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Athletes' Funk: A Study of Bacterial Communities at Saint John's University Athletic Facilities

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Abstract

For our research project, bacterial swabs were taken in both the baseball locker room at the Saint John's Palaestra facility and hockey locker room at the SCSU Herb Brooks National Hockey Center. The bacterial samples were then isolated and subsequently tested for antibiotic resistance. PCR was done in an attempt to identify the most heavily antibiotic resistant cultures. The goal of this research was to identify bacteria that pose danger to athletes upon exposure. Due the nature of the different sports in question as well as the separate facilities, we wanted to assess the comparative risk of exposure between the different groups of athletes.

Introduction

Bacteria in athletic facilities pose a threat to athletes who may be exposed through open wounds and ingestion. Due to rapid adaptability, these bacterial populations can become resistant to multiple antibiotics, increasing the threat to humans. Multidrug resistant bacterial strains can be life threatening due to difficulty of treatment. A primary bacteria of concern is Staphylococcus aureus. In recent years this bacterial strain has become methicillin resistant, as well as increasingly resistant to other antibiotics. MRSA as it has come to be known, accounts for the majority of Staphylococcus infections and in serious cases has caused death (2). For this reason, the microbiome of healthcare and athletic facilities has garnered much interest over the last two decades. MRSA is transmitted direction through contact person-person, object-person, and person-object (5). Methicillin is a disruptor of cell wall synthesis, thus a bacteria that is resistant to methicillin has an increased likelihood to be resistant to other antibiotics whose mechanism of action is inhibition of cell wall synthesis. Indoor environments increasingly pose a greater risk due to optimal growth environments for bacteria. As humans continue to spend more time in these environments, individuals put themselves at greater risk for infection (7). Different surfaces in indoor environments pose unique threats to individuals based on the interactions that humans have with each surface. For example, research shows that while floors are the most distinct surface due to presence of foreign material transported from the external environment, while surfaces that come in direct contact with skin tend to be most similar in bacterial makeup (7). Interactions with skin thus shape much of the microbiological community on all surfaces. This research focuses on the microbiome of comparable athletic facilities at Saint John's University to assess the risk of exposure of these athletes to potentially harmful bacteria. Understanding that each surface poses a different threat based on human interactions is important to assessing microbiome and its risk to athletes.

Methods

For our data collection, standard practices for bacterial swabbing were used to obtain samples from the Saint John's University baseball locker room at the Palaestra facility and hockey locker room at the SCSU Herb Brooks National Hockey Center . The swabs were taken from different locations within the facilities including the water fountain, shower, stall/locker, and the sink. Equivalent pieces of equipment were also sampled including helmet/baseball cap and

gloves. The collection took place between post-practice. Immediately after sampling took place the swabs were transported to the lab for plating of the samples. After 1 week of incubation time the samples were observed for bacterial growth. Characteristics of bacterial colonies in locations with bacterial growth were recorded for color, shape, and quantity. Cultures of interest based on characteristic shape, size, color, and growth pattern were then marked for isolation. They were subsequently scraped off of the old DB agar plates and isolated onto fresh DB agar plates. After a week incubation period, the cultures from the hockey and baseball facility were plated separately onto lawns with 5 antibiotic disks of different types spread out on the plates (4). After a week of incubation the cultures were observed for antibiotic resistance. Cultures found to exhibit resistance to more than one drug were then once again isolated. Ampicillin immobilized plates were prepared, and the cultures found to be resistant to multiple drugs were plated into lawns on separate AMP plates. Disks of the other 4 antibiotics used previously were spread out on the plates (4). The plates were observed after a week of incubation, resistance, as well as susceptibility data including zones of inhibition were recorded. The 2 most resistant strains were then put through 16s rRNA PCR using standard methods.

Results and Discussion

Initial bacterial colony analysis results are listed in tables 1 and 2. Pictures of these colonies and the colonies isolated can be seen in figures 1 and 2. A wide variety of cultures were found and there was a high level of variance in the appearance of cultures collected from various locations within each facility. Two of the locations within the baseball locker room showed no bacterial growth, these were the sink and the stall. The samples collected from the hockey locker room all had significant bacterial growth and wider variety of colonies present.

