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Hospital Delivery Room versus Outdoor Birthing Place: Differences in Airborne Microorganisms and Their Impact on the Infant

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ABSTRACT

The incidence of allergic airway diseases continues to increase in industrial countries while remaining much more stable in developing countries. Allergens inhaled are eventually also swallowed and evidently the gastrointestinal immune system has a role in regulating allergic responses in the pulmonary as well as the GI system. While some studies have pointed out the role of probiotic bacteria as a supplementary protection against the early development of various allergies, little attention has been paid to the composition of the airborne microflora first and continuosly inhaled by newborns and infants. This study compares the composition of two airborne microbial communities, one from hospital delivery rooms and the other from a nature reserve, evidently in use as a birthing place as early as 7500 B.C. around the air from the outdoor birthing place was marked by a far greater variation in microbial composition and a much higher representation of fungi than the air from the hospitals. The dominant bacterial species from the delivery rooms were *Staphylococcus areus* and *Micrococcus luteus*, originating from the staff and the hospital environment; the outdoor flora, however, was dominated by *Pseudomonas* spp. and *Bacillus* spp. In addition, 56% of all the bacterial isolates from the delivery rooms were most closely related to strains previously associated with clinical infections, whereas only 15% of isolates in the outdoor bacterial sample had such relationships. The role of airborne microorganisms could be important to infants with developing immune systems considering the microbial bias of hospital air presented in this study.

Keywords: Allergy; Newborns; Airborne Microflora; Delivery Rooms; Outdoor Birthing Place

1. Introduction

Over the past 40 years the incidence of allergic airway disease has risen in industrialized countries and has remained stable in developing countries [1]. Several researchers have suggested that the higher incidence of asthma in industrialized countries may be attributed to environmental changes [2-4], while other studies support the possibility that lack of early microbial stimulation results in abnormal immune response to antigens later in life [5-7]. Repeated exposure of airways to antigens is thought to lead to decreased responsiveness and the development of immunologic tolerance of the antigens [8-11]. Animal studies show that inhaled aeroallergens such as microorganisms may also be swallowed [12], and antigens delivered orally may also lead to the development of antigen-specific immunologic tolerance [13-15]. In addition, oral tolerance to an allergen can block responses outside of the gastrointestinal tract (GIT), including the allergic response to that allergen in the lungs [16-18]. Hence, the immune systems of the lungs and the GIT are connected and dependent on each other. Both the lungs and the GIT of infants are sterile, but after delivery the microbiota of the GIT is acquired from the environment [19]. The GIT of an infant is initially colonized by the mother's vaginal, perinal, and skin flora. Major factors affecting the nature of the early microbial populations are vaginal delivery versus cesarian delivery, antibiotic use in the mother, and bottle feeding versus breastfeeding [2,20,21]. Several studies have shown that the GIT of infants are first inhabited by facultative anaerobes such as *Enterobacteriaceae*, *Enterococcus*, *Streptococcus*, and *Staphylococcus* [22-24]. Later on, but still in the first week of life, *Bifidobacterium*, *Bacteroides*, and *Clostridium* are also established [25].

Several studies demonstrate evidence of different microbiota among lower income and middle income individuals from industrial versus developing countries [26-31]. Estonia has a markedly lower incidence of allergic disease than Sweden. It was demonstrated that the microbiota of children from poor Estonian societies had a

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different composition from those of Swedish children. The analyses revealed that the microbiota of the Swedish children was associated with increased aerobic microbes and decreased anaerobic microbes, especially *Lactobacillus*, in fecal samples [26]. In another study [27], it was discovered that infants with developed allergies showed fewer *Bifidobacterium* and *Enterococcus* but more *Clostridium* spp.

Hospitals harbour microorganisms, many of which are multiply resistant to antibiotics and cause hospital-acquired infections. Thorough and continuous cleaning can significantly disturb the normal niches of microorganisms found on patients and staff and in the hospital environment [32-35]. Many hospital-acquired infections are due to well-known pathogens that seem to survive easily in the harsh hospital environment. Well-known examples are *Clostridium difficile*, linked with antibiotic-associated colitis [36,37], and *Bacillus cereus*, with infections recorded in maternity, surgical, and intensive care units [38, 39]. Additionally, bacterial infective genera such as multiresistant *Klebsiella* [40], *Enterobacter* as a source of septicaemia [41], *Escherichia* in operating theaters [42], *Serratia* in an intensive therapy unit [43], and *Pseudomonas* in a special care baby unit outbreak [44], have been found in hospitals. Furthermore, *Acinetobacterradioresistens* was found in an increasing number of hospital-acquired infections and outbreaks [45], and *A. baumani* was the cause of an epidemiological outbreak [46]. Better known organisms from hospital environments are methicillin-resistant *Staphylococcus aureus* (MRSA) [47], and vancomycin-resistant *Enterococcus* (VRE) [48]. Finally the hospital-acquired infection organisms *Legionella* spp. and *Aspergillus* spp. are known to survive the hospital environment [49,50].

