ORIGINAL ARTICLE

ASSESSMENT OF INDOOR AIRBORNE MICROORGANISMS IN A DENSELY POPULATED MALAYSIAN PUBLIC UNIVERSITY

Eric Tzyy Jiann Chong, Khairul Atikah Khairul Faizin, Lucky Poh Wah Goh and Ping-Chin Lee*

Biotechnology Programme, Faculty of Science and Natural Resources, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia

***Corresponding author:**
Lee Ping-Chin
E-mail address: leepc@ums.edu.my

ABSTRACT

Indoor air quality is an essential aspect for occupational health including in a densely populated university. This study aimed to assess the indoor airborne microorganisms via biochemical and molecular approaches in five enclosed workplaces, and their resistance towards six commonly used antibiotics. Cfu/dm^2/h for five enclosed workplaces was determined using settle plate technique with 1/1/1 scheme and Gram staining was performed for all pure strains isolated. Two strains with the highest count and with different morphologies were identified using biochemical test as well as 16S rRNA amplification and direct sequencing. Minimum inhibitory concentration for antibiotics was carried out for these two strains. In this study, 27 microbial strains with different morphologies were obtained from all workplaces and 2 strains with the highest count were strain J in café and strain M in library, which were identified as Bacillus cereus and Staphylococcus cohnii, respectively. Both of them were highly susceptible to ampicillin and tetracycline. With resistance up to 0.78 µg/mL; B. cereus was less sensitive to kanamycin and neomycin whereas S. cohnii was less sensitive to streptomycin. In conclusion, antibiotics resistant B. cereus and S. cohnii were two of the microorganisms showing the most abundance in the café and library of a Malaysian public university, respectively. This study may serve as the baseline for the prescriptions of antibiotics to airborne microbial related infections especially to the community in the university who seek for medical treatments; particularly for respiratory and digestive infections which often associated with indoor microenvironment.

Keywords: *antibiotic resistance, Bacillus cereus, indoor air quality, minimum inhibitory concentration, Staphylococcus cohnii*

INTRODUCTION

The National Human Activity Pattern Survey reported that more than 90% of humans spend their time in an enclosed environment by breathing on about fourteen cubic meter of air daily[1,2]. Hence, indoor air quality is an essential aspect for occupational and public health especially in a densely populated university. Numerous scientific publications have been reported regarding the air quality in universities in recent years[3-5]. Indoor environment is abundantly populated with microorganisms that primarily originated from human (i.e. through talking, coughing, sneezing, and walking), pets, and outdoor air sources. Inhaled of air-borne microorganisms may influence the health condition of humans, especially in those who are with weak immunity and may lead to diseases such as asthma, allergies, and pneumonia[6-8].

Antibiotics are chemical compounds which are produced from synthetic chemicals or secondary metabolites that use to treat diseases caused by microorganisms. However, several studies reported the utilization of antibiotics had lead to the antibiotic resistance airborne microorganisms that posed as a health hazard for individuals[9-11]. Once antibiotic resistance in airborne microorganisms has been established, the removal of resistance in microorganisms is difficult and resistance persists for long period of time[12]. Hence, the determination of minimum inhibitory concentration (MIC) of different antibiotics in airborne microorganisms is crucial[13].

Besides, health condition of students and staffs in a university has been known as the common factor that affects their efficiency and productivity for carry out their daily tasks[14,15]. There is a high incidence of sickness reported in the studied university which is of approximately 25,000 population. Majority of the cases were with symptoms like sore throat, headache, eye irritation, and diarrhea. This attracts our concern on whether indoor airborne microorganisms contribute to the sickness in the university community. Since investigation to ascertain the cause of sickness is recommended according to the Industry Code of Practice on Indoor Air Quality 2010 guidelines[16], therefore this study was conducted to assess the indoor airborne microorganisms via biochemical and molecular approaches in five enclosed workplaces that are most occupied in the university, and their MICs towards six common antibiotics were also evaluated. This study uncovered an emerging antibiotic resistant *B. cereus* and *S. cohnii*

microbial dominant in the café and library of a Malaysian public university, respectively, and serves as a recommendation for airborne microbial related infections.

