Potential Pathogens in the School Environment

Zhicong Wang
Undergraduate, Biology

Key Words: Microbiology, School Health and Sanitation, Bacterial and Non-bacterial Pathogens

Abstract
Pathogenic microorganisms are potent threats to school health. In this experiment, Colony Forming Unit (a viable bacterial colony count) samplings were taken, in various regions of a school, of microorganisms (Staphylococcus aureus, various aerobic bacteria, and molds) in order to find a pattern of distribution between the colony count and the environment. Fifteen hall passes were sampled from three regions of a school, and then categorized into groups A, B, and C (each of five hall passes). It was hypothesized that regions near entranceways would contain more molds (Group A), regions in the vicinity of lavatories would contain more mold and yeast (Group B), and regions with most students would contain more Staphylococcus aureus and aerobic bacteria (Group C). Data overall supported the hypothesis: Group A registered a large count of mold, and Group B surpassed all other regions in the count of both mold and yeast colonies. Furthermore, Group C showed significantly more Staphylococcus aureus and other aerobic bacterial colonies than Group A or B.

Introduction

Pathogenic microorganisms are serious concerns in schools, where contact with various bacterial strains and other microorganisms occur frequently throughout the school day (Whitaker, 2005). Unlike non-pathogens, pathogens can cause disease in humans, whether bacterial or non-bacterial. Though only a small fraction of the thousands of species of bacteria and fungi are pathogenic, serious diseases can result if proper prevention and treatment do not take place. Therefore, schools must monitor strict microorganism counts in order to prevent serious outbreaks. In this experiment, the counts separate into two categories: bacterial pathogens (*Staphylococcus aureus* and aerobic bacteria) and non-bacterial pathogens (molds and yeasts).

Bacterial pathogens include *Staphylococcus aureus* bacteria and some species of aerobic bacteria. Commonly found in air and water and on human skin, *S. aureus* is known to cause pneumonia, septicemia, and toxic shock syndrome, as well as wound infections and food poisoning. As the number of drug-resistant strains continues to grow at an alarming rate—present supplies of viable antibiotic and anti-bacterials that can be used to treat and prevent infections are being exhausted. According to research by Wertheim and Verbrugh (2006) of the Erasmus University Medical Center, "In the USA, community-acquired MRSA (meticillin-resistant *S. aureus*—bacteria resistant to meticillin, a semisynthetic penicillin) have outrun susceptible *S. aureus* infections as causes of skin and soft tissue infections (p. 368)." Furthermore, in another study conducted at the University of Celal Bayar, Turkey, three of four popular drugs (dexmedetomidine, etomidate-lipuro, and propofol) were shown to be ineffective in inhibiting the growth of *Staphylococcus aureus* isolated from hospital patients (Keleş et al., 2006). In contrast to *S. aureus,* aerobic bacteria are characterized by their need of atmospheric oxygen in metabolism for survival; they are the most prevalent form of microorganism present both around the human environment and on the human body, including organisms such as *E. coli*

or *Salmonella spp*. In fact, four out of the top five illnesses that keep students from school are caused by bacterial pathogens: stomach flu (gastroenteritis), ear infection (otitis media), pink eye (conjunctivitis), and sore throat from streptococci bacteria (Mayo Clinic, 2006). Together, *S. aureus* and pathogenic species of aerobic bacteria constitute a large percentage of pathogenic microorganisms.

Non-bacterial pathogens include species of mold and yeast (fungi). Molds tend to be external parasites of humans, causing ringworm, athlete's foot, and jock itch, while yeasts invade internal tissues, infecting the genital tract or activating allergies and other respiratory diseases. Commonly found in moist and dark areas, mold and yeast proliferate in entrances around the school: to hallways, lavatories, and classrooms. According to a hypothetical "safe [mold] contamination remediation project," contamination by fungi through airways and entranceway surfaces are highlighted as two of the most prevalent forms of transmission (Wayne, 2006). Also to be closely monitored, molds and yeasts make up much of the remaining percentage of pathogenic microorganisms.