In a study [51] of the bacterial flora on the hands of 119 nurses working in two neonatal intensive care units and found *Acinetobacterbaumani*, *A. iwoffi*, *Enterobacteragglomerans*, *E. cloace*, *Klebsiellaoxytoca*, *K. pneumonia*, *Pseudomonas aeroginosa*, *P. flourescens*, *Staphylococcus aureus*, *S. warneri*, and *S. epidermis*, with the last two most dominant. In another study [52], sampling the bacterial aerosol in various areas of a dental clinic *Staphylococcus epidermis* was most frequently found, closely followed by *Micrcoccus* spp. and *Corynebacteria* spp., and *Staphylococcus aureus*, *Pseudomonas* spp., and fungi were also found.

To our knowledge no previous studies have compared the airborne microbiota inhaled by newborns after delivery in hospitals with the microbiota of outdoor air in more natural settings. In this study we wanted to discover the microbial composition of the hospital air in some Swedish delivery rooms because the airborne microorganisms first inhaled by newborns might have an impact on their developing immune systems. As a control we used the air of a possible outdoor birthing place in a Swedish primeval forest, with supposedly conserved fauna and flora and records of human activities circa 7500 B.C. [53].

2. Materials and Methods

2.1. Samples Collection

Once each month for one year, the airborne microorganisms from Solviken in the Kullaberg Nature Reserve (Skåne, Sweden) were sampled. On each occasion the sampling was performed with a Trypton Soy Broth agar (TSB, Oxoid Ltd., Basingstoke, Hampshire, England) on a surface plate 14.0 cm in diameter, for a total of 12 plates. The plates were circled manually in the air, without lids, sweeping to 0.5 m above the ground for 1 min to collect both airborne and ground surface microorgansims. Sampling was also performed in eight hospital delivery rooms in one single day during the summer. In each of eight delivery rooms in the University Hospital of Malmö (Malmö, Sweden) one uncovered TSB surface agar plate, 14 cm in diameter was placed by a nurse on a shelf and left for 1 h. Some rooms were empty at the time but most rooms were occupied with mothers giving birth. Plates from both Kullaberg and the delivery rooms were incubated aerobically at 22˚C for 5d. One of every morphologically different colony of bacteria, yeasts, and molds were selected and re-cultivated for purity as isolates. The recultivation was performed on TSB agar surface plates, incubated aerobically at 22˚C for 5d.

2.2. PCR of Bacterial Isolates

One colony from the purified isolates was placed in 2.0 ml Eppendorf tubes together with 0.25 ml sterile water and 10 - 15 glass beads (2.0 mm). Cells were disintegrated by shaking for 45 min in an Eppendorf mixer 5432 (Eppendorf, Hamburg, Germany). After centrifugation (20,200 × g for 5 min), 1 µl of the supernatant was used in the following PCR reaction.

Amplification was conducted with primers designed to anneal to conserved regions of bacterial 16S rRNA genes. The forward primer ENV1 (5'-AGA GTT TGA TII TGG CTC AG-3') corresponded to positions 8 - 27 in *Escherichia* coli 16S rRNA, and the reverse primer ENV2 (5'-CGG ITA CCT TGT TAC GAC TT-3') corresponded to positions 1511-1492 [54]. The PCR reaction contained 5 µl 10× PCR buffer (100 mMTris-HCl, 15 mM $MgCl_2$, 500 mMKCl, pH 8.3), 200 $\mu mol \cdot 1^{-1}$ of each deoxyribonucleotide triphosphate, 2.5 U of Taq DNA polymerase (Roche Diagnostics, Mannheim, Germany), 10 pmol of each primer and 1 - 10 µl template in a total volume of 50 µl. Amplification was performed with a T-personal (WhatmanBiometra, Goettingen, Germany)

as follows: 30 cycles at 95˚C for 15 s, 48˚C for 30 s, and 72˚C for 90 s followed by an elongation step at 72˚C for 10 min. The PCR product was stored at –20˚C for sequencing.