METHODOLOGY

Sampling and colony forming unit

Five replicates of nutrient agar plate (9 cm in diameter) was left open to indoor air environment and settled in each workplace including: (i) a centre that provide services to staffs and students, (ii) an office of top management, (iii) a tissue culture laboratory, (iv) a café, and (v) the university's library (Figure 1) twice in one day (at 10 a.m. and 3 p.m.) using 1/1/1 scheme (for 1 hour, 1 m from the floor, and at least 1 m away from walls or any obstacle) as recommended[17]. Airflow velocity (ft/min) in the selected workplaces was measured using Large Vane CFM/CMM Thermo-Anemometers (Extech Instruments, Nashua, USA). All nutrient agar plates were incubated at 37°C for 24 hours and morphology for all growing colonies was recorded. The colony forming unit per decimeter square in 1 hour (cfu/dm^2/h) was also obtained for all replicates.

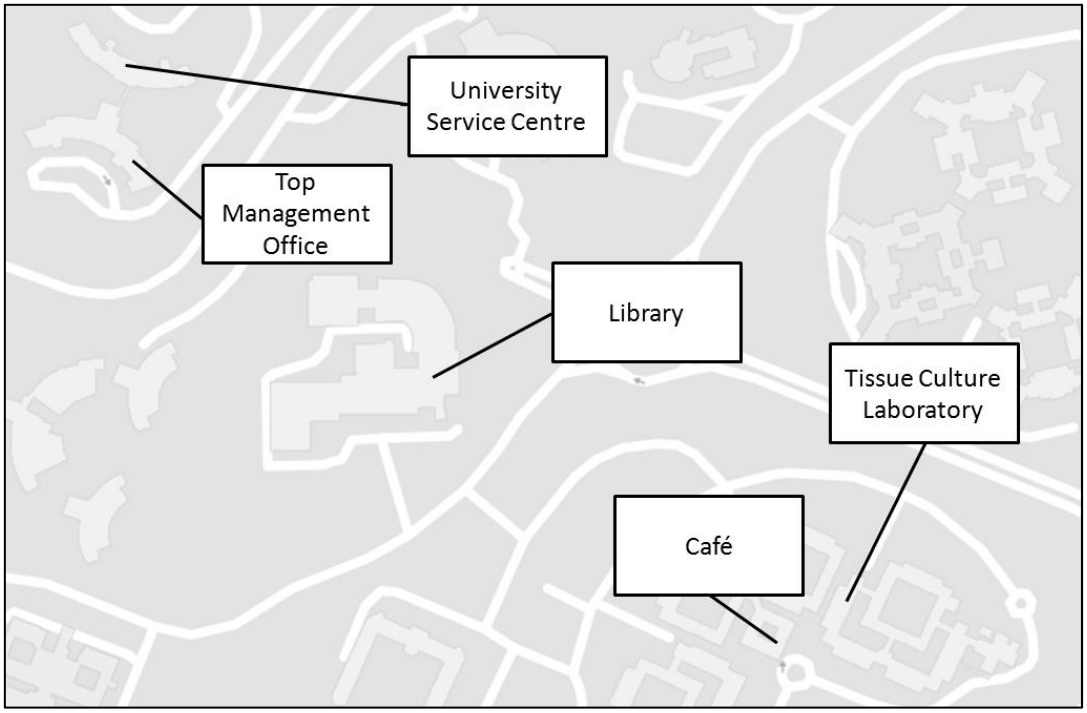

Figure 1: Sampling site of five different workplaces in this study

Gram stain and biochemical test

Gram stain was performed for all pure strains with different morphologies following the protocols previously described[18]. However, only two strains with the highest count and with different morphologies (strain J from café and strain M from library) were subjected to subsequent analysis. BiOLOG GEN III MicroPlate™ (BiOLOG, Hayward, CA) was used for biochemical test following the manufacturer's instructions and was visualized using BiOLOG MicroStation™ ID System (BiOLOG, Hayward, CA) for microbial species identification.