Table 1: This table displays quantitative and qualitative information regarding the bacterial swabs taken on January 25th from the hockey locker room at SCSU Herb Brooks National Hockey Center.

Bacterial Colony Analysis			
Location	**Size**	**Color**	**Number**
Helmet	mostly small	yellow and white	500+
Shower	all small	yellow, white, and orange	1000+
Water Fountain	very small	transparent	1000+
Sink	large	yellow and white	10-15
Stall	small and medium	yellow, and white	200+
Gloves	small and medium	yellow, white, and brown	200+

Table 2: This table shows quantitative and qualitative information regarding the bacterial swabs taken on January 25th from the baseball locker room at the St. John's University campus.

Bacterial Colony Analysis			
Location	**Size**	**Color**	**Number**
Helmet	medium	yellow	50
Shower	small	yellow and red	200+
Water Fountain	small and medium	transparent/yellow	1
Sink	-	-	-
Stall	-	-	-
Gloves	small	yellow	50

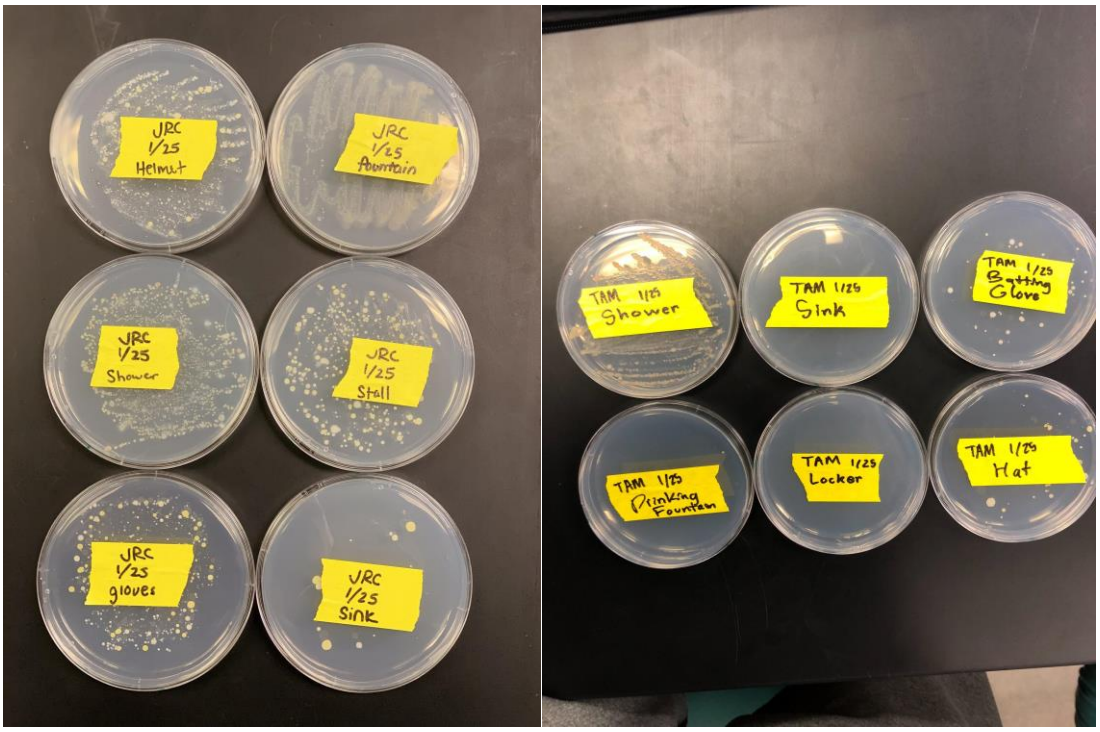

Figure 1: Swab cultures from the 6 different locations within the Saint John's University athletic facilities (left:hockey, right:baseball).