2.3. Sequencing and Identification of Isolate DNA

PCR products were sequenced by a sequencing company (MWG Operon Eurofins, Ebersberg, Germany) with universal primer ENV1. These partial 16S rDNA sequences were searched against GenBank (National Centre for Biotechnology Information, Rockville Pike, Bethesda, MD) using the Advanced BLAST similarity search option [55], accessible from the homepage of the National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). For comparison, sequences were also searched against another software, The Ribosomal Database Project II [56], accessible from the homepage (http://rdp.cme.msu.edu/).The partial sequences were mostly around 550 - 900 base pairs (ranging from the 8th bp). A bacterial isolate was defined as a specific phylotype when it diverged by 5 bases or more from the closest related isolate. The identification of mold isolates was performed to the morphological genus level. The identification of yeasts was performed visually by noting differences in colony appearances, and by microscope, but never to genus or species level.

3. Results

A total of 355 bacterial sequences were identified and all showed a similarity of >91% to type strains in the RDP. In addition 28 colonies of yeast were morphologically identified and 105 colonies of mold were identified to the level of genus. A total of 242 microorganisms (142 bacterial sequences, 75 mold colonies, and 25 yeast colonies) originated in the Kullaberg Nature Reserve, and 246 microorganisms (213 bacterial sequences, 30 mold colonies, and 3 yeast colonies) originated in the delivery rooms. The bacterial diversity was larger in the samples from Kullaberg, constituting 88 different phylotypes among 41 different genera and 76 different species of the 142 isolated bacteria **Table 1**. From the delivery rooms 213 bacteria were isolated, representing 80 different phylotypes among 35 different genera and 68 different species **Table 2**. A number of possibly novel bacterial species with <97% sequence similarity to the nearest type strain were found from both Kullaberg (n 14) and the delivery rooms (n 10). Bacterial isolates constituted 142 (59%) of the isolated microorganisms from Kullaberg and 213 (87%) from the delivery rooms. Kullaberg had 75 (31%) mold isolates representing 12 genera **Table 3** compared with 30 (12%) representing 5 genera from the hospital **Table 4**. Finally, yeasts were found to be more dominant in the samples from Kullaberg, with 25 isolates (10%), than in the hospital samples, with 3 isolates (1.4%). The dominant bacterial genera from Kullaberg were *Pseudomonas* (25 isolates; 18%), *Bacillus* (16 isolates; 11%), *Rhodococcus* (14 isolates; 10%), *Arthrobacter* (10 isolates; 7%), *Micrococcus* (9 isolates; 6%), *Microbacterium* (6 isolates; 4%), *Curtobacterium* (6 isolates; 4%), *Sporosarcina* (6 isolates; 4%), and *Paenibacillus* 4 isolates; 3%) (**Figure 1**). The dominant bacterial genus from the delivery rooms were markedly different with 47 isolates of *Staphylococcus* (22%), 36 isolates of *Micrococcus* (17%), 17 isolates of *Arthrobacter* (8%), 14 isolates of *Acinetobacter* (7%), 13 isolates of *Microbacterium* (6%), 13 isolates of *Bacillus* (6%), 9 isolates of *Paenibacillus* (4%), 8 isolates of *Kocuria* (4%), and7 isolates of *Rhodococcus* (3%) (**Figure 2**). The percentage of isolated bacterial species previously found in clinical samples were much higher in the delivery room samples (51%) **Table 1** than in the samples from the nature reserve Kullaberg (18%) **Table 2**.

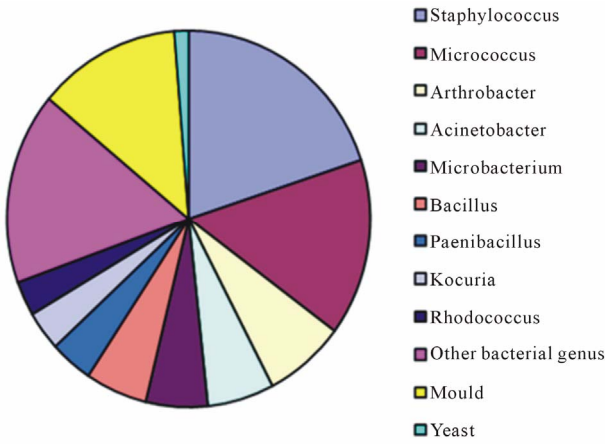

Figure 1. The dominant airborne microorganism flora from the Kullaberg Nature Reserve, Sweden.