Methods

Materials

*Yeast and Mold (YM) Petrifilm: 10 (1 for each sample)
*Aerobic Count (AC) Petrifilm: 10 (1 for each sample)
*Rapid Staphylococcus aureus (RSA) Petrifilm: 10 (1 for each sample)
Standard Colony Counter (Quebec Counter): 1
Micropipette (1000 μm): 1
Micropipette tips: 10 (1 for each sample)
5 mL Sterile Water Tubes with Hall Pass Swab Samples: 10 (1 for each sample)

Design

In this experiment, four different surfaces were tested: wooden hall passes, students' hand, and a bathroom door and sink. Each sample area was swabbed in a 2 $in.^2$ region using sterile swabs and then plated onto three kinds of Petrifilm. The three different Petrifilm (culture mediums containing nutrients and indicators that assist colony enumeration) used were AC, for detecting aerobic bacteria; RSA, for detecting *Staphylococcus aureus*; and YM, for detecting yeasts and molds. After incubation (48 hours at 35ºC for AC, 4 days at 25ºC for YM, and 24 hours at 35ºC for RSA), the samples were retrieved for analysis.
* Petrifilm used was a courtesy of the local 3M Corporation.

In the assistance of a Quebec Counter, the Petrifilm samples were counted. Following standard colony count guidelines (3M), four grids of each Petrifilm sample were hand counted. The four respective numbers were averaged, and then multiplied by 20 (the number of grids on each Petrifilm sample) to arrive at the number in 1 mL in sample, and then by 5, to get the number in 5 mL (volume of the original sample), and finally by 22 (the ratio of original surface area of 2 $in.^2$ to total surface area of 44 $in.^2$ to arrive at the final count of the CFU (Colony Forming Units).

Procedure

When replicating the procedure, first, collect all samples using aseptic swabs and 10 mL tubes of sterile water. Use a sterile swab and new water container for each sample. Wearing hand gloves, remove the swab from its sterile wrap. After dipping into the tube of 10mL of sterile water, move the swab first horizontally across the hall pass. Repeat the procedure vertically across the same sample medium while slightly turning the swab, to assure greatest amount of

contact. Take the swab and break the cotton end along with about 2-3 cm of the wooden stalk off. Place the swab into the 10mL container of sterile water. Do not keep samples longer than 24 hours without plating.

Second, prepare the sample for plating onto Petrifilm. Place each 10mL container sample into a centrifuge so that bacteria will settle on the bottom of the container for easy extraction. Meanwhile, arrange the needed Petrifilm: Aerobic Count Plate (AC), Rapid *S. aureus* Plate (RSA), and Yeast and Mold Plate (YM). When finished, attach a sterile tip onto the end of a micropipette. Verify that the increment on each micropipette is 1000μm, and withdraw that amount from each sample, using a new tip for each sample. Remember to continue plating onto a Petrifilm until all contents from the micropipette tip is emptied. After plating, use the marked Petrifilm spreaders (separate for AC, YM, and RSA) to spread the pipetted sample evenly upon the Petrifilm.

Third, incubate each Petrifilm sample as indicated. AC plates are to be incubated for 48 hours at 35ºC. YM plates for 4 days at 25ºC. The procedure for incubation of RSA plates involves several steps. After the initial 24 hours of incubation at 35ºC, remove from the incubator and keep at 62ºC for 2 hours. During this time, prepare to insert the TNase reactive disk. With sterile forceps, remove one round TNase disk from its outer frame. Lift the top layer of the Petrifilm and place the disk in the well of the Petrifilm. Gently apply pressure across the reactive plate area, minimizing any air bubbles and ensuring uniform contact. Finally, incubate RSA plates with disk inserts for another 2 hours at 35ºC.

Lastly, count each AC, YM, and RSA plate. Adhere to the following guidelines for legal microorganism counting: *Staphylococcus aureus* should have a distinct surrounding pink hallow and be in individual round colonies, aerobic bacteria should be red in appearance and be in round clusters, yeast should be green and appear in distinct round colonies, and molds should be distinctly black or green. After identifying respective colonies, use the Standard Colony Counter (Quebec Counter) to count the number of microorganisms in four grids. Then average the four counts (divide by four) and multiply first by 20 to find the number of microorganisms present on one Petrifilm sample. Next, multiply the figure again by 5 to arrive at the number present in the original 5mL sterile water tube. Finally, multiply the number by 22, the ratio of the surface area swabbed (2 square inches) to the total surface area (44 square inches).

