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Bacterial constituents of indoor air in a high throughput building in the tropics

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Abstract. Airborne bacteria are significant biotic constituents of bioaerosol. Bacteria at high concentrations in the air can compromise indoor air quality (IAQ) and result in many diseases. In tropical environments like Malaysia that extensively utilize air-conditioning systems, this is particularly significant due to continuous recirculation of indoor air and the potential implications for human health. Currently, there is a lack of knowledge regarding the impact of airborne bacteria on IAQ in Malaysia. This study was prompted by a need for reliable baseline data on airborne bacteria in the indoor environment of tropical equatorial Malaysia, that may be used as a reference for further investigations on the potential role played by airborne bacteria as an agent of disease in this region. It was further necessitated due to the threat of bioterrorism with the potentiality of release of exotic pathogenic microorganisms into indoor or outdoor air. Before scientists can detect the latter, a gauge of the common microorganisms in indoor (as well as outdoor) air needs to be ascertained, hence the expediency of this study. Bacterial counts from the broad-based and targeted study were generally n the order of 10^2 colony-forming units (CFU) per m^3 of air. The most prevalent airborne bacteria found in the broad-based study that encompassed all five levels of the building were Gram-positive cocci (67.73%), followed by Gram-positive rods (24.26%) and Gram-negative rods (7.10%). Gram-negative cocci were rarely detected (0.71%). Amongst the genera identified, *Kytococcus* sp., *Micrococcus* sp., *Staphylococcus* sp., *Leifsonia* sp., Bacillus sp. and Corynebacterium sp. predominated in indoor air. The most dominant bacterial species were Kytococcus sedentarius, Staphylococcus epidermidis and Micrococcus luteus. The opportunistic and nosocomial pathogen, *Stenotrophomonas maltophilia* was also discovered at a high percentage in the cafeteria. The bacteria isolated in this study have been increasingly documented to cause opportunistic infections in immuno-compromised patients, sometimes with fatal outcomes. Furthermore, some of them are becoming increasingly resistant to antibiotics. Hence, we propose that indoor reservoirs of these bacteria and their associated clinical and more subtle health effects, if any, be investigated further.

INTRODUCTION

Bacteria are ubiquitous micro-organisms (Aydogdu *et al.*, 2005). The air, with its lack of nutrients and water, and filled with deadly ultraviolet (UV) rays, seems probably the last place that a microbe would wish to live in. Quite surprisingly however, a new genetic census of some air samples from Austin and San Antonio, Texas, found on average at least 1800 divergent bacteria present in the air we breathe in on any particular day. There is also the consistent presence of bacterial families with pathogenic members including bio-terrorism-relevant bacteria and their spores (Brodie *et al.*, 2007).

The sources of bacteria in indoor air include building occupants, outdoor air and microbial growth in certain building structures such as heating-ventilation-air-conditioning (HVAC) systems (Nevalainen & Seuri, 2005; Stetzenbach, 2007). If physical

conditions, such as moisture level, temperature and the presence of certain organic and inorganic substrates, are met, bacteria easily proliferate in a building (JWT, 2009). Moreover, with the construction of airtight buildings to achieve energy efficiency, with the interior air being changed less frequently, there is build up in the numbers of bacteria in the air. This places the human occupants at greater health risk because the enclosed space confines and protects bio-aerosols.

It has become increasingly evident that micro-organisms in the indoor environment have a significant role in causing human health disorders and resultant disability, and contributing to unacceptable air quality in the workplace. According to Beggs (2003), we should not doubt the potential of airborne bacteria in the transmission of disease. Bacteria are now a recognized contributory factor to irritation, allergies, diseases and other toxic effects indoors (Lopez & Salvaggio, 1985; Susan & Zummo, 1996; Saravanan, 2004; Fabian *et al.*, 2005). Exposure to a diverse array of non-cellular agents associated with bacteria, such as endotoxin, glucans, polypeptides and allergenic proteins may also result in adverse health effects (Rylander, 1999; Alexis *et al.*, 2001; Michel *et al.*, 2003; Nilsson *et al.*, 2004; Kalogerakis *et al.*, 2005). Elevated concentrations of airborne bacteria are implicated with higher probability of epidemics and food pollution. There are reported cases of food poisoning due to the presence of the soil bacterium *Bacillus cereus* in the indoor air of schools and childcare centers (Kotiranta, 2000). It may also be the primary cause of a number of dermatological and respiratory infections and diseases (Kim *et al.*, 2007; Fang *et al.*, 2007).

Pastuszka *et al.* (2000) report on epidemiological studies associating 'sick building syndrome' (SBS) and hypersensitivity diseases with exposure to high concentrations of airborne microbes. In another study, Daisey *et al.* (2003) measured airborne bacteria and fungi in the indoor air of schools and reported on, amongst others, asthma and common symptoms of 'SBS'. *Legionellae* isolated from water contained in cooling towers of air-conditioning systems have also been implicated as causative agents of SBS (O'Mahony *et al.*, 1989). The United States Environmental Protection Agency (USEPA), has defined SBS as *situations in which building occupants experience discomfort and acute health effects that appear to be linked to time spent in buildings* (USEPA, 1991). SBS indicators encompass complaints of discomfort, including headache, eye, nose or throat irritations, dry cough, skin irritation, dizziness and nausea, fatigue and sensitivity to odour. Relief from the discomfort is frequently felt immediately upon exiting the building (USEPA, 1991).

Despite the interest in the role of bioaerosols to health and perception of IAQ in Malaysia, there is currently no consensus on regulatory limits for airborne bacterial concentrations in indoor air. Moreover, data on the species, concentration and distribution of airborne bacteria in the indoor environment is lacking. There is, therefore, a need for comprehensive surveys on indoor airborne bacteria. This investigation will provide baseline data on bacterial pollutants of indoor air in a five-level multistory tertiary educational institution, which in turn can aid health authorities in formulating good guidelines/standards with regards to Indoor Air Quality (IAQ).

MATERIALS & METHODS

Study design and sampling sites

From June 2009 to September 2009, culturable airborne bacteria were collected from five levels of a tertiary educational institution in Kuala Lumpur, that carried at its centre an atrium with a transparent glass roof. Tropical sunlight, whenever present, easily accessed the indoors of the building through the roof of the atrium.