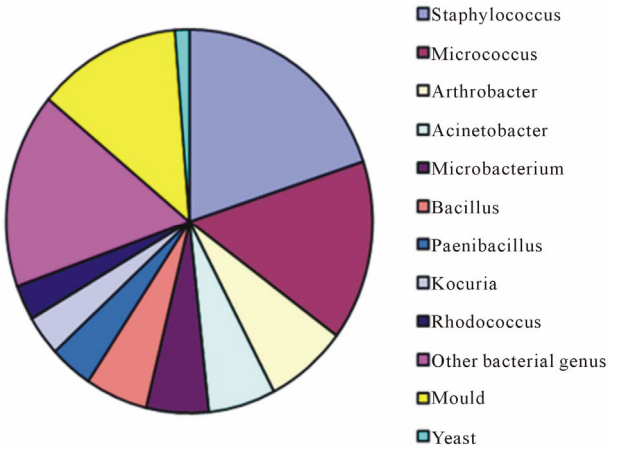

Figure 2. The dominant airborne microorganism flora from five hospital delivery rooms.

Table 1. Thebacterial flora of air from the Kullaberg Nature Reserve. Identity of 16S rRNA gene sequences generated from isolates selected for morphological differences after cultivation. Isolates are presented with the clinically recorded type strains to which they are most closely related.

Number of isolates[a]	Most closely related type strain[b]	Compared sequence similarity	References to studies with species associated to clinical cases
6 [2]	*Pseudomonas koreensis* Ps 9-14; AF468452	(98 - 99)	
3 [2]	*Pseudomonas umsongensis* Ps 3-10; AF468450	(98)	
2 [2]	*Pseudomonas graminis* DSM 11363; Y11150	(98 - 99)	
2 [2]	*Pseudomonasasplenii* LMG 2137T; Z76655	(98 - 99)	
2	*Pseudomonas congelans* LMG 21466	(98)	
2 [2]	*Pseudomonas jessenii* CIP 105274; AF068259	(98 - 99)	
2	*Pseudomonas amygdali* LMG 2123T; Z76654	(99)	
1	*Pseudomonas rhizosphaerae* IH5; AY152673	(98)	
1	*Pseudomonas fulva* IAM1529; D84015	(98)	
1	*Pseudomonashibiscicola* ATCC 19867T; AB021405	(98)	
1	*Pseudomonas orientalis* CFML 96-170; AF064457	(99)	
1	*Pseudomonasantarctica* CMS 35; AJ537601/ *Pseudomonas meridiana* CMS 38; AJ537602	(99)	
1	*Pseudomonas costantinii* HAMBI 2444; AF374472	(99)	
8	*Bacillus mycoides* ATCC6462; AB021192/ *Bacillus weihenstephanensis* DSM11821; AB021199	(99)	
3 [3]	*Bacillus muralis* LMG 20238; AJ628748	(96)	
2	*Bacillus flexus* IFO15715; AB021185	(98)	
1	*Bacillus psychrodurans* 68E3; AJ277984/ *Bacillus psychrotolerans* DSM 11706; 3H1; AJ277983	(98)	
1	*Bacillus silvestris* HR3-23; AJ006086	(97)	
1	*Bacillus sphaericus* IAM 13420; D16280	(98)	[85]
5 [2]	*Rhodococcus erythreus* (T); X79289	(99)	
4 [2]	*Rhodococcus erythropolis* DSM43188; X80618	(99)	
3	*Rhodococcus globerulus* NCIMB 12315; X81931	(99)	[86]
2 [2]	*Rhodococcus fascians* DSM 20669; X79186	(98 - 99)	
3	*Arthrobacter arilaitensis* Re117; AJ609628	(99)	
1	*Arthrobacter flavus* CMS-19Y; AJ242532	(98)	
1	*Arthrobacter ramosus* DSM 20546; X80742/ *Arthrobacter pascens* DSM 20545; X80740	(99)	
1	*Arthrobacter kerguelensis* KGN15; AJ606062	(99)	
1	*Arthrobacter globiformis* DSM 20124; X80736	(99)	[87]
1	*Arthrobacter psychrolactophilus* (T); AF134179	(98)	
1	*Arthrobacterchlorophenolicus* A-6; AF102267	(98)	
1	*Arthrobacter rhombi* F98.3HR69; Y15885	(99)	
9 [2]	*Micrococcus luteus* DSM 20030; AJ536198	(99)	[74]
3	*Microbacterium phyllosphaerae* DSM 13468; P 369/06; AJ277840	(99)	