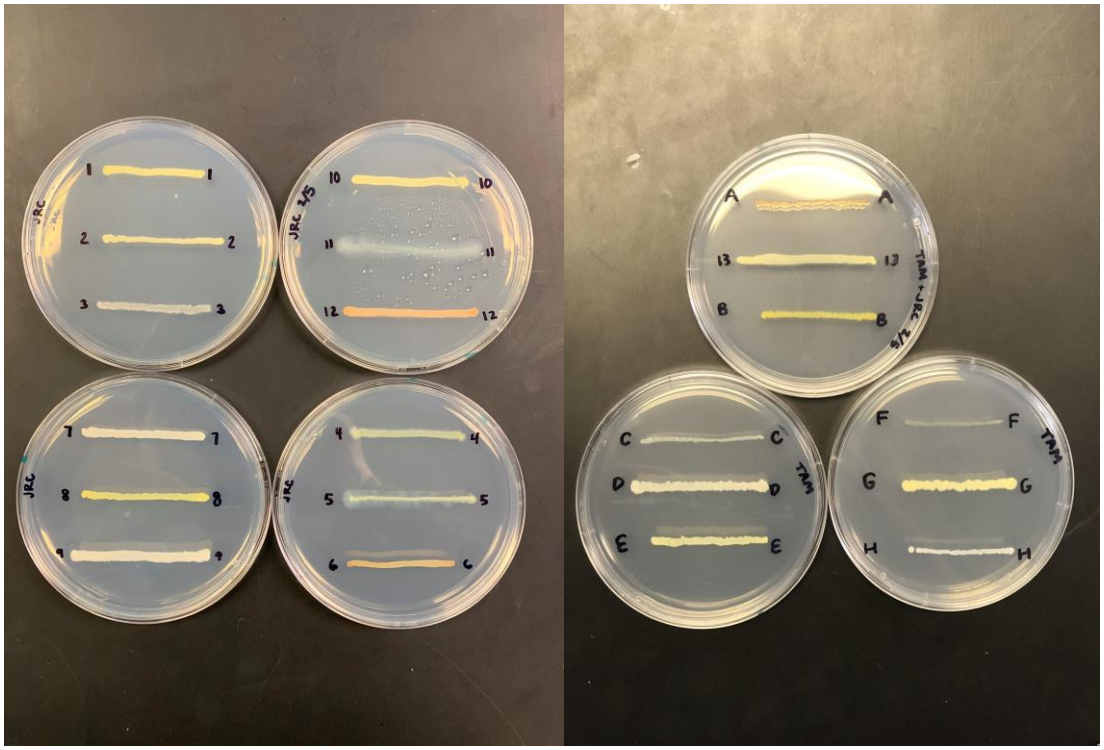

Figure 2: Isolated cultures of interest from swabs taken at the Saint John's University athletic facilities (Left: hockey, Right: baseball)

Table 3: Listed in this table are the antibiotics used on the various bacterial cultures. These antibiotics were administered as circular discs and were equally distributed among the agar plate.

Drugs Tested	Method of Action	Dosage (ug/disk)
Ciprofloxacin (CIP)	DNA synthesis inhibitor	5
Sulfamethoxazole Trimethoprim (SXT)	Metabolic inhibitor/DNA systhesis inhibitor	23.75 **S** 1.25 **T**
Ampicillin (AM)	Cell wall synthesis inhibitor	10
Cefotaxime (CTX)	Cell wall synthesis inhibitor	30
Cephalothin (CF)	Cell wall synthesis inhibitor	30

Drugs were chosen based on availability to the researchers. Three of the drugs had very similar methods of action as cell wall inhibitors, while the two others had different methods of action (table 3). Use of different drug types enabled drug synergy to be observed between different drugs.

Table 4: This table displays bacterial cultures taken from various locations within the hockey locker room and shows their resistance and susceptibility to several different antibiotics.