Polymerase chain reaction

A single pure colony for strain J and M was cultured in 3 ml of fresh LB broth at 37°C overnight with shaking (~220 rpm). Microbial DNA was extracted using phenol-chloroform method. Polymerase chain reaction (PCR) amplification of microbial 16S rRNA gene was performed using primers 5'-AGA GTT TGA TCC TGG CTC AG-3' (forward) and 5'-GTT ACC TTG TTA CGA CTT-3' (reverse) in a total volume of 20 uL containing 100 ng of extracted microbial DNA, 1X reaction buffer, 1.5 mM $MgCl_2$ solution, 0.2 mM of dNTPs mixture, 0.2 µM of each primers and 1 unit of *Go Taq*® Flexi DNA polymerase (Promega, USA). The PCR conditions were set at: initial activation for 4 min at 94°C, 35 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 2 min, and a final elongation step at 72°C for 5 min. The PCR products were electrophoresized in 1% agarose gel stained with ethidium bromide.

Gel extraction and direct sequencing

PCR products were isolated and purified from agarose gel using QIAquick Gel Extraction Kit (Qiagen, Germany) following manufacturer's recommendations and were subjected to direct sequencing using ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, USA). The sequencing outputs were blasted and compared to National Centre for Biotechnology Information (NCBI) database.

Minimum inhibitory concentration test for antibiotics

MIC assay was used to test resistance of strain J and M to six commonly used antibiotics including ampicillin, gentamicin, kanamycin, neomycin, streptomycin, and tetracycline with different concentrations (0.00, 0.10, 0.20, 0.39, 0.78, 1.56, 3.13, 6.25, 12.50, 25.00, 50.00, and 100.00 µg/mL). OD_{600} was measured using Multiskan™ GO Microplate Spectrophotometer (Thermo Scientific Inc., USA) after 24 hours incubation at 37°C. Heat-map of relative growth was generated using TreeView 3.0 software (available online at http://jtreeview.sourceforge.net/).

Statistical analysis

In statistical analysis, Pearson's correlation test was used to determine the correlation between airflow velocity (ft/min) and mean of cfu/dm^2/h in all workplaces. The mean difference for cfu/dm^2/h between morning and afternoon was also compared using Mann-Whitney *U*-test. In addition, two-way ANOVA was used to examine the interacting factors for mean of cfu/dm^2/h. SPSS Software V17.0 (SPSS Inc, Chicago, Illinois, USA) was used for all statistical testing in this study and all tests were considered as statistical significant when the *p*-value was less than 0.05.

RESULTS

Twenty-seven microbial strains with different morphologies were obtained from all workplaces and two strains with the highest count were strain J from café and strain M from library (Figure 2). Overall, air quality in the library had the highest microbial count with mean of 44 cfu/dm^2/h, followed by café (28 cfu/dm^2/h), tissue culture laboratory (20 cfu/dm^2/h), university service centre (14 cfu/dm^2/h) and top management office (8 cfu/dm^2/h). The lowest airflow velocity was obtained in café with only 43 ft/min, followed by library (50 ft/min), tissue culture laboratory (51 ft/min), university service centre (59 ft/min) and top management office (60 ft/min) (Table 1).

Table 1: Mean of cfu/dm^2/h obtained from different workplaces in the morning and afternoon

Workplaces	Airflow velocity (ft/min)	Morning		Afternoon		Overall	
		Range of colony/plate	Cfu/dm^2/h[a]	Range of colony/plate	Cfu/dm^2/h[a]	Range of colony/plate	Cfu/dm^2/h[a]
University Service Centre	59	6-18	19	3-7	8	3-18	14
Top Management Office	60	0-11	9	2-5	6	0-11	8
Tissue Culture Laboratory	51	3-10	11	15-22	28	3-22	20
Café	43	12-33	35	11-17	24	11-33	28
Library	50	24-31	44	19-31	44	19-31	44

Note: [a] = Did not correlate to airflow velocity (p > 0.05)

Statistical analysis showed that no significant correlation was found between airflow velocity and mean of cfu/dm^2/h using Pearson's correlation test. Besides, Mann-Whitney *U*-test revealed no significant mean difference for cfu/dm^2/h between morning and afternoon in all workplaces (p = 0.757). Interestingly, sampling workplace and sampling time were significantly showed as two interacting factors to influence the mean of cfu/dm^2/h in two-way ANOVA test (p < 0.001).