Continued

2	*Microbacterium liquefaciens* DSM 20638; X77444	(98)	
1	*Microbacterium arborescens* DSM 20754; X77443	(98)	
6 [2]	*Curtobacterium flaccumfacienspv. flaccumfaciens* LMG 3645; AJ312209	(99)	
3	*Sporosarcina aquimarina* SW28(T); AF202056	(98)	
2	*Sporosarcina globispora* DSM 4; X68415	(97 - 98)	
1	*Sporosarcina psychrophila* IAM 12468; D16277	(99)	
2	*Paenibacillusa mylolyticus* NRS-290T; D85396	(99)	
1	*Paenibacillus borealis* KK19; AJ011322	(98)	
1	*Paenibacillus antarcticus* 20CM; AJ605292	(96)	
3	*Sanguibacter inulinus* ST50; X79451	(99)	
3	*Flavobacterium hibernum*ATCC51468; L39067	(97 - 98)	
2	*Acinetobacter johnsonii* DSM 6963; X81663	(98)	[75]
1	*Leifsonia aurea* CMS 81; AJ438586	(98)	
1	*Leifsonia poae* Ac-1401; AF116342	(97)	
2	*Subtercolaboreus* (T); AF224722	(97)	
1	*Agromycess alentinus* 20-5; AY507129	(98)	
1	*Rathayibacter tritici* DSM 7486; X77438	(98)	
1	*Frigoribacterium faeni* 801; Y18807	(99)	
1	*Agrococcus jenensis* DSM 9580; X92492	(97)	
1	*Kocuria rosea* ATCC 187T; Y11330	(99)	[88]
1	*Streptomyces virginiae* IFO 3729; D85119	(98)	
1	*Dietziamaris* (T); X79290	(98)	[89]
1	*Kytococcus sedentarius* DSM; X87755	(99)	[90]
1	*Oerskovia enterophila* DSM 43852; X83807	(99)	
1	*Beutenbergia cavernosa* DSM 12333; Y18378	(95)	
1	*Patulibacter minatonensis* KV-614; AB193261	(99)	
1	*Staphylococcus equorum subsp. equorum* ATCC 43958T; AB009939	(99)	
1	*Listeria welshimeri* ATCC35897; X98532	(97)	
1	*Carnobacterium viridans* MPL-11; AF425608	(99)	
1	*Pedobacter africanus* DSM 12126T; AJ438171	(94)	
1	*Chryseobacterium formosense* CC-H3-2; AY315443	(95)	
1	*Raoultella planticola* ATCC 33531T; Y17659	(98)	
1	*Yersinia kristensenii* ATCC 33638; AF366381	(98)	[91]
1	*Erwinia persicina* ATCC 35998; U80205	(98)	
1	*Stenotrophomonas rhizophila* e-p10; AJ293463	(100)	

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1	*Xanthomonas cynarae* CFBP4188; AF208315/ *Xanthomonas campestris* LMG 568-T; X95917/ *Xanthomonas hortorum* LMG 733T; Y10759/ *Xanthomonas vasicola* LMG 736 T; Y10755/ *Xanthomonas arboricola* LMG 747 T; Y10757s	(97)	
1	*Psychrobacter maritimus* KMM 3646; AJ609272	(99)	
1	*Variovorax paradoxus* DSM 66; AJ420329	(97)	
1	*Massili atimonae* (T); timone; U54470	(98)	[92]
1	*Acidovorax defluvii* BSB411; Y18616	(98)	
1	*Brevundimonas diminuta* (T); M59064	(98)	[93]
1	*Sphingomonas aurantiaca* (T); MA101b; AJ429236	(99)	
1	*Rhizobium giardinii* (T); H152; U86344	(98)	

[a]Among the number of isolates found closely related to a specific type strain, the number of phylotypes (if more than one) are shown in brackets; [b]Type strain numbers are followed by GenBank accession numbers.

Of 213 bacterial isolates from the delivery rooms, 120 (56%) were most closely related to type strains previously associated with clinical samples. From the Kullaberg Nature Reserve, of 142 bacterial isolates, only 22 (15% of the isolates) were most closely related to type strains previously associated with clinical samples.