Results

Table 1

Microbiological Counts of AC, RSA, and YM in Groups A, B, C of 15 Hall Pass Surfaces

		AC	**RSA**	**Mold**	**Yeast**
Group	**Hall Pass**	CFU	CFU	CFU	CFU
	1	2200	3300	4950	1100
	2	2750	1650	6050	0
A	3	2200	2200	6600	550

	4	3300	1100	7700	0
	5	2750	1650	7150	0
	A Average	**2640**	**1980**	**6490**	**330**
	6	4400	6050	1650	4950
	7	7700	4400	4950	3300
B	8	8800	4950	5500	4400
	9	11000	6600	3850	2750
	10	7150	6050	4400	3850
	B Average	**7810**	**5610**	**4070**	**3850**
	11	11000	16500	550	0
	12	16500	14300	1100	550
C	13	22000	13200	1650	0
	14	19800	11000	550	0
	15	20900	10450	0	0
	C Average	**18040**	**13090**	**770**	**110**

Note: All of the figures above are in CFU units and are the finalized tabulations.

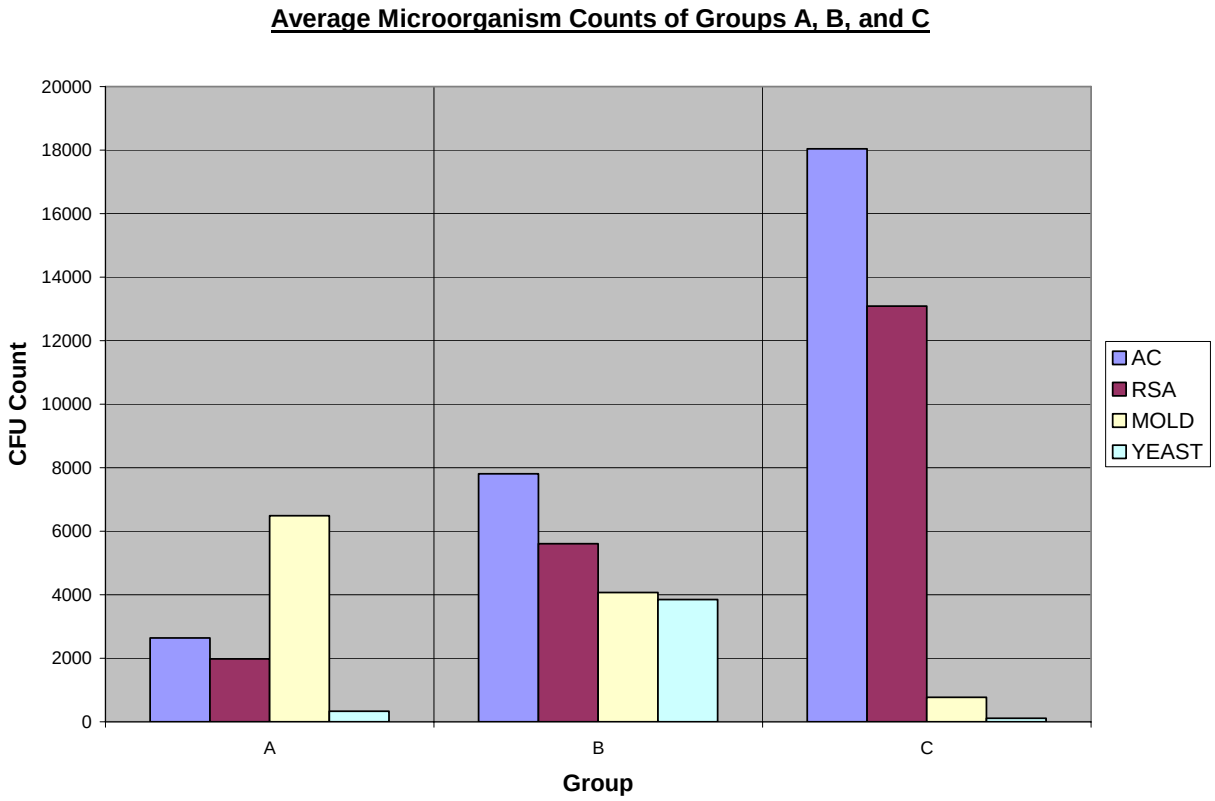

Figure 1. Bar graph of average microorganism counts of all groups.

