

**Microbiological Assessment of Selected Laboratories at a
Local University in Malaysia**
(Penilaian Tahap Pencemaran Mikrobiologi di Makmal Terpilih
Sebuah Universiti Awam di Malaysia)

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Abstract

Microbiological contaminations in the laboratories create not only diagnostic issues but also pose a major health risk to lab users. This study was conducted to determine the airborne microbial contamination in seven selected laboratories (KA, KP, PB, NA, BP, CR and MB) at a local public university in Malaysia and to assess the level of contamination on the contact surfaces of the studied laboratories. Two types of sampling methods were used in this study; passive air sampling and contact surface swab sampling. The total microbial counts were determined using Tryptone Soya agar (bacterial count) and Potato Dextrose agar (fungal count). Results showed that NA laboratory had the highest level of total microbial contamination (20.33 ± 3.35 CFU/m³). Most laboratories had significantly higher ($p < 0.05$) air fungal contamination level compared to bacterial contaminations except for PB and NA laboratories. Significant differences were observed for airborne bacterial contamination readings between sampling time (during working hours vs after hours) for all laboratories except for BP and CR. Overall, bacterial contamination was the highest for incubator door handles' samples from MB laboratory with an average reading of 93.00 ± 1.43 CFU/cm² whereas the highest fungal contamination level was obtained from door knobs and work benches, both from CR laboratory with an average reading of 73.33 ± 6.67 CFU/cm² and 73.33 ± 0.58 CFU/cm² respectively. Findings from this study could assist in monitoring the efficacy of the existing laboratory management systems namely on the good laboratory practices including aseptic techniques, care for laboratory hygiene and cross-contamination control practices by the laboratory users to ensure that the microbiological contaminations in the laboratories are minimized.

Keywords: Microbiological contamination; indoor air quality; laboratory; contact surfaces; passive air sampling

Abstrak

Kontaminasi mikrobiologi di dalam makmal bukan sahaja mewujudkan isu atau permasalahan dalam menjalankan ujian diagnostik tetapi juga menimbulkan risiko kesihatan utama kepada pengguna makmal. Kajian ini dijalankan untuk menentukan tahap kontaminasi mikrobiologi di tujuh makmal terpilih (KA, KP, PB, NA, BP, CR dan MB) di sebuah universiti awam di Malaysia. Dua jenis kaedah persampelan digunakan dalam kajian ini, iaitu kaedah pendedahan secara pasif dan kaedah calitan permukaan sentuhan yang terdapat di dalam makmal. Kehadiran dan jumlah mikroorganisma pada sampel ditentukan menggunakan kiraan plat jumlah melalui teknik plat sebaran di atas agar tripton soya (kiraan jumlah bakteria) dan agar dekstrosa kentang (kiraan jumlah fungus). Hasil kajian menunjukkan bahawa makmal NA mempunyai tahap pencemaran mikrobial jumlah yang paling tinggi (20.33 ± 3.35 CFU/m³). Kebanyakan makmal mempunyai tahap pencemaran fungus persekitaran udara dalaman yang lebih tinggi secara signifikan ($p < 0.05$) berbanding dengan pencemaran bakteria kecuali bagi makmal PB dan NA. Terdapat perbezaan yang signifikan pada tahap pencemaran mikrobial di antara waktu persampelan (semasa waktu operasi vs selepas waktu operasi) bagi semua makmal kecuali makmal BP dan CR. Secara umumnya, pencemaran bakteria yang paling tinggi diperolehi daripada sample pemegang pintu inkubator makmal MB dengan purata bacaan sebanyak 93.00 ± 1.43 CFU/cm² manakala tahap pencemaran fungus yang tertinggi dijumpai pada tombol pintu (73.33 ± 6.67 CFU/cm²) dan meja kerja (73.33 ± 0.58 CFU/cm²) daripada makmal CR. Dapatan daripada kajian ini diharapkan dapat membantu dalam permonitoran keberkesanan pengurusan makmal sedia ada terutama berkenaan dengan amalan makmal yang baik termasuk teknik aseptik, penjagaan higen makmal dan kawalan kontaminasi silang bagi memastikan pencemaran mikrobiologi di makmal dapat diminimumkan.

