International Journal of Medical, Medicine and Health Sciences
ISSN: 2517-9969
Vol:6, No:12, 2012

Detection of Airborne Bacteria and Mildew in the Shanghai Metro System

Feng Zhou, and Yuyan Wang

***Abstract*—**This study aimed to detect and to identify the main strains of airborne microorganisms present in the Shanghai Metro system. Samples were collected using agar plates exposed to the air and microorganisms were identified using catalase, plasma coagulase and hymolytic analysis. The results show that the concentration of mildew present within a newly opened metro line was significantly higher than for other lines. Differences among underground and elevated stations can be attributed to differences in passenger flow and the environment surrounding the stations. Additionally, the investigation indicated that bacteria reached maximum levels at different times on weekdays and weekends. The bacteria in the Metro stations were identified as primarily Gram positive, consisting mainly of *coagulase-negative staphylococcus* strains (*CNS*).

***Keywords*—**Bacteria, environment, Metro system, mildew, passenger flow.

I. Introduction

A number of infectious diseases can be passed through crowds, resulting in cross-contaminations, and one of the most common routes of transmission is via respiratory passages. It is, therefore, important to investigate air quality in public areas, especially those with high visitor flow rates. As the primary means of public transportation in over 120 megacities, Metro systems tend to be subject to high scrutiny in terms of efficiency, safety and capacity. However, considerable importance should also be attached to conditions of hygiene, since pollutants such as sulfurous anhydride, particles and airborne microorganisms can accumulate in the closed environment of the Metro system [1]. Only a small number of countries, including the United States [2], Egypt [3], Japan [4], [5], Hungary [6], [7] and Russia [8], undertake regular investigations of bacterial populations in their cities' Metro systems. With nearly 7 million people boarding daily and a peak passenger flow of 7.5 million [9], the Shanghai Metro system is now the largest in the world. However, since the first line opened in 1995, no investigations into the level of airborne microorganisms have been carried out and no formal procedures exist in China to monitor air quality in Metro systems [10]. In the course of this study, we investigated the concentration and distribution of airborne microorganisms, namely bacteria and mildew, in the Shanghai Metro system and the probable conditions that affect the abundance of such microorganisms. The aim of this study was, therefore, to provide a scientific basis to inform policy decisions on public health and the construction of future Metro systems to improve passenger safety and comfort.

F. Zhou is with School of Basic Medical Sciences, Fudan University, Shanghai 200032, P. R. China, phone: +86-021-54237543-333; fax: +86-021-54237122; Email: 09301010100@fudan.edu.cn.

Y. Wang (Corresponding author) is with the Department of Medical Microbiology and Parasitology, School of Basic Medical Sciences, Fudan University,Shanghai, P. R. China, Email: yuyanss@fudan.edu.cn.

II. Methods and Materials

A. Sample Collecting and Cultivation

Bacteria and mildew were collected using nutrient agar and Czapek's medium plates, respectively. Bacterial samples and mycotic samples were collected using the settling process, according to the Chinese Ministry of Public Health protocol [11]. The nutrient agar plates and Czapek's medium plates were placed 1.2 meters above ground level and exposed to the air in the center of the carriage or the station waiting room for 5 min. For consistency across the results, all collections were conducted on the same day. Nutrient agar and Czapek's medium plates were cultivated at 37°C for 48 hours and at 28°C for 5 days, respectively. Cell colonies were counted beginning on the third day of cultivation.

B. Group Allocation

The study involved the detection and classification of airborne microorganisms in two types of location: within Metro carriages and within Metro stations. The former included carriages on four metro lines. Line one experienced the highest passenger flow, reaching over 1 million people per day, and Line ten had been opened just 3 days previously. The other two lines were selected at random. In order to avoid the influence of peak passenger flow rate during rush hour, all collections were conducted in the early afternoon on the same day.

The station group included samples taken in five underground stations and five over-ground stations. Three of the underground stations were interchange stations where at least two Metro lines crossed. Subsequently, these stations had higher passenger flow. Two of the elevated stations were near trunk roads and subject to large amounts of dust produced by vehicles. Similarly to the carriage group, all collections were carried out in the early afternoon on the same day.