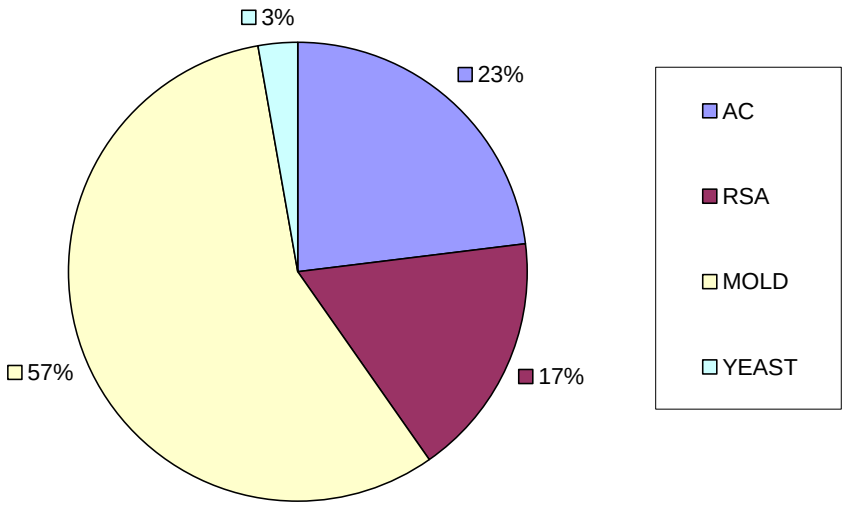

Figure 2. Pie chart of microorganism distribution for Group A hall passes.

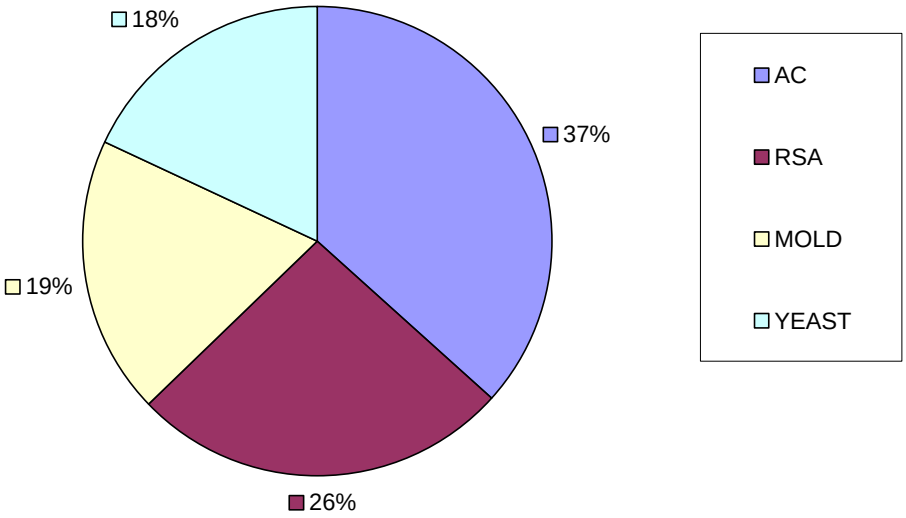

Figure 3. Pie chart of microorganism distribution for Group B hall passes.

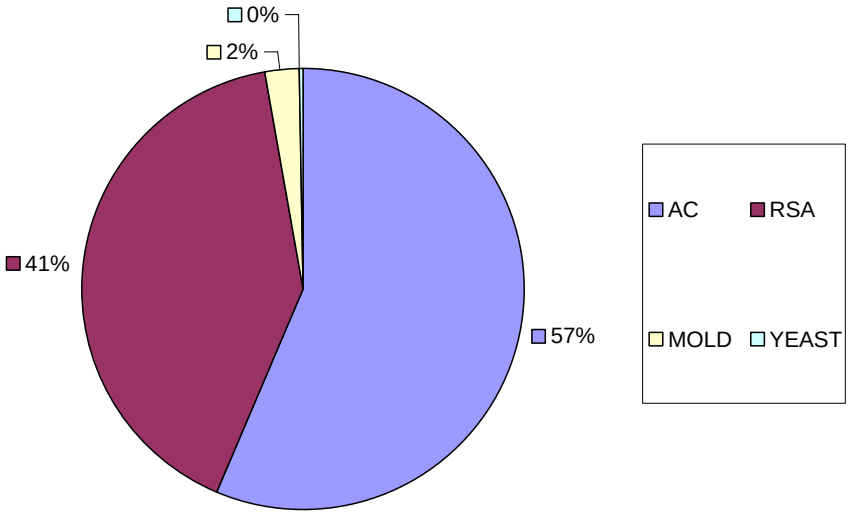

Figure 4. Pie chart of microorganism distribution for Group C hall passes.