Kata kunci: Kontaminasi mikrobiologi, kualiti udara dalaman, makmal, permukaan sentuhan, persampelan udara pasif.

INTRODUCTION

Good indoor air quality (IAQ) in the laboratory is required for a healthy worker's environment. Poor indoor air quality in the laboratory can cause various short-term and long-term health problems. Health problems commonly associated with poor IAQ are including allergic reactions, respiratory problems, eye irritation, sinusitis, bronchitis and pneumonia. IAQ problems are normally due to presence of air pollutant or inadequate air ventilation. IAQ issues commonly occur in buildings with mechanical ventilation and air conditioning systems (MVACs) including split air conditioning systems especially when these systems are not well-maintained thus affecting its operations (Department of Occupational Safety & Health (DOSH) 2010).

Exposure to indoor air microbial contaminants is a serious public health issue as it has been estimated that people spend 90% of their time indoors including at home, school and workplaces (Cincinelli & Martellini 2017). These airborne contaminants include infectious materials such as bacteria, yeast, mould, fungi, virus, prions, protozoa or their toxins and their by-products (Wong et al. 2009). Indoor air contamination is one of the parameters studied to determine the quality control implemented in a laboratory in accordance with the Occupational Safety and Health Management System (Anuar 2014). Laboratory workers especially medical diagnostic or microbiological laboratory are highly at risk for airborne infections. Laboratory associated microbial infection is a well-documented occupational hazard for staff working in these laboratories (Sewell 2000).

Environmental contaminants within microbiology laboratories create not only diagnostic issues but also pose a major health risk for the workers (Konar & Das

2013). Good aseptic techniques and good laboratories practices are essential to prevent the spread of the microorganisms being handled to the laboratory environment and to avoid cross contaminations by the surrounding microorganisms to the works carried out by the workers. This includes the use of manipulation techniques that minimize the likelihood of aerosol production and to ensure that the occurrence of airborne microbial contamination in laboratories are minimized (Ghayoor et al. 2015).

Most epidemiological studies on laboratory associated infections only emphasizes on airborne contaminations. Besides the presence of harmful pathogens floating in the indoor air, these airborne

MATERIALS AND METHODS

Sample collection

Samples were collected from 7 laboratories in a local university. Descriptions of the works commonly carried out in the selected laboratories are as described in Table 1. Two methods of sampling; passive sampling method to determine the airborne microbiological contaminations, and swab samples of selected contact surfaces were conducted in the selected laboratories from April to November 2017

Passive sampling method

Passive sampling method with settle plates were conducted by exposing prepared Tryptone Soya agar conducted by exposing prepared Tryptone Soya agar (TSA; Oxoid, United Kingdom) and Potato Dextrose agar (PDA; Merck, Germany) for determination of total bacterial counts and total fungal counts respectively (Agbagwa & Onyemaechi 2014). Exposed plates in duplicates were stationed at the corners of each sampled laboratory and sampling activity was performed during working hours' period

TABLE 1. Laboratories and contact surfaces sampled in the study

Laboratory ID	Works commonly carried out in the laboratory	Contact surfaces sampled in the study
KA	Multipurpose laboratory	Door knob, work benches, incubator door handles & fridge handles
KP	Multipurpose laboratory	Door knob & work benches,
PB	Biological and microbiological analysis	Door knob, work benches, incubator door handles, fridge handles & biosafety cabinet/laminar flow hood.
NA	Microbiological and molecular analysis	Door knob, work benches, incubator door handles, fridge handles & biosafety cabinet/laminar flow hood.
BP	Biological and microbiological analysis	Door knob, work benches & incubator door handles.
CR	Cold storage area for biological samples	Door knob & work benches.
MB	Microbiological analysis	Door knob, work benches, incubator door handles, fridge handles & biosafety cabinet/laminar flow hood.

(between 7 am to 6 pm) and during after working hours' period (between 6 pm to 7am). Plates were then sealed and incubated for 24 hours at 37°C (TSA) and for 7 days at 25°C (PDA).