C. Bacterial Isolation and Identification

Primary identification was carried out based on the results of Gram-staining and microscopic examination. After microscopic confirmation of the purification of populations based on Gram-staining, further identification was performed using catalase and plasma coagulase tests.

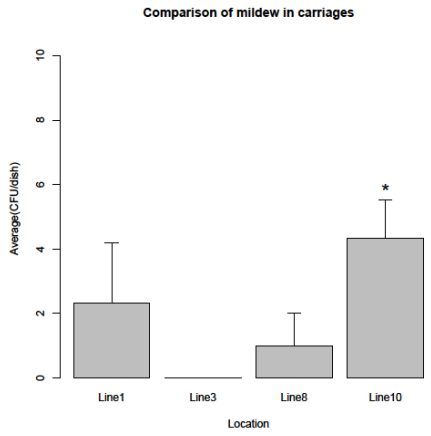
The catalase test distinguishes between *streptococcus* and *staphylococcus* by observing the decomposition of hydrogen peroxide solution. Bacterial samples were obtained after cultivation for 17 h and mixed evenly with 3% hydrogen peroxide solution. Bubbles observed in the solution revealed the bacteria to be *staphylococcus*.

The plasma coagulase test distinguishes *Staphylococcus aureus* from *CNS* strains based on the absence or presence clot-lysis ability, respectively. Rabbit blood was added to a 3.8% solution of citrate sodium anticoagulant. This was then added to the bacterial samples, having been cultivated for 18 h at 37°C, and the presence or absence of clots was recorded. Normal saline was also used as a control.

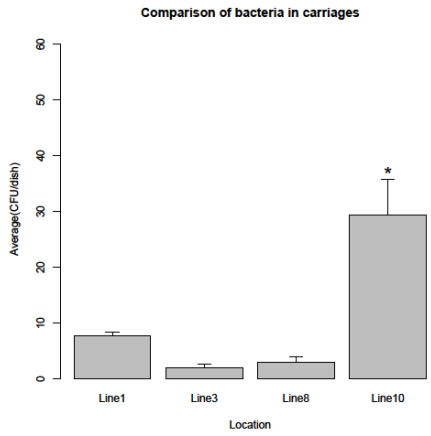
The hemolytic test was performed to distinguish *Staphylococcus haemolyticus* from other *Staphylococci*. Streak cultivation of bacterial samples was conducted on nutrient agar containing goat blood and the presence or absence of hemolysis was recorded after incubation for 18 h at 37°C.

III. Results

A. Detection of Bacteria and Mildew in Carriages



(a)



(b)

Fig. 1 Comparison of (a) mildew and (b) bacterial concentrations in Metro carriages *p < 0.05

Fig. 1 shows a comparison of mildew and bacterial concentrations in the Metro carriages tested. Line ten was found to have the highest concentration of bacteria and mildew, with a statistically significant difference compared to other lines ($p < 0.05$). Line one had the next highest bacterial concentration. However, the difference in the amount of bacteria and mildew compared to Line three and Line eight was not found to be statistically significant ($p > 0.05$).

B. Detection of Bacteria and Mildew in Stations

Table I shows the concentrations of bacteria and mildew in the different stations sampled. The concentration of bacteria and mildew varied considerably across the different stations. The highest content of bacteria appeared at South Changjiang Road with a value of 101.67 CFU/dish, which was 22 times than the result at Zhongxing Road with a value of 4.67 CHF/dish. People's Square, Century Avenue and Zhongshan Park underground interchange stations, where at least two lines intersect, were found to have the highest concentrations of bacteria of the underground stations ($p < 0.01$). However, the difference in the amount of mildew in these stations was not found to be statistically significant ($p > 0.05$). The elevated stations Gongfuxincun and South Changjiang Road had significantly higher concentrations of bacteria and mildew than any other station ($p < 0.05$).