The study was divided into two parts, namely, a broad-based study and a targeted study. In the broad-based study, air samples were collected at a strategic site at each of five levels in the building. The air collected

was taken to reflect the general air quality at that level. The selected sampling site at each level had the following criteria: they had the highest people movement and they were within 10-15 metres from the toilets. As there were five levels in the building, one level was sampled each day of the working week and the choice of levels to be sampled across the week was randomised. The time of air sample collection each day was fixed at 10.30 am. In the targeted study, five specific locations were targeted for sampling i.e. air-conditioning vents, laboratories, lecture theatres, a cafeteria and the toilets. These sites were selected because they were postulated to harbour specific niches of bacteria (e.g. Gram negative rods in the toilets) that were related to the activities carried out at the sites; bacteria which may have been missed altogether in the broad-based study or may have been present at low levels would then be detected in the targeted study. Once air-borne, these activity-related niche bacteria may also pose a risk to the occupants of the building.

Collection of air samples

Air sampling was undertaken using two air samplers. The first air sampler was the Reuter Centrifugal (RCS) High Flow Microbial Airborne Sampler. It employs centrifugal force for the impaction of airborne microbes onto an agar surface. This gentle and efficient collection mechanism ensures that even damaged microbes can be detected. It uses culture media in the form of packaged strips for sampling of air. The second air sampler utilised was the Biomerieux™ Air IDEAL Sampler, which is a handheld air sampler designed for portable air sampling. The machine has a compartment which fits in a culture plate. Under the compartment is a vacuum, which sucks in air through the cover of the compartment. This air suction allows particles to be impacted onto the culture plate.

Three combinations of media and incubationary conditions were utilised for this study. The first used was Trypticase Soy Agar (TSA) with ambient air incubation (TSA-ambient). Reports in the literature suggest that this medium with ambient air incubation yields the best recoveries of aerosolised cultures (Shahamat *et al.*, 1997). Second, TSA supplemented with 5 µg/ml of haemin and NADH with CO_2 enhanced incubation (TSA-plus-CO_2). Various sources of literature indicate that certain fastidious pathogens which can be transmitted by air e.g. *Haemophilus influenzae* and *Neisseria meningitidis* can be cultured on this medium with carbon dioxide enhanced incubation (Bergeron *et al.*, 1987). This has been confirmed in our laboratory tests using *Haemophilus influenzae* ATCC 9006 and ATCC 9007 and *Neisseria meningitidis* ATCC 35561 and ATCC 13113. Third, TSA without growth supplements but with CO_2 enhanced incubation (TSA-CO_2). Our laboratory tests have shown that the latter conditions can be used to culture the respiratory pathogen, Streptococcus pneumoniae (ATCC 6301), which could not be cultured on TSA with growth supplements and CO_2 enhancement. All cultures were incubated at 37°C.

A total of 240 air samples were collected with both air samplers throughout the entire study. After sampling, the test strips and plates were sealed and transported back to the laboratory to be incubated at 37°C with and without CO_2 enhancement and observed for colonies three days later.

During the sampling of air, both air samplers were held at breathing level (1.5 m from the ground) such that it could be predicted to generally sample the air or aerosols that people breathed in and out. Air was sampled directly beneath the ceiling air – conditioning vents. A facemask was worn throughout sampling. Great care and caution had been taken to ensure that the air samplers were thoroughly disinfected between sampling events so that there was no carry over of contaminants from one sampling event to the next.

Sample analysis

Colony forming units (CFU) on each plate were counted three days after incubation, and concentrations of bacteria were expressed as CFU per cubic meter of air (CFU/m^3) for

comparative analysis. A representative number of bacterial isolates from each sampling event were selected randomly from the culture media for Gram-staining and bacterial identification. The bacterial isolates were classified as Gram-positive cocci, Gram-positive rods, Gram-negative cocci and Gram-negative rods, according to their microscopic Gram morphology.

Once classified by Gram response, a representative number of isolates were again randomly selected for identification at the genus or species level. BBL Crystal Identification systems, which consist of three separate kits, were used to identify the bacteria. The BBL Crystal Gram-positive Identification (ID) Kit was used for the identification of aerobic Gram-positive bacteria. The BBL Crystal Enteric/Non-fermenter Identification (ID) Kit was used for the identification of aerobic Gram-negative bacteria belonging to the family Enterobacteriaceae in addition to some of the more commonly isolated glucose fermenting and nonfermenting Gram-negative bacilli (Micklewright & Sartory, 1995). The BBL Crystal *Neisseria / Haemophilus* Identification (ID) Kit was used for identification of the genera *Neisseria* and *Haemophilus* as well as several other fastidious bacteria. The BBL Crystal ID Systems are miniaturized biochemical identification systems that provide a metabolic profile of test bacteria. The three systems cumulatively have the capacity to identify more than 300 species of bacteria.

For Gram identity, a total of 282 isolates were analysed in the broad-based study and a total of 300 isolates were analysed in the targeted study. For genus / species identification, a total of 150 isolates each were analyzed in the broad-based study and targeted study, respectively.

Statistical analysis

All experimental data was analyzed using Excel 2003 and SPSS Version 16.0 (SPSS. Inc., Standard Version). One way analysis of variance (ANOVA) using SPSS Version 16.0 was used to compare the bacterial concentrations at different sampling sites. p values of <0.05 were considered significant.

RESULTS

Culturable Bacterial Concentrations

Culturable airborne bacteria were investigated on three combinations of media and incubationary conditions i.e. trypticase soy agar with ambient air incubation (TSA-ambient), trypticase soy agar supplemented with hemin and NADH with CO_2 enhanced incubation (TSA-plus-CO_2) and trypticase soy agar without supplements but with CO_2 enhanced incubation (TSA-CO_2). The distribution of culturable airborne bacteria on these three combinations of media and incubatory conditions are expressed as mean colony-forming units (CFU) per m^3 of air.