The 16S rRNA gene for strain J and M was successfully amplified with significant band at ~1500 bp (Figure 3). Strain J was Gram-positive rods with colony morphology of round, raised surface, and yellowish color. BiOLOG's biochemical test for strain J did not match with any species identity (similarity < 0.300) but 16S rRNA sequencing revealed as *Bacillus cereus*. For strain M, it was Gram-positive cocci with colony morphology of round, smooth surface and milky white color, and was identified as *Staphylococcus cohnii* in both BiOLOG's biochemical test (similarity = 0.762) and 16S rRNA sequencing.

In MIC test, both strain J and M were highly susceptible to ampicillin and tetracycline with addition of gentamicin for strain M (Figure 4). Strain J was less sensitive to kanamycin and neomycin antibiotics with resistance up to 0.78 µg/mL. For strain M, it was most less sensitive to streptomycin (resistance up to 0.78 µg/mL) followed by kanamycin with resistance up to 0.39 µg/mL.

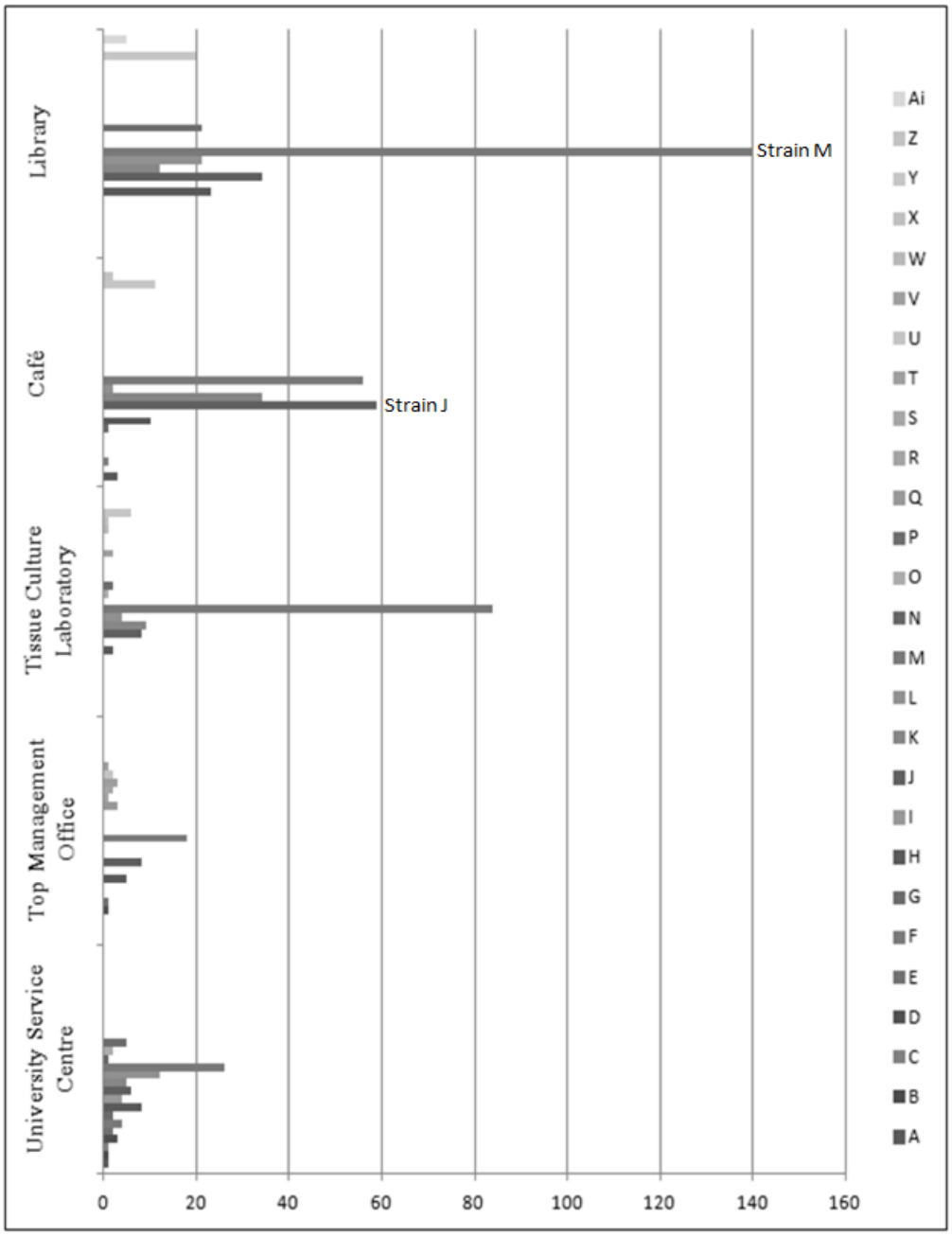

Figure 2: Number of colonies for all twenty-seven microbial strains with different morphologies obtained in five different workplaces in this study

DISCUSSION