Contact surface swab sampling

Surface swab sampling was conducted to determine the level of microbiological contamination on selected contact surfaces in the sampled laboratories. Composite surface samples were collected from door knobs, fridge handles, incubator door handles, biosafety cabinets/laminar flow hood and work benches. Sampling was conducted based on the methods by Zulfakar et al. (2017) with slight modifications. A pre-moist sterile cotton swab with buffered peptone water (BPW) (Merck, Germany) was used to swab the test surfaces. Swabbing on the same test area was then repeated with a dry sterile swab. A 10 cmX 10 cm template was used for swabbing the work benches whereas the whole area of the surface was swabbed for other contact surface samples and the surface area of the items were recorded. After swabbing, each sample was placed in a sterile tube containing 10 ml BPW. Samples were then kept at 4°C and transported back to the laboratory for further analysis.

Microbiological analyses

Microbiological analyses were performed according to the methods reported by

Zulfakar et al. (2017) with slight modifications. All tubes containing swab samples were vortexed and serially diluted in BPW for determination of bacterial counts. One hundred microliters of appropriately diluted samples were plated in duplicates on TSA (Merck, Germany). Vortexed samples for total fungal counts were directly plated on PDA without serial dilutions. All plates were incubated for 24 hours at 37°C for TSA and for 7 days at 25°C for PDA. For negative controls, 100 µl of BPW were plated onto the media. Bacterial and fungal counts from passive sampling activity were enumerated using the Omeliansky formula (Awad & Mawla 2012) and expressed as mean CFU/m³, whereas results for contact surface swab sampling were enumerated using the standard colony count formula and expressed as mean CFU/cm². Bacterial colonies from all samples were further characterized based on Bergey's Manual of Determinative Bacteriology (Williams 2000) and then samples were subjected to Gram-staining procedures for bacterial isolates whereas for fungal isolates, lactophenol cotton blue staining procedures were conducted (Bier et al. 2001).

Statistical Analysis

Statistical analysis was conducted using IBM SPSS Statistic Version 23.0. Data were analyzed using one-way analysis of variance (ANOVA) with post-hoc

comparison using Tukey test to determine the difference in means for microbial contamination level between laboratories and to determine the differences in means of microbial contamination level between contact surfaces. Comparisons between bacterial and fungal contamination level were analyzed using T independent test whereas comparison between microbial contamination level sampled during office hours and after office hours for each laboratories and contact surfaces were analyzed using paired sample T-test. Results were considered significant when $p < 0.05$.

RESULTS

Overall results of total microbial contamination at the sampled laboratories (Figure 1) obtained from the passive air sampling activity showed that the microbial levels ranged from 10 – 21 CFU/m³ with NA laboratory had the highest level of contamination (20.33 ± 3.35 CFU/m³) as compared to KP, BP and CR laboratories ($p < 0.05$). However, this reading was not significantly different when compared to KA, PB and MB laboratories ($p > 0.05$). Figure 2 presents the comparison between bacterial and fungal contamination in the sampled laboratories. From this figure, it can be concluded that most laboratories had significantly higher ($p < 0.05$) air fungal contamination level compared to bacterial contaminations except for PB and NA laboratories. NA had a significantly higher ($p < 0.05$) airborne bacterial contamination at an average of 22.88 ± 2.23 CFU/m³ whereas there was no significant difference ($p > 0.05$) between bacterial and fungal contamination level for PB laboratory. It can also be clearly observed that KA laboratory had the highest air fungal contamination level (26.55 ± 5.98 CFU/m³) whereas CR laboratory had the lowest air bacterial contamination level (1.31 ± 0.85 CFU/m³) as compared to other laboratories.

The levels of airborne bacterial and fungal contamination based on sampling time were summarized in Table 2. Sampling of airborne bacteria during working hours showed that PB laboratory had the highest contamination level with an average of 21.30 ± 0.32 CFU/m³ whereas CR had the lowest reading at mean 0.86 ± 0.31 CFU/m³. There were significant differences ($p < 0.05$) in bacterial contamination levels during working hours between the laboratories except between PB and NA and BP and KP laboratories. Meanwhile for readings obtained from after working hours sampling time, showed that NA had the highest airborne bacterial contamination with an average of 24.75 ± 0.72 CFU/m³ as compared to other laboratories. PB laboratory was the second highest with an average reading of 16.97 ± 1.24 CFU/m³ which was significantly lower than NA.