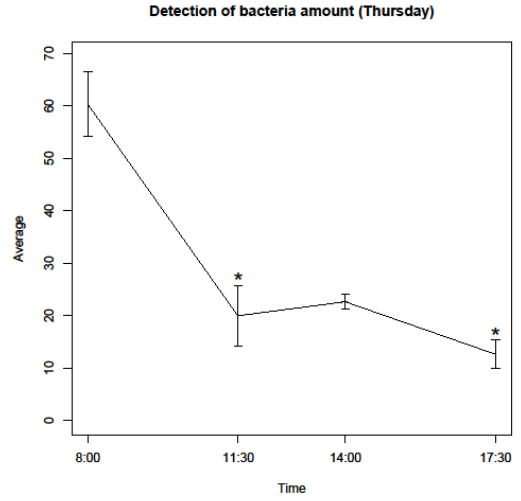
TABLE I
THE CONCENTRATIONS OF BACTERIA AND MILDEW IN DIFFERENT METRO STATIONS

Location	Bacteria (CFU/dish)	Mildew (CFU/dish)
Interchange/underground stations		
Line 1 - People's Square	15.33 ±4.03	2.33 ±1.25
Line 2 - Century Avenue	21.67 ±3.30	1.33 ±0.47
Line 8 - Hongkou Football studium	8.00 ±0.82	1.67 ±1.25
Line 8 - Zhongxing Road	4.67±1.70	3.00 ±1.41
Line 2 - Zhongshan Park	25.00 ±3.56	2.33 ±0.47
Elevated stations		
Line 3 - Chifeng Road	14.00 ±4.97	3.67 ±1.70
Line3 - East Dongbaoxing Road	7.67±3.79	2.67±1.15
Line 3/4 - Hongqiao Road	16.00 ±3.56	3.67 ±1.25
Line 3 - South Changjiang Road	101.67±27.06	34.67±11.93
Line 1 - Gongfuxincun	95.67 ±8.18	13.67±2.87

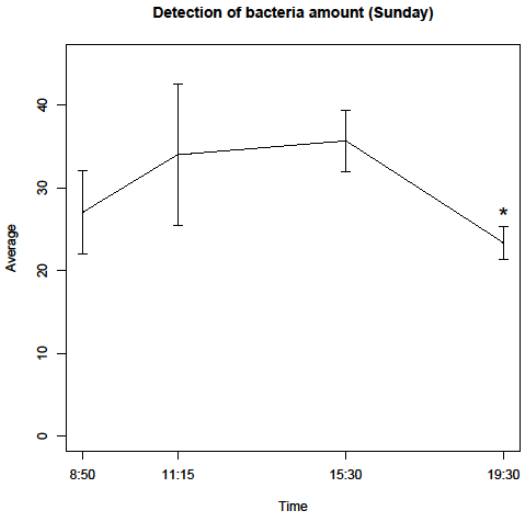
C. Daily Variation in Bacterial Concentration

Fig. 2 shows the daily variation in bacterial concentration at a single station on two different days, Thursday and Sunday. Fig. 2a shows that bacterial concentration in the station peaked in the morning on Thursday, and it can be assumed that this is the general pattern characteristic of workdays. The statistical result showed that the amount of bacteria dropped significantly at noon and then again in the evening ($p < 0.05$). Fig. 2b shows that the bacterial concentration in the station peaked in the afternoon on the Sunday. However, the differences between the

morning, noon and afternoon measurements were not found to be statistically significant ($p > 0.05$). Bacterial concentration then dropped significantly to reach its lowest point in the evening ($p < 0.05$).



(a)



(b)

Fig. 2 Daily variation in bacterial concentration for a single station on a (a) weekday and (b) the weekend *p < 0.05

D. Identification of Bacteria in Metro Stations

Of the 163 bacterial samples obtained from the Metro stations, 135 (82.82%) were identified as Gram-positive. Catalase, plasma coagulase, and hemolytic tests revealed that *CNS* strains were most prevalent (66.26%) in the Metro stations (Fig. 3). Streptococcus spp. was identified at low frequency (1.74%) in samples from the Metro stations. Further study showed that the *staphylococcus*-positive samples mainly consisted of *Staphylococcus epidermidis*, *Staphylococcus lentus* and *Staphylococcus saprophyticus*. However, the study took nutrient agar to collect the bacteria. The result is probably different if another medium was used.