On TSA-ambient-plates in the broad-based study, bacterial concentrations ranged from 313 – 475 CFU per m^3 of air (Figure 1). Level 3 had the highest bacterial concentration of 475 CFU per m^3 of air, followed closely by Level 2, which had a bacterial concentration of 467 CFU per m^3 of air. Level 4, Ground level and Level 1 were ranked third, fourth and fifth, having bacterial concentrations of 407 CFU per m^3 of air, 360 CFU per m^3 of air and 313 CFU per m^3 of air respectively. The differences in mean bacterial concentrations (in CFU per m^3 of air), on TSA with ambient air incubation, between the different levels, were statistically significant ($p < 0.05$).

On TSA-plus-CO_2,plates in the broad-based study, bacterial concentrations ranged from 228 – 357 CFU per m^3 of air. Level 4 had the highest bacterial concentration of 357 CFU per m^3 of air, followed closely by Level 2 with a bacterial concentration of 352 CFU per m^3 of air. Level 3, Ground level and Level 1 were ranked third, fourth and fifth, having bacterial concentrations of 335 CFU per m^3 of air, 293 CFU per m^3 of air and 228 CFU per m^3 of air respectively. The differences in mean bacterial concentrations between the levels, were not statistically significant ($p = 0.475$).

On TSA-CO_2 plates in the broad-based study, bacterial concentrations ranged from 195 – 416 CFU per m^3 of air. Level 2 had the highest bacterial concentration of 416 CFU per m^3 of air, followed by Level 3 with a bacterial concentration of 353 CFU per m^3 of

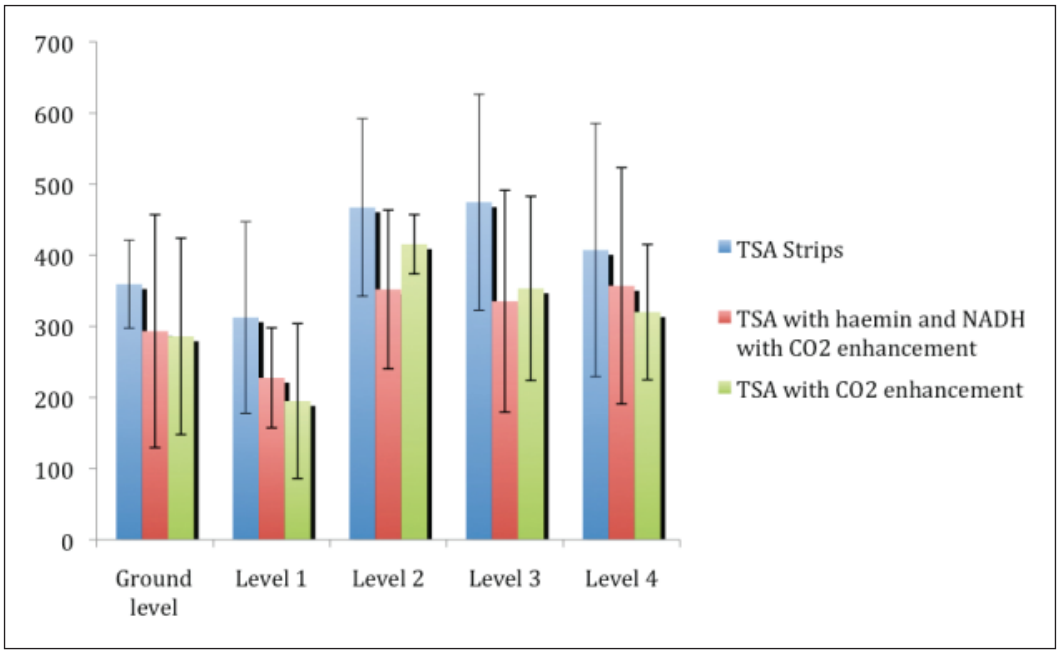

Figure 1. Comparison of bacterial concentrations (in CFU/m^3 of air), by level, in the broad-based study

air. Level 4, Ground level and Level 1 were ranked third, fourth and fifth, having bacterial concentrations of 320 CFU per m^3 of air, 286 CFU per m^3 of air and 195 CFU per m^3 of air respectively. The differences in mean bacterial concentrations between the levels, were not statistically significant ($p = 0.116$).

Results of the targeted study of air-conditioning vents, laboratories, lecture theatres, cafeteria and toilets are depicted in Figure 2. On TSA-ambient plates, mean bacterial concentrations ranged between 98 – 481 CFU/m^3 of air. The lecture theatre had the highest bacterial concentration of 481 CFU per m^3 of air, followed by the toilets, which had a bacterial concentration of 425 CFU per m^3 of air. The cafeteria, air-conditioning vents and laboratories were ranked third, fourth and fifth, having bacterial concentrations of 283 CFU per m^3 of air, 103 CFU per m^3 of air and 98 CFU per m^3 of air respectively. The differences in the mean bacterial concentrations (in CFU per m^3) between the five different sites were statistically significant ($p < 0.001$).

On TSA-plus-CO_2 plates, the mean bacterial concentrations ranged between 89 – 711 CFU per m^3 of air. The toilets had the highest mean bacterial concentration of 711 CFU per m^3 of air, followed by the lecture theatres with a mean bacterial concentration of 381. CFU per m^3 of air. The cafeteria, laboratories and air-conditioning vents were ranked third, fourth and fifth, having bacterial concentrations of 353 CFU per m^3 of air , 112 CFU per m^3 of air and 89 CFU per m^3 of air respectively. The differences in mean bacterial concentrations (in CFU per $1m^3$) between the five different sites were statistically significant ($p < 0.05$).

On TSA-CO_2 plates, mean bacterial concentrations ranged between 75. – 737 CFU per m^3 of air. The toilets had the highest bacterial concentration of 737 CFU per m^3 of air, followed by the lecture theatres with a bacterial concentration of 448 CFU per m^3 of air. The cafeteria, laboratories and air-conditioning vents were ranked third, fourth and fifth, having bacterial concentrations of 291 CFU per m^3 of air, 129 CFU per m^3 of air and 74 CFU per m^3 of air respectively. The variations in mean bacterial concentrations between the five different sites were not statistically significant ($p = 0.06$).