4. Discussion

We hypothesised that the air from the delivery rooms would have less variation in microorganisms than that from the nature reserve and would also have a higher incidence of microorganisms with clinical origin. There are several reasons to believe that Kullaberg, the first piece of land to emerge in Sweden after the last ice age [57], hosts a greater diversity of microorganisms than hospital delivery rooms. First, the soil of the forest of Solviken in the Kullaberg Nature Reservehas been uncultivated since the first leafed trees immigrated from the south around 7500 B.C. [57]. Second, 70% of all the plant species recorded in Sweden can be found in Kullaberg. Third, according to an inventroy of plant species in Kullaberg, the particular locale of Solviken supported the most plant species of 70 locales investigated [58]. Finally, fauna and flora undisturbed by human settlement or agriculture will in time create a unique environment [57].

The total number of microbial isolates was about the same from each of the two different locales investigated, but the percentages of mold and yeast were higher and the overall diversity of microorganisms was greater in the air of the nature reserve than in the delivery rooms. To our knowledge this has not been reported before, even though it seems reasonable considering the higher biological activity in the nature reserve. A general picture indicates a higher frequency and diversity of molds and yeasts in the air from the Nature reserve compared to the hospital. Both environments had a high incidence of molds, although molds were found twice as frequently in air from the nature reserve. Molds were identified to only the genus level and the results showed 12 different genera in the samples from Kullaberg compared to 5 different genera from the hospital. Yeasts were clearly represented in the samples from the nature reserve but almost absent in the delivery rooms. Yeasts were not identified to species or genus level but differentiated morphologically through observation of their colonies on plates and cells under the microscope. Nevertheless, 25 different types of yeast colonies were found in the samples from Kullaberg compared to only 3 different colonies from the hospital.

Species level identification of the bacterial flora needs to be clarified. In the present study, partial sequences above 550 bases were evaluated which was in agreement with other studies [59,60]. The relationships of the isolates to described taxa in the database are given in **Tables 1** and **2**. It has been suggested that a similarity in 16S rDNA of >97% indicates closely related species. However, in one study [59], it was shown that there was only 50% chance for two 16S rDNA to belong to the same species if the similarity was >99.8 %. Thus, the descriptions in **Tables 1** and **2** are not definite; the taxa defined are not necessarily true species but should be called phylotypes [61], or molecular species [60].

The bacterial flora of the delivery rooms was largely consistent with previous reports of hospital environments, e.g. *Staphylococcus*, which made up a major part of the hand flora of housekeeping staff and neonatal intensive care unit nurses and was also abundant in aerosols [51; 52]. Two of its species, *Staphylococcus epidermidis* and *S. capitis*, were found to be two of the first colonizers of an infant GIT [24]. *Micrococcus* spp. occurred frequently in the hospital air in this study, as previously described in

Table 2. Thebacterial flora of air from hospital delivery rooms. Identity of 16S rRNA gene sequences generated from isolates selected for morphological differences after cultivation. Isolates are presented with the clinically recorded type strains to which they are most closely related.

