moisture problems but seldom in homes where no complaint has been made. Their occurrence evidently indicates a moisture problem in the house, and remedial actions are recommended. In farmhouses, however, actinomycetes belong to the normal flora because of the specific sources in the farming environment.

This work has raised several questions that should be studied in the future. Experimental studies on the specific sources of bacteria in indoor air, as well as the effects of cleaning habits and other everyday routines, would help in constructioning models that illustrate the characteristics of these particles. The effect of air temperature and relative humidity on bacterial survival in air should also be studied with typical airborne bacteria.

In future research emphasis should be placed on studying the airborne actinomycetes that seem to indicate moisture problems in houses with no specific sources such as farming. Actinomycetes are an important group of airborne disease agents in occupational environments, and more specific knowledge of the effects of their spores and their chemical metabolic products are needed. Further studies on the occurrence of *Pseudomonas* in air could bring about better understanding of their possible role as agents of humidifier fever or in hospital infections.

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Appendix 1. The characteristics of the problem houses.

No.	type of house	area m^2	ventil. system	built in (year)	material
1	townhouse	81	mech ->	1980	wood
2	townhouse	100	mech ->	1983	concrete
3	townhouse	74	mech ->	1983	concrete
4	townhouse	60	mech ->	1979	wood
5	townhouse	94	mech ->	1979	wood
6	townhouse	82	mech ->	1979	concrete
7	townhouse	82	mech ->	1980	concrete
8	townhouse	111	mech ->	1979	wood
9	townhouse	68	mech ->	1979	concrete
10	townhouse	68	mech ->	1979	concrete
11	townhouse	68	mech ->	1979	concrete
12	townhouse	76	mech - >	1974	concrete
13	1-family house	180	mech <-> with recirc.	1985	wood
14	1-family house	80	natural	1937(-67)[1]	wood
15	1-family house	149	mech ->	1952(-80)[1]	wood
16	1-family house	96	mech ->	1975	wood
17	townhouse	71	natural	1982	wood
18	townhouse	58	natural	1982	wood
19	apartment	59	natural	1977	concrete
20	townhouse	64	mech ->	1985	wood
21	townhouse	62	mech ->	1985	wood
22	townhouse	63	mech ->	1985	wood
23	1-family house	80	natural	1923(-56)[1]	wood
24	apartment	73	mech ->	1975	concrete
25	apartment	59	mech ->	1985	wood
26	1-family house	120	mech <-> with recirc.	1983	wood
27	apartment	60	mech - >	1980	concrete

[1](renovation year)
mech -> mechanical exhaust ventilation
mech <-> mechanical supply and exhaust ventilation
recirc recirculation
1-family single-family