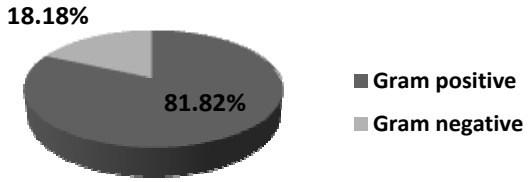


Fig. 3 The characterization of airborne bacteria in Metro stations. The airborne bacteria in metro stations were mainly consisted of Gram positive strains. Further study showed that *CNS* strains predominated in metro stations (66.26%). *Streptococcus* was identified at low frequency from samples in metro stations (1.47%). The *Staphylococcus* samples mainly consist of *Staphylococcus Epidermidis*, *Staphylococcus Lentus* and *Staphylococcus Saprophyticus*

IV. Discussion

The results from the collections carried out in Metro carriages showed that Line ten had significantly higher concentrations of bacteria and mildew than other lines, despite the fact that the passenger flow was lowest. However, as our study was conducted in the early afternoon; the influence of passenger flow is not considered the main condition affecting the concentration of airborne microorganisms. A recent study showed similar results with respect to airborne bacteria in the Shenzhen Metro system. In newly opened Metro lines, bacterial content was found to be higher and dropped after several years [12].

In our study, underground stations and elevated stations were treated separately since the conditions that affect the abundance and distribution of airborne microorganisms are different. Soil is one of the primary natural sources of bacteria and mildew. Since the stations of Line ten were all underground, the presence of microorganisms is likely to originate from the soil exposed when the line was opened. When the metro system is operational, the amount of bacteria and mildew is likely to be modified by such factors as the ventilating system, disinfection activities and passenger flow [13]. However, the higher concentration of bacteria and mildew in Line ten can be attributed to it having been opened only recently and being underground at all points along its length. To improve conditions and guarantee the comfort and safety of passengers, it is recommended that regular air sterilization practices should be carried out on this particular line.

The results in underground stations revealed that interchange stations had higher concentrations of bacteria and mildew, indicating that passenger flow may influence the abundance of airborne microorganisms. Previous studies have demonstrated the effect of several conditions on the amount of airborne bacteria. In Seoul underground stations, deeper underground stations and stations with higher passenger flow were found to have higher bacterial contents than those shallow ones [14]. In London, similar results showed that deep underground stations had higher fungal counts than others [15]. This is attributed to the fact that exchange with the external environment is less

frequent in such closed stations, allowing airborne microorganisms to accumulate. In the present study, the environment surrounding elevated stations was found to be the primary condition influencing the concentration of microorganisms. In addition to passenger flow, it is suggested that particles produced by vehicles travelling on the trunk road affect the abundance of bacteria and mildew in elevated stations.

The results suggest that the time of day at which bacterial concentration reaches a peak differs between weekends and weekdays. Only one day was studied since it was deemed that the temperature and humidity were unlikely to change significantly at the same station during this period. However, the concentration of microorganisms is likely to vary considerably between seasons due to the influence of temperature and humidity.

Gram-positive cocci were identified as the dominant strain of microorganism in the Metro stations. Extracellular enzyme tests indicated the predominant airborne bacteria in Metro stations to be *CNS* strains, including *Staphylococcus saprophyticus, Staphylococcus epidermidis* and *Staphylococcus haemolyticus. Staphylococcus epidermidis* and *Staphylococcus saprophyticus* are easily detected on human skin and mucous membranes as normal flora [16]. The huge passenger flow in Metro systems provides ideal conditions for the widespread circulation of bacteria. However, the invasive capacity of bacteria depends on characteristics such as adhesiveness and extracellular enzyme profiles. Identification of coagulase activity constitutes important evidence of bacterial pathogenicity. All samples investigated in this study were coagulase-negative, and the proportion of *Staphylococcus haemolyticus* was low, suggesting that no highly pathogenic strains exist in the studied Metro stations.

This study represents a significant step forward in terms of identifying and measuring the concentration of airborne bacteria in the Shanghai Metro system, as well as shedding light on the potential factors that influence distribution patterns. It should be noted that the nutrient agar used in the present study is commonly used and can cultivate most bacteria, but not all. A valuable continuation of the present work could, therefore, include comparisons of the results obtained using different culturing media.