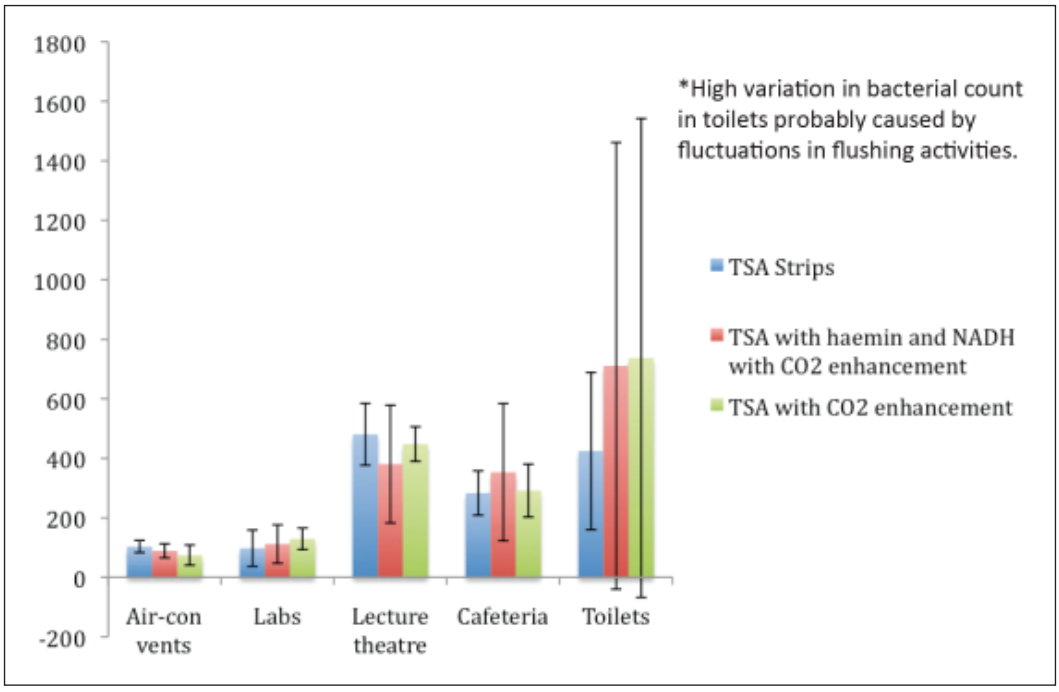

Figure 2. Comparison of bacterial concentrations (in CFU/m^3 of air) in the targeted study (Air-con : Air-conditioning vents ; Labs : Laboratories ; LT : Lecture Theatres)

Table 1. The distribution and percentage (%) of Gram positive cocci, Gram positive bacilli, Gram negative cocci and Gram negative rods in the broad-based study

Bacteria	Ground Level (CFU/m^3)	Level 1 (CFU/m^3)	Level 2 (CFU/m^3)	Level 3 (CFU/m^3)	Level 4 (CFU/m^3)	Percentage (%)
Gram-positive Cocci	35	42	43	42	29	67.73
Gram-positive Bacilli	16	10	13	11	19	24.46
Gram-negative Cocci	0	0	0	1	1	0.71
Gram-negative Bacilli	4	3	1	4	8	7.10
Total	55	55	57	58	57	100.00

Gram identity

For the broad-based study, a total of 282 isolates, randomly selected from all the levels of the building, were Gram-stained. Gram-positive cocci were present at the highest level i.e. at 67.73% of the total isolates. Gram-positive rods were ranked second at 24.26%, followed by Gram-negative rods at 7.10%. Gram-negative cocci were ranked fourth at 0.71%. This trend was manifested across all five levels in the building, albeit with slightly different percentages (Table 1).

For the Targeted Study, a total of 60 isolates, randomly selected from all study sites, were Gram-stained. Gram-positive cocci were present at the highest levels in the laboratories at 77.97% of the total isolates, and lowest levels in the cafeteria at 42.59% of the total isolates (Figure 3). Gram-positive rods were present at the highest levels in the lecture theatres at 31.03% of the total isolates, and lowest levels in the cafeteria at 14.82% of the total isolates. Gram-negative cocci were absent in the laboratories, lecture theatres and toilets. They were present at the relatively low percentages of 1.72% and 1.85% in the air-conditioning vents and cafeteria respectively.

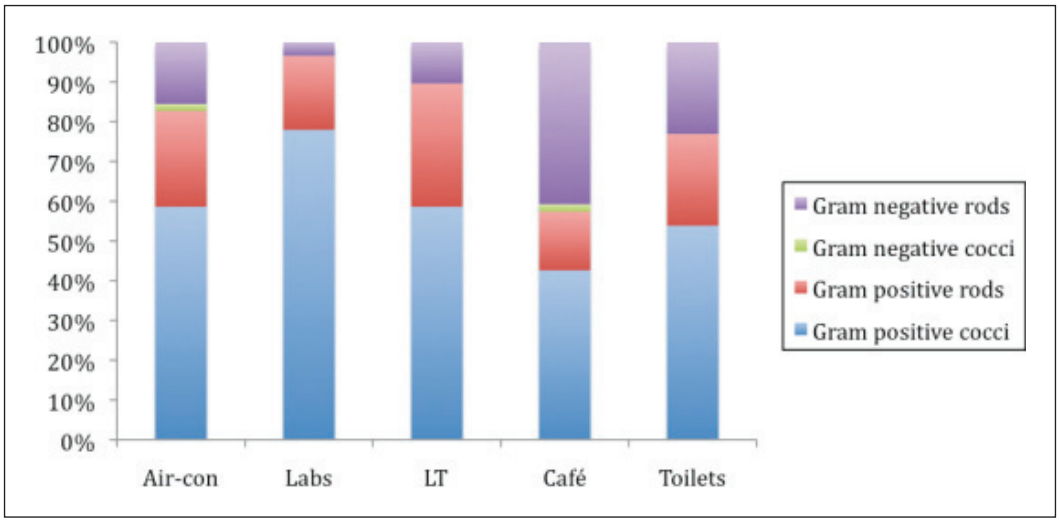

Figure 3. Comparison of bacterial isolates obtained, by Gram properties, in the targeted study

Gram-negative rods were most abundant in the cafeteria at 40.74%, and they were least abundant in the laboratories at 3.39%. They were present at the fairly high percentage of 23.08% in the toilets.

