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Biology 110: Morrison

Bacteria Found on Frequently Used Surfaces on Campus

Introduction:

Humans come into contact with a multitude of different surfaces on a daily basis. When we touch these surfaces, we simultaneously come into contact with the various types and amounts of bacteria thriving on them. Fortunately, most bacteria are not harmful to human health and some are even needed for the proper functioning of the body, such as those that make up the skin and intestinal flora (Podesta 2010). However, infectious bacteria reproduce quickly within the body and have the potential to cause a wide variety of illnesses (National Institutes of Health 2014).

It is imperative to take special precautions, such as disinfecting wounds and frequent hand washing, in places where bacteria flourish. Such places include locations open to the public and equipment in athletic facilities because they offer an optimal habit and plentiful nutrients for bacteria to thrive (Collins et al. 2012). It is important for humans to study bacteria encountered on these surfaces because a number of these bacterial species can be dangerous to human health (Memis 2013). It is additionally important to continually conduct studies on bacteria because new or evolving pathogens are always in the process of developing, posing potentially significant health risks (Collins et al. 2012).

Gram-negative bacteria are responsible for many of the more dangerous infections in humans because their structure makes them resistant to many antibiotics. They possess a double lipid bilayer that encloses a peptidoglycan layer, plus an outer lipopolysaccharide layer. This results in a low degree of permeability and makes it hard for antibiotic molecules to enter the bacterial cell. Gram-positive bacteria possess a single lipid bilayer and a porous peptidoglycan layer, which makes them more susceptible to antibiotics and easier to treat (Sperandio et al. 2013). Because of the differences in susceptibility to various antibiotics, it is important to know if bacteria are Gram-positive of Gram-negative when trying to determine the best treatment for a bacterial infection (Morrison and Newman 2014).

Certain surfaces provide an optimal habitat for bacterial life, while others inhibit bacterial growth. Hydrophobic, nonpolar materials, such as many types of plastics, are more likely to contain bacteria than hydrophilic, polar substances, such as glass and metals (Donlan 2002). The objective of the experiment is to determine which of our commonly used surfaces contain the most bacteria and to study various characteristics of our bacterial samples, such as shape, size, color, colony morphology, and whether they are Gram positive or Gram negative.

Our hypothesis is that the playground window, the soccer field turf, and the shin guards will contain the most bacteria because they are not frequently disinfected. To test this, we will swab various locations and place the bacteria onto agar plates to grow. We will then count the number of colonies and observe the various characteristics of the bacteria. Since each colony represents a single bacterial cell that was present when the surface was first swabbed, the number

of colonies on the plate is proportional to the number of bacteria that came from the original surface (Brooker et al. 2014).

Methods:

Three lysogeny broth agar plates were obtained and divided into four quadrants each, using a Sharpie marker. The plates were labeled on the bottom as opposed to the lid in case the lids were accidentally switched. A sterile swab, moistened with Luria broth in order to allow for the adhesion of the bacteria, was used to lightly rub *E. coli* bacteria, the positive control, onto one of the quadrants. It was labeled + Control. A wet sterile swab containing no bacteria was applied to another quadrant for the purpose of a negative control. It was labeled – Control.

Next, our group traveled around campus to swab various locations that are known to contain copious amounts of bacteria. At each location, we used a new sterile swab dipped in Luria broth to procure the bacteria. We gently rubbed the wet swab on each surface to pick up the bacteria, and then we gently rolled the swab onto the labeled quadrant of the agar plate in order to place the bacteria in its designated quadrant.

When swabbing each location, we took special care to use sterile technique, including keeping the plates and the Luria broth closed as much as possible, and being careful not to touch or breathe on the plates, which would contaminate them with unwanted bacteria. The locations that we obtained bacteria from included a playground window, playground monkey bars, car keys, the Heim building computer lab keyboard, the door handle to the men's soccer locker room, an outside door handle from Morrone's Pub, synthetic grass from the soccer field, turf pellets from the soccer field, soccer shin guards, and the field house elevator button.