Exposure to unhealthy indoor air causes sensory irritation or stimulation which gives discomfort to humans. This affects the human's health which subsequently causes a decrease in workforce efficiencies. It is hypothesized that airflow rate plays an important role in sick building syndrome and directly relates to cfu. The Industry Code of Practice on Indoor Air Quality 2010 recommended an airflow rate of 29.5 ft/min to 98.4 ft/min for ideal occupational health[16]; since the airflow rate for all workplaces in this study fell within the recommended range, this could support that airflow rate did not correlate with the mean of cfu/dm^2/h and no significant difference of the mean of cfu/dm^2/h between morning and afternoon was observed in the present study. According to the index of microbial air contamination (IMA) classifications[19], the air quality in the top management office is considered "very good" (0-9 cfu/dm^2/h) while the university service centre, tissue culture laboratory and café are categorized in the "good" category (10-39 cfu/dm^2/h). Library is the only facility that fell in the "fair" category (40-84 cfu/dm^2/h) in this study. However, the overall air quality in all five workplaces is satisfactory as they did not exceed the empirically defined maximum acceptable level of IMA for facilities which is 124 cfu/dm^2/h[19].

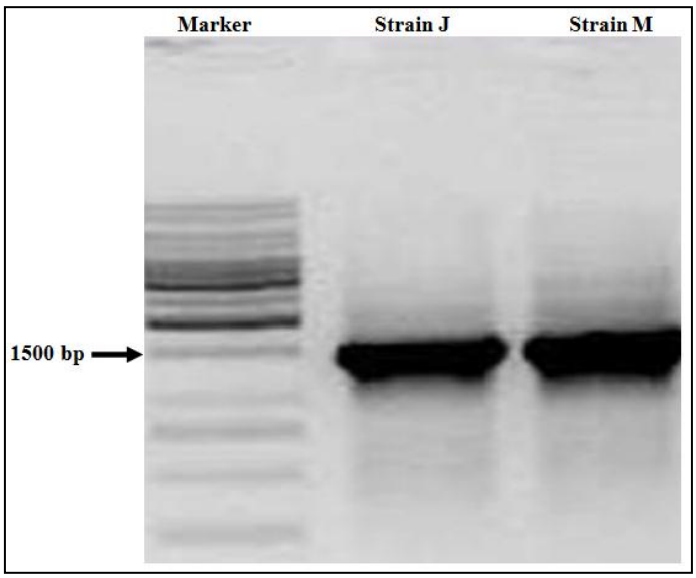

Figure 3: PCR amplification of 16S rRNA gene for strain J and M with expected band at ~1500 bp. Marker = GeneRuler™ 1kb DNA Ladder (Thermo Scientific, USA)