Bacterial genera and species identified

In the Broad-Based Study, 21 genera, comprising 43 culturable species of bacteria, were identified from the sampling sites (Table 2 : Additional file 1). The prevailing isolates at genus level were *Kytococcus* (Gram positive coccus, 16.67% of isolates), *Staphylococcus* (Gram positive coccus, 13.33% of isolates), *Micrococcus* (Gram positive coccus, 12.67% of isolates) and *Leifsonia* (Gram positive pleomorphic rod, 7.33% of isolates). These genera could be found in the indoor air at all levels of the building. Together, they accounted for 50% of all culturable bacteria, of which the most dominant species was *Kytococcus sedentarius*. Other genera which were present at relatively significant percentages were *Corynebacterium* (Gram positive pleomorphic rod) and *Bacillus* (Gram positive rod). They were isolated at 6.67% and 6% respectively, and they could be found in four out of five levels of the building. *Moraxella* (Gram negative diplococcus) was isolated at 2.67%, and *Aerococcus* (Gram positive coccus) and *Gemella* (Gram positive coccus) were both isolated at 3.33%. These latter three genera were present in three out of five levels of the building. *Leuconostoc* (Gram positive coccus), *Brevibacillus* (Gram positive rod), *Kocuria* (Gram positive coccus), *Pseudomonas* (Gram negative rod) and *Streptococcus* (Gram positive coccus) were present at lower percentages i.e. < 2%, and they could only be found in two of five levels of the building. Finally, *Enterococcus* (Gram positive coccus), *Globicatella* (Gram positive coccus), *Lactococcus* (Gram positive coccus), *Myroides* (Gram negative rod), *Pantoea* (Gram negative rod), *Pediococcus* (Gram positive coccus) and *Stenotrophomonas* (Gram negative rod) were the least prevalent isolates, each present at a level of 0.67% of total isolates in the indoor air of the building .

In the Targeted Study, 22 genera, including 46 culturable species of bacteria, were identified from the sampling sites (Table 3 : Additional file 2). The predominant genera and species were Staphylococcus sp. and Kytococcus sedentarius, which could be found in all sampling sites. They accounted for approximately 40% of the isolates randomly selected for identification. The dominant isolates that prevailed, however, varied from site to site. In the air-conditioning vents, the dominant genera were *Staphylococcus* (23.33%), *Micrococcus*

Table 2. Composition of airborne bacteria in the broad-based study (the highest counts are given in bold)

Groups	Ground Level (%)	L1 (%)	L2 (%)	L3 (%)	L4 (%)
Gram positive					
Aerococcus urinae	6.67	3.33	6.67	–	–
Bacillus	**13.33**	6.66	6.67	–	3.33
Bacillus cereus	–	–	3.33	–	–
Bacillus circulans	3.33	–	–	–	–
Bacillus licheniformis	–	3.33	–	–	–
Bacillus megaterium	–	–	–	–	3.33
Bacillus sphaericus	10.00	–	3.33	–	–
Bacillus subtilis	–	3.33	–	–	–
Brevibacillus brevis	–	–	–	3.33	3.33
Corynebacterium	10.00	–	3.33	10.00	10.00
Corynebacterium bovis	–	–	–	3.33	–
Corynebacterium genitalium	–	–	–	–	3.33
Corynebacterium jeikeium	3.33	–	3.33	3.33	3.33
Corynebacterium renale	3.33	–	–	3.33	3.33
Corynebacterium sp	3.33	–	–	–	–
Enterococcus solitarius	–	–	–	3.33	–
Gemella morbillorum	–	3.33	3.33	–	10.00
Globicatella sanguinis	–	–	–	3.33	–
Kocuria kristinae	3.33	–	–	–	–
Kocuria rosea	–	–	–	3.33	–
Kytococcus sedentarius	**13.33**	13.33	**23.33**	13.33	**20.00**
Lactococcus lactis sp	–	–	–	3.33	–
Leuconostoc citreum	–	–	3.33	–	–
Leuconostoc lactis	–	–	–	–	3.33
Leuconostoc pseudomesenteroides	–	–	3.33	–	–
Leifsonia aquaticum	3.33	10.00	10.0	10.00	3.33
Micrococcus luteus	3.33	**23.33**	10.0	3.33	13.33
Micrococcus lylae	–	3.33	–	3.33	–
Micrococcus sp	3.33	–	3.33	–	–
Myroides odoratus	–	3.33	–	–	–
Pantoea agglomerans	–	–	3.33	–	–
Pediococcus sp	3.33–	–	–	–	–
Staphylococcus	**13.33**	**20.00**	10.00	**16.67**	6.67
Staphylococcus epidermidis	3.33	6.67	3.33	6.67	3.33
Staphylococcus haemolyticus	–	–	6.67	–	3.33
Staphylococcus hominis	6.67	6.66	–	–	–
Staphylococcus saccharolyticus	–	3.33	–	3.33	–
Staphylococcus saprophyticus	–	–	–	6.67	–
Staphylococcus sciuri	3.33	–	–	–	–
Staphylococcus simulans	3.33	3.33	–	–	–
Streptococcus gordonii	–	3.33	–	–	–
Streptococcus sanguinis	3.33	–	–	–	–
Gram negative					
Moraxella	3.33	3.33	–	6.67	–
Moraxella osleonsis	3.33	3.33	–	–	–
Moraxella phenylpyruvica	–	–	–	6.67	–
Pseudomonas stutzeri	3.33	–	–	–	3.33
Stenotrophomonas maltophilia	3.33	–	–	–	–
Unidentified species	13.33	10.00	13.33	20.00	23.33

Table 3. Composition of airborne bacteria in the targeted study (Air-con: Air-conditioning vents; Labs: Laboratories; LT: Lecture Theatres; the highest counts are given in bold)