The surface that the bacteria were obtained from was the independent variable in this experiment and the bacterial growth was the dependent variable. The research hypothesis was that the playground window, the soccer field turf, and the shin guards would contain the most bacteria because they are not frequently disinfected. The alternative hypothesis was that different surfaces would contain varying amounts, types, sizes, and shapes of bacteria. The null hypothesis was that there would be no difference in amount, types, or sizes of the bacteria taken from the different locations.

The samples were left in the lab at room temperature for two days and in a refrigerator for two days so they could have a chance to feed on the nutrients of the agar plate and multiply in number at various temperatures. After the bacteria were given ample time to multiply, we observed the number of colonies and their morphology and color for each of the surfaces.

Each group member performed a Gram stain on bacteria from two different surfaces. This was accomplished through a series of steps which can be found in the Bio 110 Lab Manual (Morrison and Newman 2014). First, a sterile wooden stick was used to smear a small amount of the bacteria on a clean glass slide. The bacteria were spread out in a thin layer in order to allow optimal viewing of the organisms. We allowed the smears to dry at room temperature for approximately two minutes.

After the smears were given time to dry, we heat-fixed them to the slides by quickly passing them through the flame of the Bunsen burner, using a metal clamp to hold the slides. We took caution when heat-fixing the slides to avoid overheating them.

Next, we placed the smears on a rack over a staining tray and covered them with crystal-violet stain. After allowing the slide to react for one minute, we used a wash bottle to lightly rinse the slide with water. Then we shook the slide to remove excess water.

Next, we covered the smears with Gram's iodide stain, allowed them to react for one minute, and washed them off with water.

In the next step of the Gram staining procedure, we held the slides at a 45° angle over the staining tray and applied 4-5 drops of alcohol at the top of the slide, allowing it to run off into the staining tray. Then we immediately rinsed the slides with water to avoid overdecolorization. The excess water was removed by shaking.

Finally, we counterstained the slides by flooding them with safranin for one minute. We rinsed the slides with water and blotted them dry by placing them between pages of a bibulous pad. In order to increase visibility of the bacteria, we wiped the bottom of the slide to remove excess stain.

We observed our Gram stains by using the 10X and 40X objective first. Then we switched to the 100X objective. In order to use the 100X objective, we placed a drop of immersion oil onto each of the slides and rotated the 100X into position, making sure that the oil was touching both the lens and the slide. After observing the shape, size, and color of the bacteria, we cleaned the 100X objective using lens tissue.

Results:

We observed the most bacterial growth in the quadrant containing bacteria from the playground window (Figure 1, bottom left corner). Large, white colonies and a few red colonies covered the entire quadrant for this sample. Upon examination under the microscope, the bacteria from the playground window were determined to be Gram-positive bacilli bacteria (Figures 4, 5, and 6). The positive control, which had a white lawn of bacteria covering most of its quadrant, also exhibited significant bacterial growth (Figure 3, bottom right corner).

The Heim computer lab keyboard, the men's soccer locker room door handle, the playground monkey bars, and the positive control all contained Gram-positive, bacilli bacteria when examined under the microscope (Figures 7, 8, 5, and 6 respectively). The morphologies of these colonies were mostly small, circular, and white (Figures 1 and 3).

The bacteria from the shin guards were present in numerous small white and orange colonies (Figure 8, right side). When examined under the microscope, they were determined to be Gram-negative cocci bacteria (Figure 9).

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The bacteria from the soccer field turf pellets were present in numerous small circular colonies (Figure 2, left side). Under microscopic examination, they were determined to be Gram-variable bacilli (Figure 8).

The soccer field grass and the negative control also exhibited bacterial growth in the form of white, circular colonies (Figures 2 and 3). Gram stains were not performed on these samples. The car key remote pad, the field house elevator button, and the pub door handle did not show bacterial growth (Figures 1, 2, and 3 respectively).

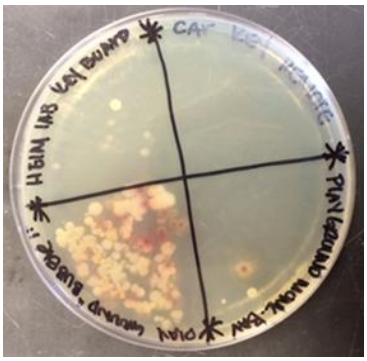

Figure 1: Plate containing bacterial growth from the Heim computer lab keyboard, a car key remote, a playground window, and monkey bars.