Two-way ANOVA test revealed that mean of cfu/dm^2/h was significantly different when sampling was carried out in different workplace and time, suggesting that occupancy might be the key factor for influencing the mean of cfu/dm^2/h in this study. This was represented by an overall high cfu/dm^2/h in the library and café in this study as there was high occupancy of people from morning until afternoon in both workplaces. Besides, previous study reported that architectural design of building contributed to indoor air microorganism communities[20]; the enclosed air-conditional environment in the café and carpeted-floor in the library may also drives to high concentration of microorganisms in both workplaces.

Food-borne diseases and poisoning due to microbial contaminations are common occurrence in any area and have been most frequently observed in restaurants, cafeterias and bars[21]. We identified high concentration of *Bacillus cereus* in the café in this study through molecular approach, but the BiOLOG's biochemical test revealed no matched identity. As most of the hospitals in Malaysia are still using biochemical test for species identification in clinical specimens, we recommend the results should be molecularly validated, especially for critical cases. *B. cereus* has been presented in an open restaurant in Malaysia and commonly correlated to the number of people indoor[22,23]. A recent study also reported that toxin-producing *Bacillus* spp. which causing food poisoning was isolated from paper towels in a kitchen[24], suggesting that *Bacillus* spp. can be spread with different mediums within the café. Therefore, all tableware should be kept sterile before using for food intake.

In this study, more than 60% of Gram-positive cocci were presented in indoor environment especially *Staphylococcus cohnii* which was abundant in the library. A large office building airborne microbial assessment carried out in the United States showed that the concentration of Gram-positive cocci was significantly higher in indoor environment when compared to outdoor[25]. Besides, Gram-positive cocci was also highly isolated in an enclosed high throughput building in Malaysia as well as hospitals in Nigeria[26-28]. *S. cohnii* is known to form reside and populates on human skin, nose and clothing[29,30], indicating that contamination of *S. cohnii* in indoor air may result from human presence. Furthermore, *S. cohnii* has been reported to perfectly adaptive to hospital environment and able to alter its heredity under effect of antibiotic pressure[31], urging the MIC of different antibiotics for *S. cohnii* should be tested before any medical treatments.

B. cereus and *S. cohnii* isolated in this study showed different tolerance levels towards different antibiotics. Although both of the isolates were highly susceptible to ampicillin and tetracycline, they were previously reported resistance to these antibiotics[32-34]. Interestingly, *B. cereus* showed resistant to kanamycin and neomycin; and *S. cohnii* was resistant to streptomycin for up to 0.78 µg/mL in this study, suggesting that they are developing resistance to these antibiotics. *Bacillus* spp. and *Staphylococcus* spp. have been intensively reported to develop resistance to methicillin in recent studies but we did not include the methicillin in the present study due to material limitation[33,35,36]. Based on the MIC results in this study, sickness reports from this university might be caused by infection of antibiotic resistant microbial spp. and this unpromising situation should be seriously monitored.