Ethical Approval

None sought.

Acknowledgment

Authors are grateful to the staff of Shanghai Medical College of Fudan University for their support. The Authors also thank them for kindly providing the essential tools used in this study. The research was financially sponsored by Fudan's Undergraduate Research Opportunities Program (FDUROP, No.10081).

References

[1] M. Dybwad, P. E. Granum, P. Bruheim, and J. M. Blatnya, "Characterization of airborne bacteria at an underground subway," *Applied and Environmental Microbiology*, vol. 78, no. 6, pp. 1917-1929, 2012.

[2] A. Birenzvigea, J. Eversoleb, M. Seaverb, S. Francesconic, E. Valdesa, and H. Kulagad, "Aerosol characteristics in a subway environment," *Aerosol Science and Technology*, vol. 37, pp. 210-220, 2003.

[3] A. H. A. Awad, "Environmental study in subway metro stations in Cairo, Egypt," *Journal of Occupational Health*, vol. 44, pp. 112-118, 2002.

[4] T. Kawasaki, T. Kyotani, T. Ushiogi, Y. Izumi, H. Lee, and T. Hayakawa, "Distribution and identification of airborne fungi in railway stations in Tokyo, Japan," *Journal of Occupational Health*, vol. 52, pp. 186-193, 2010.

[5] K. Seino, T. Takano, K. Nakamura, and M. Watanabe, "An evidential example of airborne bacteria in a crowded, underground public concourse in Tokyo," *Atmospheric Environment*, vol. 39, pp. 337-341, 2005.

[6] L. Szam, I. Nikodemusz, L. Csatai, I. Vedres, and M. Dakay, "Airborne microflora found in some stations of the metro in the Hungarian capital of Budapest," *Zentralbl Bakteriol Mikrobiol Hyg B*, vol. 170, pp. 199-208, 1980.

[7] L. Szam, I. Vedres, L. Csatai, and I. Nikodemusz, "Further microbiological studies of the air in a newly built (under the pavement) section of the underground railway in Budapest," *Zentralbl Bakteriol Mikrobiol Hyg B*, vol. 177, pp. 312-318, 1983.

[8] E. Bogomolova and I. Kirtsideli, "Airborne fungi in four stations of the St. Petersburg underground railway system," *International Biodeterioration and Biodegradation*, vol. 63, pp. 156-160, 2009.

[9] Xinmin Net: The daily boarding in Shanghai metro is closing to 7 million. (2012, March). [Online]. Available: http://news.xinmin.cn/domestic/gnkb/2012/03/30/14245511.html

[10] Zhang Z : The consultation of metro air, Focus on Every Month, 2005, 12:46-48

[11] Ministry of Health Law and Oversight Division. Disinfection technical specifications [S]. Beijing: Ministry of Health, pp. 194-196, 2002.

[12] A. H. Lin, B. Y. Ye, F. F. Zeng, and R. Zhang, "Investigation of biological pollution in Shenzhen metro before opening," *Practical Preventive Medicine*, vol. 1, pp. 134-136, 2006.

[13] Z. C. Zhang, S. Y. Yu, Y. L. Wang, X. Y. Wang, J. S. Feng, and B. Y. Ye, "Ambient air quality of subway trains in Shenzhen," *Chinese Journal of Public Health Engineering*, vol. 6, pp. 343-344, 2007.

[14] S. H. Hwang, C. S. Yoon, K. N. Ryu, S. Y. Paik, and J. H. Cho, "Assessment of airborne environmental bacteria and related factors in 25 underground railway stations in Seoul, Korea," *Atmospheric Environment*, vol. 44, pp. 1658-1662, 2010.

[15] S. B. Gilleberg, J. L. Faull, and K. A. Graeme-Cook, "A preliminary survey of aerial biocontaminants at six London underground stations," *International Biodeterioration and Biodegradation*, vol. 41, pp. 149-152, 1998.

[16] F. Koksal, H. Yasar, and M. Samasti, "Antibiotic resistance patterns of coagulase-negative staphylococcus strains isolated from blood cultures of septicemic patients in Turkey," *Microbiological Research*, vol. 164, pp. 404-410, 2009.