Figure 2: Plate containing bacterial growth from soccer turf pellets, soccer turf grass, soccer shin guards, and the field house elevator button.

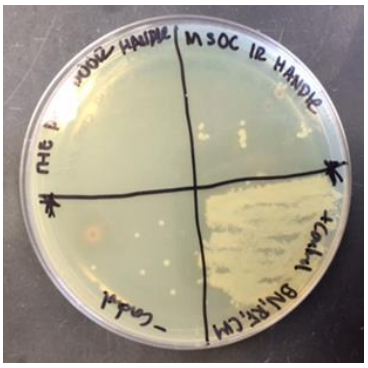

Figure 3: Plate containing bacterial growth from the door handle at Morrone's Pub, the men's soccer locker room door handle, and the positive and negative controls.

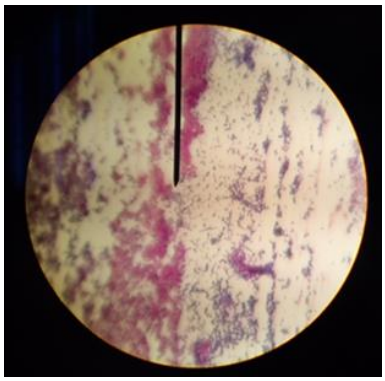

Figure 4.1: Gram-stained bacteria from the playground window under 100X magnification.

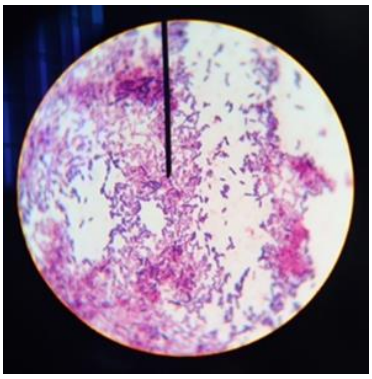

Figure 4.2: Gram-stained bacteria from the playground window under 400X magnification.

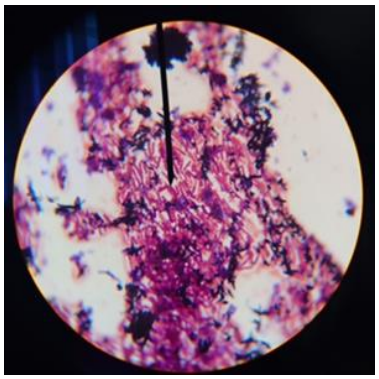

Figure 4.3: Gram-stained bacteria from the playground window under 1000X magnification.

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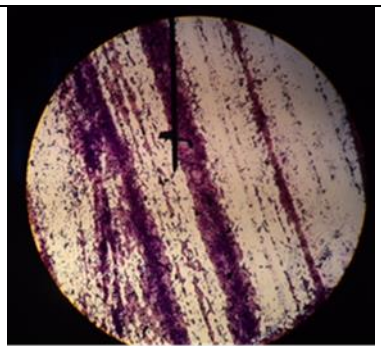

Figure 5.1: Gram-stained bacteria from playground monkey bars under 1000X magnification.

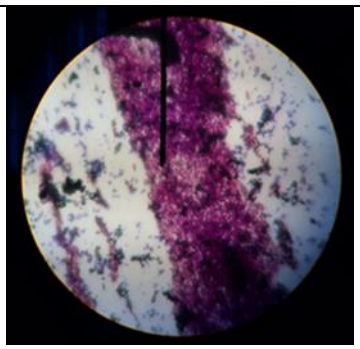

Figure 5.2: Gram-stained bacteria from playground monkey bars under 1000X magnification.

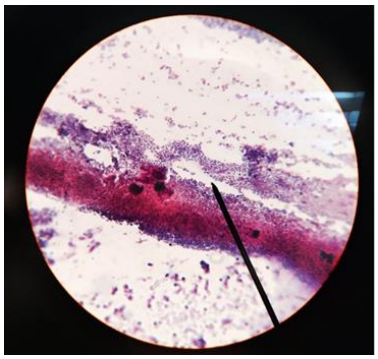

Figure 6: Gram-stained bacteria from the positive control under 400X magnification.