Number of isolates[a]	Most closely related type strain[b]	Compared sequence similarity	References to studies with species associated to clinical cases
16	*Staphylococcus epidermidis* ATCC 14990T; (D83363)	(99 - 100)	[73]
9	*Staphylococcus haemolyticus* ATCC 29970T; D83367	(100)	[71]
6	*Staphylococcus hominis* (T); L37601	(100)	[94]
7 [2]	*Staphylococcus saprophyticus* (T); L37596	(99 - 100)	[95]
4	*Staphylococcus caprae* DSM 20608; Y12593/ *Staphylococcus capitis* ATCC 49326T; AB009937	(99)	[72,96]
1	*Staphylococcus pasteuri* ATCC 51129T; AB009944	(99)	[97]
3 [2]	*Staphylococcus warneri* (T); L37603	(99 - 100)	[98]
1	*Staphylococcus cohnii* ATCC 29974T	(99)	[99]
34 [2]	*Micrococcus luteus* DSM 20030; AJ536198	(99)	[74]
3	*Micrococcus antarcticus* T2; AJ005932	(99)	
10	*Arthrobacter globiformis* DSM 20124; X80736	(99)	[87]
3 [3]	*Arthrobacter flavus* CMS-19Y; AJ242532	(98)	
1	*Arthrobacter agilis* DSM 20550; X80748	(99)	
3	*Arthrobacter oxydans* DSM 20119; X83408	(99)	
10	*Acinetobacter lwoffii* DSM 2403; X81665	(99)	[100]
3	*Acinetobacter radioresistens* DSM 6976; X81666	(99)	[101]
1	*Acinetobacter schindleri* LUH5832T; AJ278311	(99)	
7 [4]	*Microbacterium terregens* IFO 12961; AB004721	(95 - 98)	
1	*Microbacterium paraoxydans* CF36; AJ491806	(98)	[102]
2 [2]	*Microbacterium phyllosphaerae* DSM 13468; AJ277840	(99 - 100)	
3	*Microbacterium arborescens* DSM 20754; X77443	(98)	
7	*Paenibacillus amylolyticus* NRS-290T; D85396	(99)	
2	*Paenibacillus antarcticus* LMG 22078; AJ605292	(98)	
5	*Bacillus muralis* LMG 20238; AJ628748	(99)	
1	*Bacillus thuringiensis* ATCC10792; AF290545	161 (98)	[103]
1	*Bacillus simplex* DSM1321; D78478	(98)	
1	*Bacillus weihenstephanensis* DSM11821; AB021199/ *Bacillus mycoides* ATCC6462; AB021192	(99)	
2	*Bacillus drentensis* LMG 21831; AJ542506	(99)	
1	*Bacillus silvestris* HR3-23; AJ006086	(98)	
1	*Bacillus gibsonii* DSM 8722; X76446	(99)	
1	*Bacillus indicus*Sd/3; AJ583158	(98)	
4 [2]	*Kocuria palustris* DSM 11925; Y16263	(97 - 98)	
2	*Kocuria rosea* ATCC 187T; Y11330	(99)	

Continued

1	*Kocuria kristinae* DSM 20032; X80749	(99)	
1	*Kocuria carniphila* CCM 132T; AJ622907	(99)	
5 [3]	*Rhodococcus fascians* DSM 20669; X79186	(99)	
1	*Rhodococcus globerulus* NCIMB 12315; X81931	(99)	[86]
1	*Rhodococcus erythropolis* DSM43188; X80618	(99)	
5	*Dietziamaris* (T); X79290	(98)	[89]
2	*Corynebacterium pseudodiphtheriticum* CIP 103420T; X81918	(99)	[78]
1	*Corynebacterium diphtheriae* NCTC 11397; X84248	(94)	[79]
3 [2]	*Sphingomonas aurantiaca* MA101b; AJ429236	(98 - 99)	
2	*Exiguobacterium aurantiacum* NCDO 2321; X70316	(97 - 98)	
1	*Massilia timonae* (T); timone; U54470	(97)	[92]
1	*Okibacteriumfritillariae* Ac-2059; AB042094	(97)	
1	*Pseudomonas luteola* IAM13000; D84002	(98)	[76; 104]
1	*Rothia nasimurium* CCUG 35957; AJ131121	(98)	
1	*Brachybacterium faecium* DSM 4810; X91032	(97)	
1	*Kytococcuss chroeteri* DSM 13884T; AJ297722	(99)	[80]
1	*Beutenbergia cavernosa* DSM 12333; Y18378	(91)	
1	*Knoellia subterranea* CIP 106776"; AJ294413	(98)	
1	*Deinococcus radiopugnans* ATCC 19172T; Y11334	(99)	
1	*Agrococcus jenensis* DSM 9580; X92492	(99)	
1	*Agromycess alentinus* 20-5; AY507129	(98)	
1	*Planomicrobiumokeanokoites* IFO 12536T; D55729	(99)	
2	*Aerococcus viridans* M58797	(99)	
1	*Herbaspirillum seropedicae* ATCC 35892; Y10146	(97)	
1	*Sporosarcina macmurdoensis* CMS 21w;	(98)	
1	*Sporosarcina globispora* DSM 4; X68415	(94)	
1	*Streptococcus dysgalactiae* NCFB1356; AB008926	(99)	[77]
1	*Macrococcus caseolyticus* ATCC 13548; Y15711	(98)	
1	*Sphingobacterium spiritivorum* (T); M58778	(99)	[81]
1	*Curtobacterium flaccumfaciens*pv. *flaccumfaciens* LMG 3645 AJ312209	(99)	
1	*Curtobacteriumherbarum* P 420/07; AJ310413	(99)	
2	*Frigoribacterium faeni* 801; Y18807	(98)	
1	*Agreia pratensis* P 229/10; AJ310412	(99)	
1	*Sanguibacter inulinus* ST50; X79451	(99)	

[a]Among the number of isolates found closely related to a specific type strain, the number of phylotypes (if more than one) are shown in brackets. [b]Type strain numbers are followed by GenBank accession numbers.