For the level of airborne fungal contaminations, MB laboratory had the highest reading at an average of 21.33 ± 0.67 when sampled during working hours. However, this reading was not significantly different ($p > 0.05$) with average readings obtained from KA (21.11 ± 0.39 CFU/m³) whereas PB recorded the lowest ($p < 0.05$) average airborne fungal contamination level (9.78 ± 0.39 CFU/m³) when sampled during working hours. Data on airborne fungal contamination level sampled after working hours showed that the highest level of contamination occurred at the KA laboratory (31.99 ± 0.66 CFU/m³) while BP showed the lowest fungal contamination level at an average of (18.00 ± 0.00 CFU/m³) ($p < 0.05$).

When comparing the airborne bacterial contamination between sampling time, it could be observed that BP and CR readings did not significantly differ ($p > 0.05$). However, significant differences were shown for the other laboratories. Only NA showed significantly higher bacterial contamination level when sampled after working hours whereas other

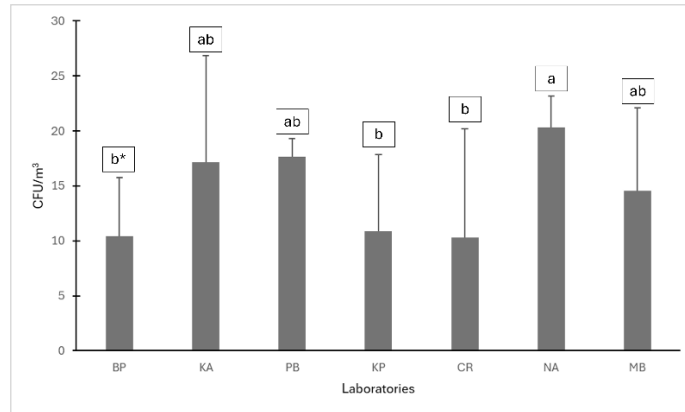


FIGURE 1. Level of total microbial contamination (Mean ± SD CFU/m³) at selected laboratories in a public university in Malaysia (n = 12). *Diferent letters indicate significant difference of microbial counts between laboratories.

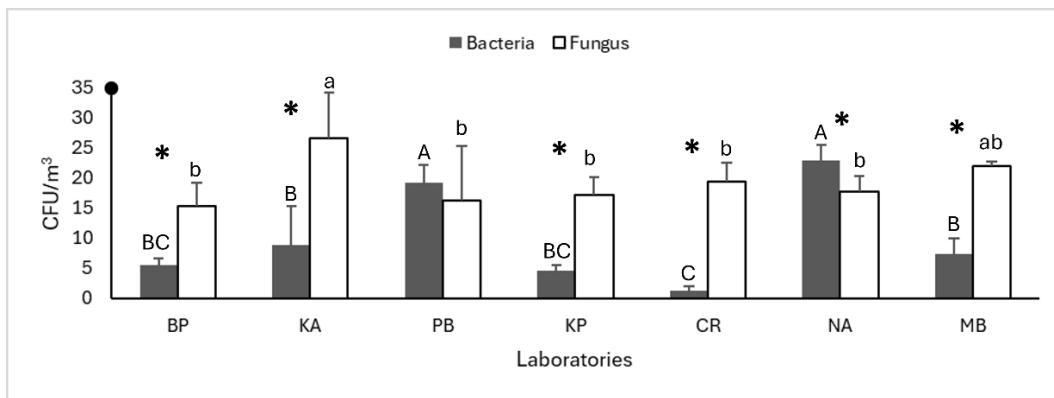


FIGURE 2. Level of bacterial and fungal contamination (Mean ± SD CFU/m³) obtained using passive air sampling activities at seven selected laboratories in a public university in Malaysia (n = 6). (*) indicates significant differences (p < 0.05) between bacterial and fungal counts for that laboratory. Different capital letters denote significant difference in bacterial contamination level between laboratories. Different lowercase letters denote significant difference in fungal contamination level between laboratories.