Discussion and Conclusion

According to the final tabulations, the original hypothesis concerning the relationship between regions and counts was supported. Group A, regions near entranceways, had a higher percentage and count of mold than Group B and C, 57% and 6490 CFU, respectively. Moreover Group B, regions near lavatories, showed greater counts and percentages of both mold and yeast: 19% and 4070 CFU for mold, and 18% and 3850 CFU for yeast. And finally, Group C, regions with the most students, displayed a significant count of *Staphylococcus aureus* and other aerobic bacteria: 41% and 13090 CFU for *S. aureus,* and 57% and 18040 CFU for aerobic bacteria. Evidently, the three different counts (AC, YM, and RSA) varied greatly depending on location and the amount of exposure to students.

Thus with the presence of hundreds and thousands of potentially pathogenic microbial colonies upon a single surface, prevention of infections and illnesses remains a serious issue in schools. But with careful considerations and stringent control methods, school microbial colony counts of bacteria and fungi and related pathogens can be kept under control.

Bacterial pathogens can be controlled by adhering to simple hygiene regulations and infection prevention guidelines. General aerobic bacteria should also be given careful consideration to prevent outbreaks. As stated by Mayo Clinic (2006) on Children's Health, "[Parents] can prevent the spread of illness by not sending a sick child to school" or treating their children with antibiotic therapy (p. 16). Moreover, good personal hygiene and proper hand-washing techniques can also diminish the possibility of aerobic bacteria outbreaks. On the other hand, *Staphylococcus aureus* infection rates can be controlled by transmission limits. Sarah Huang, a Harvard Medical School researcher, found that even staying in a clean room previously occupied by someone with MRSA (a widespread strain of *S. aureus* bacteria) may increase the odds of acquiring such bacteria (as cited in Myron, 2006). Thus in schools, according to the Illinois Department of Public Health on prevention of *Staphylococcus aureus*, "high-touch surfaces (e.g., doorknobs, light switches, drinking fountains, faucet handles, and surfaces in and around toilets) [should be] cleaned daily" (Whitaker, 2005). Additionally, items that are visibly soiled with blood or other body fluids are to be cleaned with chlorinated bleach (1:10 dilution) or other germicidal products.

Fungal pathogens and mold contamination can also be solved. Under a method approved by the Centers for Disease Control and Prevention, effective mold remediation can be carried out with sanitization and a thorough air conditioning system using a high-efficiency particulate air (HEPA) filter (Wayne, 2006). Also, yeast infections may be managed by treatment with certain antibiotics, such as those derived from the bacteria *Streptomyces norsei*, which contains antifungal properties that are used to treat vaginal yeast infections.

Because of the growing presence of microorganisms and pathogens in the school environment, schools today must be vigilant for pathogenic microorganisms. As more pathogens mutate and countless new strains are created, students and faculty are more at risk than ever. But as research continues to explore new venues of treatment and prevention, the prospect of minimizing pathogenic presence remains bright.

References

Keles, G. T. Kurutepe, S., Tok, D., Gazi, H., & Dinc1 G. (2006). Comparison of antimicrobial effects of dexmedetomidine and etomidate-lipuro with those of propofol and midazolam. *European Journal of Anaesthesiology*, *23*, 1037-1040.

Mayo Clinic Staff. (2006, Aug 1). Children's illness: Top 5 causes of missed school. Retrieved January 6, 2007, from http://www.mayoclinic.com/health/childrens-conditions/CC00059

Myron, K. (2006). Transmission of antibiotic-resistant bacteria linked to previous intensive care unit room occupants. *JAMA and Archive*, *166*, 1945-1951.

Wayne, H. (2006). Mastering mold. *Health Facilities Management*, *19*, 33-37.

Wertheim, H. & Verbrugh, H. (2006). Global prevalence of meticillin-resistant Staphylococcus aureus. *Lancet, 368*, 1866.

Whitaker, E.(2005). Recommendations for the prevention of Staphylococcal infections for schools. Retrieved December 9, 2006, from http://www.idph.state.il.us/health/infect/schoolstaphrecs.htm