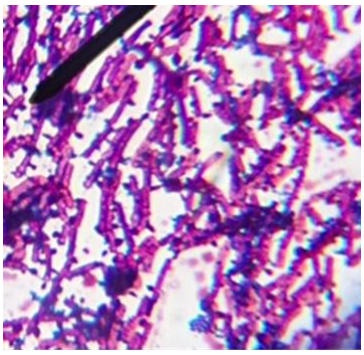

Figure 7: Gram-stained bacteria from a keyboard in the Heim computer lab under 1000X magnification.

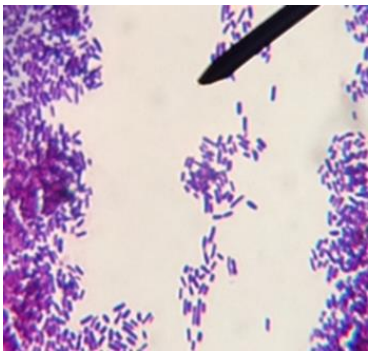

Figure 8: Gram-stained bacteria from the men's soccer locker room door handle under 1000X magnification.

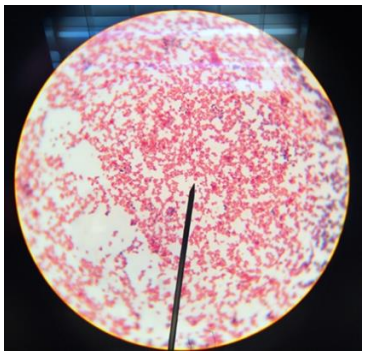

Figure 9: Gram-stained bacteria from a soccer shin guard under 1000X magnification.

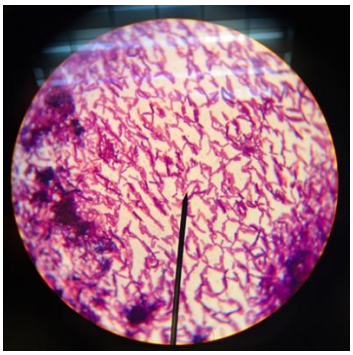

Figure 10: Gram-stained bacteria from the soccer field turf pellets under 1000X magnification.

Table 1: Bacterial Growth Results

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Negative Control
Positive Control

Table 1 contains the results of the bacterial growth from each of the surfaces, including the colony morphology, Gram stain results, shape, and size in um.

	Location	Colony Morphology	Gram (+/-)	Shape	Size (um)
Nga	Heim computer lab keyboards	1 small round yellowish, 10 white fuzzy	Positive	bacillus	length: 4.444, width: 0.444
Nga	Men soccer locker room handles	20 smooshed colonies, 1 big reddish	Positive	bacillus	length: 1.429, width: 0.476
CM	Playground Monkey Bars	White; Smooth, Round edges; 3 colonies	Positive	Baccilli	5.0 uM (length), 1.0 (width)
CM	Playground Window Bubble	White; Dry, Rough edges; About 157 colonies	Positive	Baccilli	2.58 uM (length), 0.5 uM (width)
RF	Postive Control	white lawn	N/A	N/A	N/A
RF	Negative Control	7 circular colonies: 6 white, 1 red	Positive	Baccili	2um long by 1um wide
BN	Shin Guard	11 very fine Colonies (white), 4 Big Colonies (orange)	Negative	Cocci	Diameter: 0.8um
BN	Artifical Turf Pellets	61 Colonies: Circular/White Glossy/Smooth. Few Red "Fuzzy"	Pos/Neg	Baccili	Length: 2.62um , Width: .32um

Discussion:

Overall, we were able to conclude that playground window and the positive control contained the most bacterial growth (Figures 1 and 3). We also discovered that the shin guards and the turf pellets both contained Gram-negative bacteria, which supports the idea in the introduction that athletic facilities contain an abundance of bacteria (Oller et al. 2010).

The results partially supported our hypothesis because the playground window, the shin guards, and the turf pellets all contained a significant amount of bacteria; however, the turf grass (which comes into contact with the turf pellets) surprisingly did not contain a large quantity of bacteria; this was the opposite of our prediction.