Groups	Air-con vents (%)	Labs (%)	LT (%)	Café (%)	Toilets (%)
Gram positive					
Aerococcus urinae	–	3.33	–	–	–
Bacillus	6.66	3.33	10.00	–	–
Bacillus cereus	3.33	–	–	–	–
Bacillus licheniformis	3.33	–	–	–	–
Bacillus megaterium	–	–	6.67	–	–
Bacillus sphaericus	–	3.33	3.33	–	–
Brevibacillus brevis	–	–	13.33	3.33	–
Brevundimonas diminuta	3.33	–	–	–	–
Corynebacterium	10.00	10.00	–	6.67	–
Corynebacterium genitalium	–	3.33	–	–	–
Corynebacterium jeikeium	6.67	–	–	–	–
Corynebacterium propinquum	3.33	3.33	–	–	–
Corynebacterium pseudodiphtheriticum	–	–	–	3.33	–
Corynebacterium pseudotuberculosis	–	3.33	–	–	–
Corynebacterium striatum	–	–	–	3.33	–
Gardnerella vaginalis	–	–	3.33	–	6.67
Gemella morbillorum	3.33	3.33	3.33	–	–
Helcococcus kunzii	3.33	3.33	–	–	–
Kytococcus sedentarius	13.33	**30.00**	**16.67**	16.67	16.67
Leuconostoc lactis	–	–	3.33	–	–
Leifsonia aquaticum	–	3.33	–	3.33	6.67
Micrococcus luteus	16.67	–	6.67	3.33	3.33
Micrococcus sp	–	3.33	–	–	–
Staphylococcus	**23.33**	**30.00**	10.00	10.00	**26.67**
Staphylococcus aureus	–	3.33	–	–	–
Staphylococcus capitis	3.33	6.67	–	3.33	10.00
Staphylococcus cohnii spp cohnii	–	3.33	–	–	–
Staphylococcus epidermidis	3.33	3.33	6.67	–	3.33
Staphylococcus haemolyticus	10.00	–	–	3.33	6.67
Staphylococcus hominis	6.67	3.33	–	–	3.33
Staphylococcus pasteuri	–	–	3.33	–	–
Staphylococcus saprophyticus	–	3.33	–	–	3.33
Staphylococcus schleiferi	–	6.67	–	3.33	–
Streptococcus sanguinis	–	–	–	–	10.00
Turicella otitidis	–	–	3.33	–	–
Gram negative					
Acinebacter iwoffii	–	–	–	–	3.33
Chryseobacterium indologenes	3.33	–	–	–	–
Chryseobacterium meningoseptum	–	–	–	–	3.33
Klebsiella pneumoniae	–	–	–	3.33	–
Moraxella	–	–	–	16.67	6.67
Moraxella atlantae	–	–	–	6.67	3.33
Moraxella osleonsis	–	–	–	6.67	–
Moraxella phenylpyruvica	–	–	–	–	3.33
Moraxella sp	–	–	–	3.33	–
Pseudomonas	–	–	3.33	13.33	10.00
Pseudomonas aeruginosa	–	–	3.33	–	6.67
Pseudomonas fluorescens	–	–	–	3.33	–
Pseudomonas putida	–	–	–	–	3.33
Pseudomonas stutzeri	–	–	–	10.00	–
Shewanella putrafaciens	–	–	–	3.33	3.33
Stenotrophomonas maltophilia	–	–	–	**20.00**	3.33
No identification	13.33	10.00	26.67	0.00	0.00

(16.67%), *Kytococcus* (13.33%) and *Corynebacterium* (10.00%). In the laboratories, the dominant genera were *Kytococcus* (30.00%) and *Staphylococcus* (30.00%). The abundance and variety of staphylococcal species isolated in the laboratories demonstrated the richest diversity of species, as compared to other sites. The dominant genera in the lecture theatres were *Kytococcus* (16.67%), *Brevibacillus* (13.33%), *Staphylococcus* (10.00%), *Bacillus* (10.00%) and *Micrococcus* (6.67%). In the cafeteria, the dominant genera were *Stenotrophomonas* (20.00%), *Kytococcus* (16.67%), *Moraxella* (16.67%), *Pseudomonas* (13.33%) and *Staphylococcus* (10.00%). Finally, the dominant genera in the toilets were *Staphylococcus* (26.67%), *Kytococcus* (16.67%), *Streptococcus* (10.00%), *Pseudomonas* (10.00%), *Leifsonia* (6.67%), *Gardnerella* (6.67%) and *Moraxella* (6.67%). Other bacteria identified in the Targeted Study are listed in Table 3 (Additional file 2).

DISCUSSION

The levels of airborne bacteria measured in the indoor environment of the tertiary educational institution were in the order of 10^2 colony-forming units (CFU) per m^3 of air. This finding is similar to published analyses of the bacteriological quality of indoor air in Turkey, Canada, United States and Singapore (Goh *et al*., 2000; Zhu *et al*., 2003; Bartlett *et al*., 2004; Aydogdu *et al*., 2005, Aydogdu *et al*., 2009).

In the Broad-Based Study, Level 3 was found to have the highest bacterial concentration (475 CFU/m^3 of air). Level 3 is the busiest place in the building, and is also associated with the highest density of people movement. Therefore, people movement and activity may account for the high bacterial concentrations seen at this level. Level 2 was ranked second in terms of bacterial concentrations (467 CFU/m^3 of air). Although the density of people movement in Level 2 was not as high as in Level 3, an interesting and likely contributory factor to the high bacterial count is the fact that the entire level is carpeted. Floor covering material is an important potential source of airborne microorganisms (Spendlove & Fannin, 1983). Findings of a study indicate that microbial concentrations on carpeted floors were up to 100 million organisms per square meter, which is about 4 orders of magnitude higher than that observed on uncarpeted floors (Anderson, 1969). An investigation relating carpeting with sick building syndrome in primary schools in Sweden found an increased prevalence of eye and airway symptoms, face rashes, headache and abnormal tiredness among the students, and these symptoms were not limited to sensitive atopic individuals only. After the removal of carpets, many of the reported symptoms decreased to the level similar to the group without the carpets (Norback & TorgÈn, 1989). Although carpeting offers advantages compared with other flooring materials, both technically and with regard to comfort, increased risk of bacterial contamination is a major hygienic disadvantage, coupled with the difficulty in removing the bacteria by ordinary cleaning. Moreover, one should also consider other hazards associated with carpeting such as the risk of accumulating fungal spores, dust mites and the risk for allergic reactions.