Bacterial Cultures		Resistance (+) vs. Susceptibility (-)					Notes
Location	Culture #	SXT	CF	CTX	CIP	AM	
Sink (H)	1	+	-	-	-	-	
	2	-	-	-	-	-	
	3	+	+	-	+	+	Lots of drug synergy
Fountain (H)	4	+	+	-	-	+	
	5	-	+	+	-	+	
Gloves (H)	6	-	+	+	-	+	Synergy b/t AM+SXT
	7	+	-	-	-	-	
	8	-	-	-	-	-	
Stall (H)	9	+	-	-	-	-	
	10	+	-	-	-	-	Synergy b/t AM+SXT and SXT+CIP
Shower (H)	11	-	+	+	-	+	Synergy b/t SXT+CIP and AM+CTX
	12	-	-	-	+	+	
Helmet (H)	13	-	-	-	-	-	

Table 5: Bacterial cultures taken from various locations within the baseball locker room and shows their resistance and susceptibility to several different antibiotics.

Bacterial Cultures		Resistance (+) vs. Susceptibility (-)				
Location	Culture #	SXT	CF	CTX	CIP	AM
Fountain (B)	C	-	-	-	-	-
Helmet (B)	D	-	-	-	-	-
	E	-	-	-	-	-
	F	-	-	-	-	-
Gloves (B)	G	-	-	-	-	-
	H	-	-	-	-	-
Shower (B)	A	+	+	-	+	+
	B	-	+	-	-	-

The cultures of the hockey locker room exhibited much greater drug resistance as indicated by the plus signs on tables 4 and 5. The hockey locker room also exhibited the greatest multiple drug resistance. In these tests, each drug was being tested individually for its ability to kill bacteria, but synergy could still be seen between drugs whose disks were positioned adjacent to one another. Zones of inhibition can be observed in figure 3 with cultures

tested from the baseball locker room. These zones indicate how close the bacteria could grow to the disk before the dosage became intolerable to the bacteria and killed it.

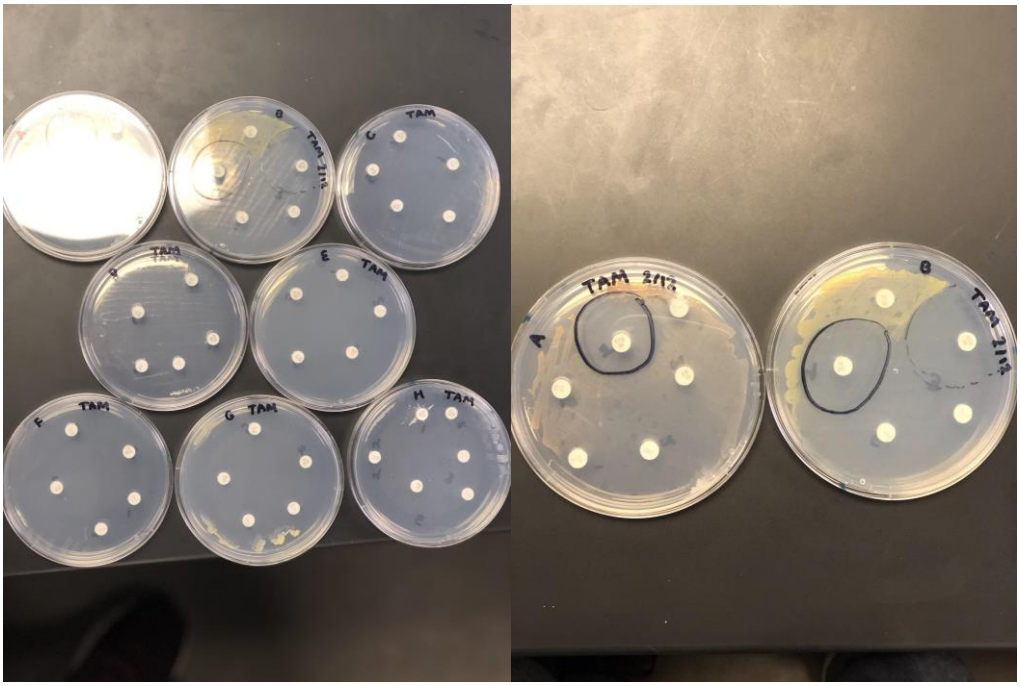

Figure 3: Isolated bacterial cultures from all locations within the baseball locker room when exposed to 5 discs of different antibiotics on DB agar plate. Zones of Inhibition were then circled.