Another anomaly that we observed in this experiment was the presence of bacterial growth in the negative control quadrant (Figure 3). The negative control should not have yielded any bacterial colonies because, in theory, it should not have contained any bacteria to start with. However, it was raining on the day that we collected out samples, so bacteria present in rain drops could have contaminated the negative control (Sattler et al. 2001). Another possible explanation for the presence of bacteria in the negative control could be that one of the group members accidentally touched or breathed on the agar and transferred bacteria onto the plate.

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We were surprised to discover the car key remote, the elevator button, and the pub door handle did not yield significant bacterial growth. Looking back to the introduction, the pub door handle probably was not a suitable environment for bacteria to live on because it was made of metal, which does not provide a suitable environment for bacterial growth (Donlan 2002). The car key remote and the elevator button, however, were expected to contain some bacteria because they come into frequent contact with human hands and they are made of plastic which is a suitable material for bacterial growth. A possible explanation for why they did not contain bacteria is that they were disinfected recently before we collected our bacterial samples.

A follow up experiment could build on our results, which showed that surfaces at playgrounds and sports facilities contain large amounts of bacteria. The follow up experiment could focus specifically on these locations and test more surfaces present at these places. It could also utilize fluorescent DNA-binding dye which is helpful when the conditions needed to grow bacteria in a laboratory are not known (Brooker et al. 2014).

References:

Brooker RJ, Widmaier EP, Graham LE, Stiling PD. (2014). "Archaea and Bacteria" in Biology (Third Edition) McGraw-Hill Higher Education Publishers, New York. p. 533-552.

Collins CJ, O'Connell B. (2012). "Infectious Disease Outbreaks in Competitive Sports, 2005-2010". *Journal of Athletic Training.* Vol. 47:516-518. (BN)

Donlan RM. (2002). "Biofilms: Microbial Life on Surfaces". *Emerging Infection Diseases*. Vol. 8:881-890. (RF)

Memis UA. (2013). "The Risk of Staphylococcus aureus Strains Isolated from Playgrounds". *Life Science Journal.* Vol. 10:3198-3203. (CM)

Morrison ME, Newman J. (2014). "Bio 110 Laboratory Manual Activity #3 – Microscopic Examination of Cell Structure". Lycoming College, Williamsport, PA. p. 36-60.

National Institutes of Health. (2014). "Bacterial Infections" http://www.nlm.nih.gov/medlineplus/bacterialinfections.html. November, 28 2014. This is a legitimate Web site because it was developed by a government agency. (RF)

Oller AR, Province L, Curless B. (2010). "Staphylococcus aureus Recovery from Environmental and Human Locations in 2 Collegiate Athletic Teams". *Journal of Athletic Training.* Vol. 45:222-229. (BN)

Podesto, M. (2010). "Infectious Agents" in The Ultimate Medical Encyclopedia Legault, MA, Batigne S, ed. Firefly Books, Buffalo, p. 282-285. (RF)

Ramos JL, Gallegos MT, Marques S, Ramos-Gonzalez MI, Espinosa-Urgel M, Segura A. (2001). "Responses of Gram-Negative Bacteria to Certain Environmental Stressors". *Current Opinion in Microbiology.* Vol. 4:166-171.

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Rutala WA, White MS, Gergen MF, Weber DJ. (2006). "Bacterial Contamination of Keyboards: Efficacy and Functional Impact of Disinfectants". http://www.jstor.org/stable/10.1086/503340. November, 27 2014. This is a legitimate Web site because it contains information regarding Bacillus Gram-positive bacteria, which we observed in some of our cultures. (ND)

Sattler B, Puxbaum H, Psenner R. (2001). "Bacterial Growth in Supercooled Cloud Droplets". *Geophysical Research Letters.* Vol. 28:239-242. (RF)

Sperandio FF1, Huang YY, Hamblin MR. (2013). "Antimicrobial Photodynamic Therapy to Kill Gram-Negative Bacteria". *Recent Patents on Anti-Infective Drug Discoveries.* Vol. 8:108-120. (ND)