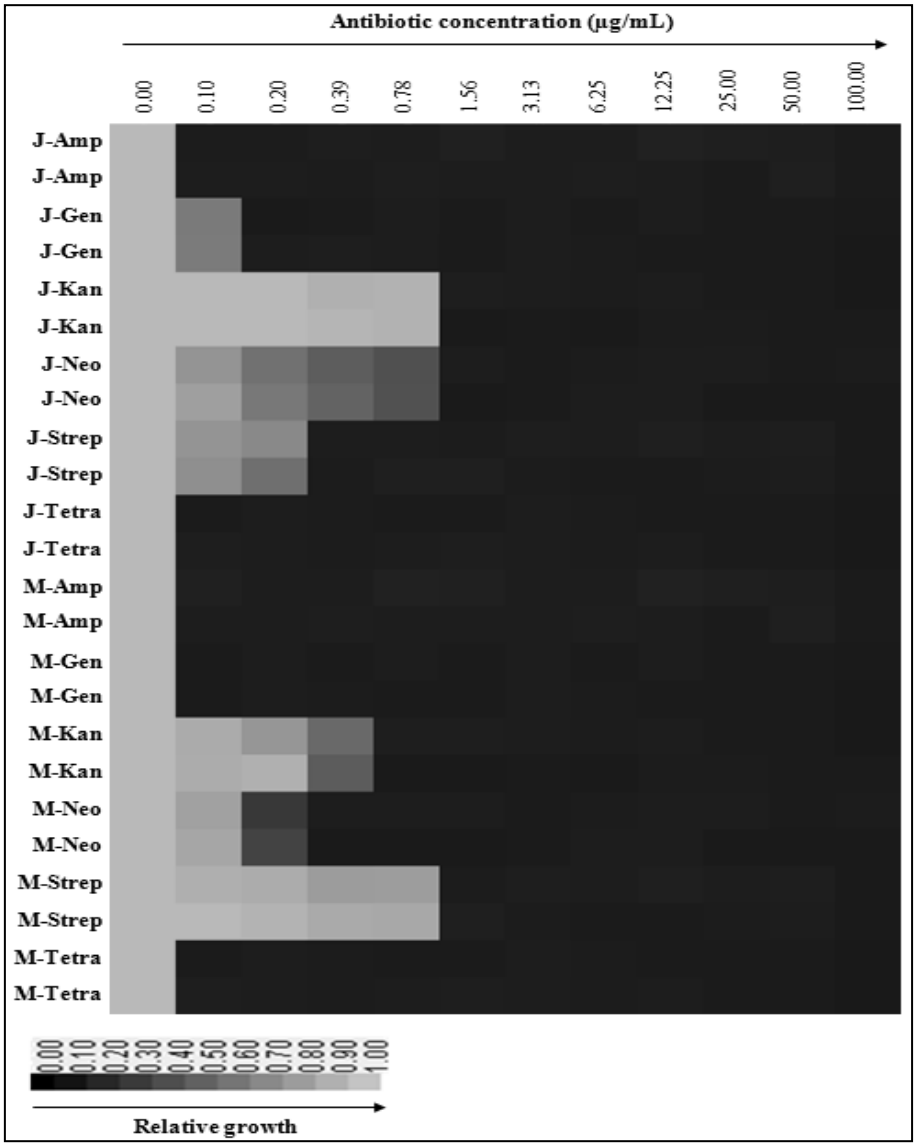

Figure 4: Heat-map of minimum inhibitory concentration (MIC) assay for six common antibiotics with different concentrations (0 to 100 µg/mL) to *B. cereus* and *S. cohnii* isolated from university's café and library, respectively. J = *B. cereus*; M = *S. cohnii*; Amp = ampicillin; Gen = gentamicin; Kan = kanamycin; Neo = neomycin; Strep = streptomycin; Tetra = tetracycline

CONCLUSIONS

The increasing evidence of occupational related health effects due to indoor microenvironment is emerging. Building design, chemical compound presence in the air and working environment that posed to worker's health[20,37,38] were not evaluated in this study as we solely focused on the airborne microorganism of indoor air to worker's health. We had highlighted antibiotic resistant *B. cereus* and *S. cohnii* were two of the microbial showing most abundance in the café and library of a Malaysian public university, respectively. Moreover, as antibiotic resistant microorganisms is emerging at a different rate across different parts of the world, antimicrobial susceptibility test conducted in this study serves as the baseline for the prescriptions of antibiotics to airborne microbial related infections. Future study should include other important parameters to be measured such as air temperature, relative humidity and particulates concentration for a greater view on how these parameters associated with microbial prevalence and characteristic in a workplace.

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COMPETING INTERESTS

The authors declare no competing interest in this study.

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