In the targeted study, the lecture theatres (481 CFU/m^3 of air) followed by the toilets (425 CFU/m^3 of air), showed highest bacterial concentrations. There are several likely reasons for this. The lecture theatres were a hive of student and teacher activities with constant generation of easily dispersed aerosols from people talking, coughing and sneezing. There is also likely to have been continuous shedding of cutaneous bacteria. Due to the barriers posed by the four walls of the lecture theatres, airborne bacteria remained within these sites and accumulated, thereby contributing to the high bacterial concentrations. In considering the toilets, a study has indicated that large numbers of bacteria remain in the toilet bowls after flushing. Even after continuous flushing, a persistent fraction of bacteria could not be removed (Gerba *et al*., 1975). The bacteria

apparently adsorbed onto the porcelain surfaces of the bowl. During flushing, bacteria became airborne and eventually settled on most surfaces in the toilets (Gerba *et al.*, 1975).

Although a direct correlation between health effects and bacterial concentrations cannot always be detected or proven, these findings in the lecture theatre and toilets do have certain implications with regard to indoor air quality control. In certain interiors with a high risk level, such as operating rooms, burn units and intensive care units (ICU), a bacterial concentration as low as possible must be striven for. Hence, there should be a limit to the number of people that can be present in these places as they are a major factor for high bacterial counts, especially in the ICU where there are often numerous doctors, nurses and other personnel. Toilets should be located a significant distance away from these wards as they could be a potential source of infection.

Air-conditioning vents had a relatively low bacterial concentration of 103 CFU per m^3 of air. Currently, there are no microbiological standards or guidelines available for air-conditioning vents. However, from the low count obtained in this study, we may infer that air-conditioning systems may generally play only a small contributory role to bacterial concentrations in the indoor environment. Contrary to reports of dissemination of hazardous bacteria via Heating, Ventilation and Air-Conditioning (HVAC) systems (Hugenholtz & Fuerst, 1992), the vents in the institution under study showed a relatively low count of bacteria. Hence, it is not a cause of concern for indoor air quality.

On a good note, the airborne bacterial concentrations in all the levels and all the sites of the tertiary educational institution were lower than standards set in the guidelines for indoor air quality formulated by the Institute of Environmental Epidemiology, Singapore, as well as the limit of the Hong Kong Indoor Air Quality Objective (HKIAQO) Level 1 standard (Singapore Institute of Environmental Epidemiology; 1996; Obbard & Fang, 2003, Chan *et al.*, 2009). Both standards specify an upper threshold not exceeding 500 CFU per m^3 of air, for an air-conditioned indoor environment to be considered 'clean'.

In terms of Gram identity, the most common microorganisms in the broad-based study were Gram-positive cocci which accounted for 67.73% of the total isolates. Gram-positive rods and Gram-negative rods were less numerous, forming 24.46% and 7.10% respectively of the total isolates. Gram-negative cocci occurred only in small proportions, i.e. 0.71% of the total isolates. The following suggestions may explain this trend. Firstly, Gram-positive bacteria - particularly the cocci, are ubiquitous micro-organisms found both in the environment and as commensalistic colonisers of the skin, mucous membranes, and other body sites in humans and animals (Koneman *et al.*, 1997). Secondly, the thick-cell-walled and spore-forming Gram-positive bacteria have better resistance and survival capabilities than Gram-negative bacteria under deleterious conditions such as desiccation, intense solar radiation (Tong & Lighthart, 1997) and exposure to aerosolised chemical pollutants (Mancinelli & Shulls, 1978). It is believed that protective mechanisms inherent in commonly airborne bacteria against ultraviolet radiation are the evolutionary result of natural selection in the atmosphere (Fang *et al.*, 2006). Moreover, the culturability of Gram-negative microflora may have been compromised by fluctuations in indoor relative humidity within the building. It has been demonstrated that relative humidity plays a significant role in the survival of Gram-negative bacteria (McDade & Hall, 1964; de Goffau *et al.*, 2009; Tang, 2009).

In the targeted study, the cafeteria had the highest percentage of Gram-negative rods as compared to all the other sites and all the levels in the building. Gram-negative rods tend to predominate in the spoilage microflora of fresh meat, fish, poultry, eggs and other high-pH/high water-activity, protein-rich foods and vegetables (Lund *et al.*, 2000). These bacteria can grow in

the presence of oxygen and in cold and ambient temperatures. They include many psychrotrophs, such as some *Pseudomonas* species, that are able to multiply below 0ºC (Lund *et al*., 2000). Toilets were also associated with a significant number of Gram-negative rods (23.08%). It can be postulated that these Gram-negative rods originated from human waste.

As a whole, *Kytococcus*, *Micrococcus*, *Staphylococcus* and *Leifsonia* predominated amongst the airborne bacteria isolated from the building. Amongst the isolated micrococci, the dominant species was *Micrococcus luteus*. There were also an abundance of staphylococcal species. These bacteria contain carotene pigments which enable them to survive exposure to solar radiation better than other bacteria (Mancinelli & Shulls, 1978; Tong & Lighthart, 1997). The staphylococci generally colonise the skin and mucous membranes of humans and other animals. In contrast, members of the genus *Micrococcus* are present in the environment and sometimes as transient flora inhabiting the skin of humans and several other mammals (Aydogdu *et al*., 2009).

The majority (>97%) of the staphylococci isolated in this study were coagulase-negative staphylococci, such as *Staphylococcus epidermidis*, *Staphylococcus haemolyticus* and *Staphylococcus saprophyticus*. Coagulase-negative staphylococci are unlikely to cause infections, except as opportunists in immuno-compromised patients. These organisms are mostly associated with endocarditis and bacteremia (Mahon *et al*., 2007). *Micrococcus luteus* is usually considered a saprophytic germ and is rarely the cause of infectious disease (Magee *et al*., 1990). It is not a recognized opportunistic pathogen of immuno-compromised patients (Adang *et al*., 1992).