TABLE 2. Total bacterial and fungal counts from passive air sampling activities during working hours and after working hours in selected laboratories at a local public university

Laboratory ID	Microbial count (mean ± SD) ¹			
	During Working Hours		After Working Hours	
	Bacteria (n = 3)	Fungus (n = 3)	Bacteria (n = 3)	Fungus (n = 3)
BP	6.37 ± 0.92 ²	12.44 ± 1.02 ^d	4.79 ± 1.33 ^c	18.00 ± 0.00 ^{4*}
KA	13.47 ± 1.38 ^{b3}	21.11 ± 0.39 ^a	4.23 ± 1.28 ^{cd}	31.99 ± 0.66 ^{a*}
PB	21.30 ± 0.32 ^{a*}	9.78 ± 0.39 ^a	16.97 ± 1.24 ^b	22.66 ± 0.66 ^{b*}
KP	5.23 ± 0.16 ^{2*}	15.11 ± 0.38 ^c	3.90 ± 0.46 ^{cd}	19.33 ± 0.67 ^{cd*}
CR	0.86 ± 0.31 ^a	17.10 ± 0.38 ^b	1.76 ± 1.06 ^d	21.55 ± 0.38 ^{b*}
NA	21.02 ± 1.25 ^a	15.99 ± 0.66 ^{bc}	24.75 ± 0.72 ^{a*}	19.55 ± 0.38 ^{c*}
MB	9.16 ± 0.22 ^{c*}	21.33 ± 0.67 ^a	5.60 ± 0.27 ^c	22.44 ± 0.38 ^b

¹Data expressed as CFU/m³.

²Different letters indicate significant differences between laboratories within the same column (p < 0.05).

³(*) indicates mean microbial count of the microorganism is significantly higher (p < 0.05) as compared to reading obtained from other sampling time.

laboratories showed the opposite ($p < 0.05$). Meanwhile, all laboratories except MB showed significantly higher fungal counts when sampled after working hours ($p < 0.05$). There was no significant difference in the mean fungal counts between sampling time for MB laboratory.

Microbial contamination levels of laboratory contact surfaces are summarized in Table 3. Bacterial contamination was highest for incubator door handles from MB laboratory with an average reading of 93.00 ± 1.43 CFU/cm² whereas the highest fungal contamination level was obtained from door knobs and work benches, both from CR laboratory with an average reading of 73.33 ± 6.67 CFU/cm² and 73.33 ± 0.58 CFU/cm² respectively. No bacterial contamination was detected on the door knob samples from NA, CR and MB laboratory and no fungal contamination detected on samples from BP, PB, KP and NA laboratories. Bacterial contamination level was significantly different ($p < 0.05$) between laboratories with the highest reading observed for samples from PB laboratory (50.50 ± 0.25 CFU/cm²).

For work benches' samples, only samples from PB showed no bacterial contamination whilst no fungal contamination was found for samples from BP, PB, KP and NA. Highest bacterial contamination was recorded for samples from CR laboratory with an average of 10.77 ± 0.25 CFU/cm² ($p < 0.05$). All incubator door samples were found to be positive with bacterial contamination with significant difference ($p < 0.05$) between laboratories except between samples from BP and NA laboratories. Only 2 laboratories (KA and MB) incubator door handles' samples were found to be contaminated with fungus, with samples from KA significantly more contaminated ($p < 0.05$) than MB. No

bacterial contamination was detected on fridge door handles' samples from MB laboratory whereas no fungal contamination was found on samples from KA laboratory. Other positive samples showed significant difference ($p < 0.05$) between laboratories for both type of microorganisms. All biosafety cabinet/laminar flow hood surface swabs samples showed significantly different bacterial contamination between laboratories with samples from PB was shown to be the most contaminated (8.05 ± 0.09 CFU/cm², $p < 0.05$) while only samples from MB showed presence of fungal contamination with an average of 1.33 ± 0.00 CFU/cm². Further characterization of bacterial isolates based on Bergey's Manual of Determination Bacteriology (Williams 2000) showed that there were 32 different types of bacterial isolates found in this study. All of the bacterial isolates were found to be Gram-positive with 51.29% of the isolates were coccus and the remaining isolates were bacillus-shaped. For the fungal isolates, 2 fungal genera were presumptively identified with 55.96% presumably *Aspergillus* sp. and 44.04% *Rhizopus* sp.