Table 3. Themold flora in air from the Kullaberg Nature Reserve. Identification to the genus level was performed using a morphology identification scheme generated from isolates selected for morphological differences after cultivation.

No. of isolates	Genus
21	*Stachybotrys*
11	*Aspergillus*
10	*Cladosporium*
7	*Penicillium*
6	*Alternaria*
4	*Wallemia*
4	*Trichoderma*
3	*Paecilomyces*
3	*Eupenicillium*
3	*Acremonium*
2	*Fusarium*
1	*Phialophora*

Table 4. Themold flora in air from hospital delivery rooms. Identification to the genus level was performed using a morphology identification scheme generated from isolates selected for morphological differences is after cultivation.

No. of isolates	Genus
9	*Penicillium*
6	*Aspergillus*
4	*Cladosporium*
3	*Curvularia*
3	*Trichoderma*

aerosols [52]. The dominant bacterial flora from the air of the nature reserve was different from those of the hospital delivery rooms in several respects. First, air from the nature reserve contained almost no *Staphylococcus* spp., much fewer *Micrococcus luteus*, and very few *Acinetobacter* spp. Instead it was dominated by more *Pseudomonas* spp. and much more *Bacillus* spp. than in the hospital. Interestingly, a study of the bioaerosol distributed in a general hospital in Korea [62], showed an almost identical result in the dominant airborne bacteria and fungi found in the delivery rooms as in our present study.

A relatively high portion of bacteria found in the delivery rooms was known to be associated with clinical infections (56% of the bacterial isolates **Table 2**). Early administration of *Lactobacillus rhamnosus* was shown to prevent small children at risk (one or more first-degree relatives with allergic disease) from developing eczema, an early manifestation of subsequent allergy [63,64], and the role of intestinal microbiota in modulating the immunological system of children and as an important factor in the prevention of various allergic diseases has been discussed [65-69]. For example, it was shown that early probiotic therapy reduced the frequency of allergies and repeated infections and this effect extended into adulthood [70] airways repeatedly exposed to antigens may lose responsiveness and may develop immunologic tolerance to the antigens [8-11]. Hence, if some bacteria have been shown to modulate the immune system reaction to allergens in the GIT, it can be speculated that the composition of the first and continuously inhaled airborne microorganisms may also influence the developing immune system and thereby potential allergic diseases. The microfloral diversity was only slightly higher at the nature reserve than in the delivery rooms; however, the sampling time in the nature reserve was only one minute versus one hour in the delivery rooms. The numbers and diversity of microorganisms in inhaled air from the nature reserve are therefore expected to be much higher than in air from delivery rooms over the same length of time, and the fewer and less varied microorganisms in hospital air might affect the developing immune system of a newborn.

What is less speculative is the fact that infants are at much greater risk of inhaling dangerous bacteria in the hospital during their first minutes, hours, and days than they would be if they were born in an open environment such as the nature reserve. Examples of such bacteria, or closely related bacteria, dominant in the passive air samples of the delivery rooms over merely one hour include *Staphylococcus spp.*, which cause septicemia in term and preterm infants[71,72]; *S. epidermidis*, which causes infections in infants [73]; *Micrococcus luteus*, which causes recurrent bacteremia [74]; *Acinetobacter johnsonii*, which causes bloodstream infections [75]; *Pseudomonas luteola*, whichcauses both cutaneous abscess and bacteremia [76]; *Streptococcus dysgalactiae* subsp. *equisimilis*, which causes vertebral osteomyelitis (spinal infection) [77]; *Corynebacterium pseudodiphtheriticum*, which is reported as a respiratory tract pathogen [78]; *Corynebacterium diphtheriae*, which causes respiratory diphtheria [79]; *Kytococcuss chroeteri*, which leads to fatal bacteremic pneumonia [80]; and *Sphingobacterium spiritivorum*, which causes extrinsic allergic alveolitis (hypersensitivity pneumonitis) [81]. The microbes acquired from the mother at the time of birth through vaginal delivery rather than cesarean section seems to be an important start to establishing a proper indigenous microbial community [21,82-84]. Further studies should be conducted to evaluate the role of newborn exposure to a

higher variety of airborne microorganisms and its influence on the development of the immune system and future allergic prevalence.

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