Kytococcus sedentarius (formerly known as *Micrococcus sedentarius*) was the most dominant species identified in indoor air in this study. Currently, three species of kytococci have been reported i.e. *Kytococcus sedentarius* (Stackebrandt *et al*., 1995), *Kytococcus schroeteri* (Becker *et al*., 2003) and *Kytococcus aerolatus* [Kampfer *et al*., 2009]. Szczerba & Krzeminski [Szczerba & Krzeminski, 2002] report *K. sedentarius* as a contaminant of human skin and mucosa. *K. sedentarius* has been reported to produce the broad spectrum oligoketide antibiotics monensins A and B [Pospisil *et al*., 1998]. Of interest, the production and antibacterial properties of the monensins may have contributed to the predominance of *K.sedentarius* in indoor air. Recent reports suggest species of kytococci as emerging opportunistic pathogens of the immuno-compromised, paediatrics and the elderly (Greene *et al*., 1980; Levenga *et al*., 2004; Lebrun *et al*., 2005; Mohammedi *et al*., 2005; Mniff *et al*., 2006; Aepinus *et al*., 2008; Renvoise *et al*., 2008; Jourdain *et al*., 2009; Hodiamont *et al*., 2010). A 55 year old neutropenic man suffering from acute myeloid leukaemia succumbed fatally to *K.sedentarius* associated haemorrhagic pneumonia (Levenga *et al*., 2004).

A high proportion of *S. maltophilia* were also found in the cafeteria. *Stenotrophomonas maltophilia* is found in a great variety of environments (Hugh & Ryschenkow, 1961). It often contaminates food sources such as frozen fish, milk, poultry eggs and lamb carcasses, which may explain the high percentage of *Stenotrophomonas* found in the cafeteria (Zhong, 1990; Denton & Kerr, 1998). Formerly, *S. maltophilia* was assumed to have limited pathogenic potential, rarely with the propensity to cause human disease, with the exception of those who are immuno-compromised or severely debilitated (Hugh & Ryschenkow, 1961; Muder *et al*., 1987). A retrospective study to determine the epidermiology of *S. maltophilia* infections in intensive-care units (ICU) of community general hospitals found that patients who are infected (commonly with pneumonia) are elderly, have a high Acute Physiology and Chronic Health Evaluation II (APACHE II) score, have been intubated for a mean of 12 days, have often had a tracheostomy or underlying respiratory disease, and have prior antibiotic exposure (Gopalakrishnan *et al*., 1999). However, more recent studies have shown that *S. maltophilia* can play the role

of a true pathogen, and community-acquired infections with this bacterium may occur more commonly than was previously assumed (McDonald & Pernenkil, 1993; Heath & Currie, 1995; Kim J-H *et al.*, 2002). As an example, in their studies of 63 patients, Laing *et al.* reported that 23.8% of cases of infection and / or colonisation with *S. maltophilia* were of a community-acquired nature (Laing *et al.*, 1995). In Korea, two endocarditis cases due to *S.maltophilia* were reported to have occurred subsequent to implantation of mitral valve prosthesis (Kim *et al.*, 2002). Other diseases that are associated with this bacterium are bacteraemia, ophthalmologic infections, urinary tract infections, and skin and soft tissue infections (Muder *et al.*, 1987; Denton & Kerr, 1998). Of a more worrying note is that *S. maltophilia* is becoming increasingly resistant to most antibiotics, such as the β-lactam class, anti-pseudomonal penicillin and quinolones (Wu *et al.*, 2006; Roscetto *et al.*, 2008). It is totally resistant to the action of imipenam, meropenam and all aminoglycoside antibiotics (Roscetto *et al.*, 2008). The predilection of *S. maltophilia* for the cafeteria and food raises the issue of whether cafeterias should be designed close to high risks environments such as Intensive Care Units (ICU). As it can behave as an opportunistic or true pathogen, its close proximity to high risk units can pose a health hazard to immuno-compromised patients.

The present study is a survey of airborne bacteria in the indoor environment of a high throughput building in Kuala Lumpur, Malaysia. Generally, the airborne bacterial counts in the indoor environment were lower than the upper limit of Singapore and Hong Kong guidelines for indoor air quality (i.e. 500 colony forming units/m^3 of air in an air-conditioned environment). The major factors which apparently influenced the bacterial count were occupancy, human activities, the presence of food and the type of floor furnishing. These factors are points to consider in building design with the aim of keeping airborne bacterial loads within acceptable limits.

In the broad-based study, airborne Gram-positive bacteria were the most abundant in the indoor environment, accounting for more than 90% of the measured populations. Gram-negative bacteria were present at less than 10% of the total isolates identified. In the targeted study, Gram-positive bacteria were present at the highest levels in the laboratories, lecture theatres and air-conditioning vents and Gram-negative bacteria were present at the highest levels in the cafeteria and toilets.

A total of 21 bacterial genera were identified in the Broad-Based Study and 22 bacterial genera were identified in the Targeted Study. We conclude that the major genera of airborne bacteria in the indoor environment were Kytococcus, Staphylococcus, Micrococcus, Leifsonia, Bacillus and Corynebacterium. The dominant bacterial species were Kytococcus sedentarius, Staphylococcus epidermidis and Micrococcus luteus. *Stenotrophomonas maltophilia* was also present in high proportion in the cafeteria. Many of these bacteria have been demonstrated to cause opportunistic diseases in immuno-compromised patients, at times with fatal outcomes. Furthermore, many are becoming increasingly resistant to antibiotics. Hence, we propose that indoor reservoirs of these bacteria and their associated clinical and more subtle health effects, if any, be investigated further.

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A list of abbreviations

NADH	Nicotinamide Adenine Dinucleotide
HVAC	Heating, Ventilation and Air-Conditioning
IAQ	Indoor Air Quality
SBS	Sick Building Syndrome
TSA	Trypticase Soy Agar
USEPA	United States of Environmental Protection Agency
UV	Ultraviolet
WHO	World Health Organisation

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