DISCUSSION

Airborne microbes are one of the main contaminants that play a role as an indicator of clean indoor air (Wong et al. 2009). Good indoor air quality is essential in maintaining a healthy indoor working environment. To date, there is no set of standards on the permissible level of microbial contaminants established specifically for laboratory indoor air in Malaysia. The current available guideline is the Industrial Code of Practice for Indoor Air Quality (ICOP IAQ) set by the Department of Occupational Safety and Health, to oversee the indoor air quality issues at the workplace namely in an office setting (DOSH 2010). The standard limit for bacterial contamination level set by this guideline is 500 CFU/m³ whereas for fungal contamination, the standard is set at 1000

CFU/m³. Nevertheless, this standard will be used as a basic guideline for comparison with the results obtained in this study. In this study, the microbial contamination levels obtained were much lower as compared to the standard limits for all samples. The readings were also low as compared to a similar study conducted on other university laboratories by Hazrin et al. (2015). Although the contamination levels are considered low, improvements need to be conducted periodically to maintain good indoor air quality.

Total microbial contamination levels in this study were found to vary between laboratories and some of the differences were significant. This was not surprising as microorganisms can be transferred through air and remains airborne depending on its size. Total volume of airborne microorganisms in an enclosed area also depends on the location, weather, structural design, relative humidity, ventilation rate, air movement rate and the number of users of that room (Muhammad Ghayoor et al. 2015; Wamedo et al. 2012; Graudenz et al 2005; Douglas Traber & Shanks 2001). CR laboratory has fewer users and is used less frequently which may explain the lower bacterial contamination level as compared to the other laboratories.

During the sampling activities, it was observed that there were several laboratories with repeated breakdowns of the central air-conditioning system thus affecting the central ventilation system of these spaces. This was observed especially in KA laboratory during the sampling activity which may have contributed to the consistently high levels of microbial contamination in this laboratory. The central air-conditioning system was also turned off after working hours allowing the microorganism and its spores to move passively in the air space and eventually accumulate. With slower air movements during this sampling time, microorganisms

will be sedimented due to gravity forces especially for bigger microorganism particles. This may also explain the higher levels of microbial contamination namely fungal contamination found on samples taken after working hours. Although absence of air flow or ventilation rate measurement is a limitation of this study, it has been established that inadequate ventilation system has been identified as one of the contributing factors causing poor indoor air quality (DOSH 2010).

Contact surface swab samples results showed that laboratory door knobs, incubator door handles and work benches were highly contaminated. Microbial contamination of laboratory contact surfaces has been reported to potentially cause health risk to the workers (Neely & Orloff 2008). Microorganisms, especially fungi, have the ability to survive on the laboratory contact surfaces for a long period of time and could serve as a continuous source of contamination (Neely & Sittig 2009). Good laboratory practices including effective aseptic techniques are crucial in prevention of unintended microbial contamination in the laboratory. Disinfection of contact surfaces using 70% propyl alcohol is commonly practiced and its effectiveness has been proven in eliminating microorganisms from contact surface when performed before and after any laboratory work especially those involving biological agent (Harding & Brandt 2012).

Good laboratory practices such as wearing gloves and other personal protective equipment (PPE) is also important in preventing the risk of exposure to the microbial agents. Incompliance of this practice will increase the risk of infection especially when good hand hygiene practices are compromised as well (WHO 2009). Good housekeeping and effective cleaning procedures must be maintained regularly to ensure smooth laboratory operations.

CONCLUSION

This study demonstrated that microbiological contamination of the selected laboratories are still in compliance with the standard guideline but it can be improved to increase the quality of laboratory environment. The importance of maintaining good central air-conditioning and air ventilation system, effective housekeeping and cleaning procedures should be emphasized by the laboratory managers. Good laboratory practices and aseptic techniques should be utilised at all times by the workers. These activities could help minimise any microbial contamination, thus ensuring a safe and healthy working environment in the laboratory

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