Table 6: Cultures of bacteria that demonstrated antibiotic resistance to any combination of multiple drugs.

Multi-drug Resistant Cultures	
Culture #	Drugs
3	CXT, CF, CIP, AM
4	SXT, CF, AM
5	CF, CTX, AM
6	CF, CTX, AM
11	CF, CTX, AM
12	CIP, AM
A	SXT, CF, CIP, AM

Multiple drug resistant cultures isolated for multi-drug resistant tests were decidedly grown on ampicillin plates due to the fact that all of the multiple drug resistant drugs shared the common characteristic of resistance to ampicillin as seen in table 6, making it the ubiquitous choice.

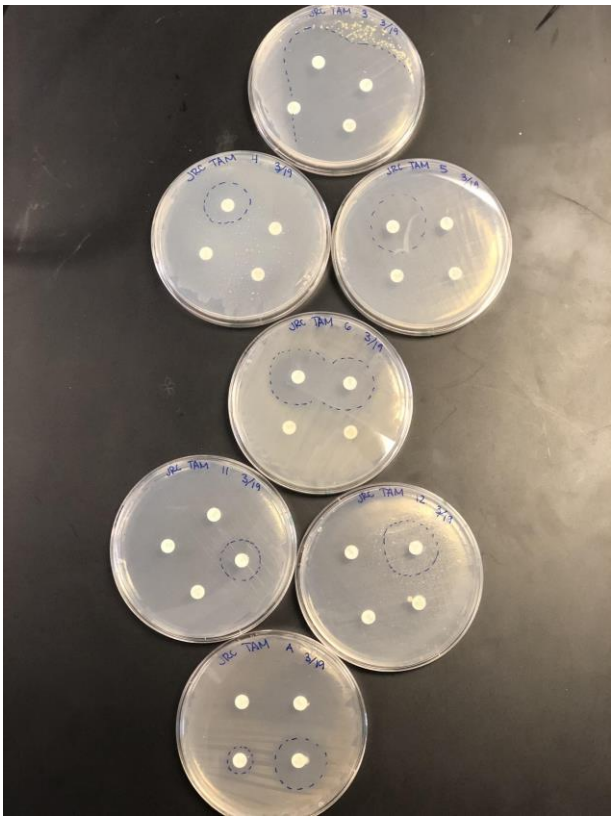

Figure 4: This figure displays all baseball and hockey cultures that demonstrated multidrug resistance. The cultures were grown on Ampicillin plates with the 4 remaining bacterial discs spaced out among the plates. Individual zones of inhibition as well as synergistic inhibition were labeled in marker.

Several bacterial cultures were deemed to be resistant to multiple drugs. Samples taken from the drinking fountain, sink, gloves, and showers associated with the hockey team all displayed multidrug resistance. Conversely, only the shower culture showed such resistance in the baseball locker room. When tested for multidrug resistance on ampicillin plates (which included CXT, CF, CIP, and SXT discs equally spaced) the cultures of every swab location experienced some zones of inhibition as well as multidrug resistance. Therefore, it can be assumed that hockey has a higher comparative risk of exposure due to having more locations of multidrug resistance, however, it was shown that specific combinations of antibiotics did inhibit bacterial growth in all cultures. Additionally, it was also determined that other combinations of drugs were ineffective in eliminating bacteria growth.

Once it was determined that multiple cultures were resistant to combinations of several antibiotics, polymerase chain reaction (PCR) was used to help identify the two cultures of bacteria (“4” and “A”) that were inferred to pose the biggest threat to athletes based on its multidrug resistance (Figure 4). PCR uses RNA segments from the bacteria and amplifies the strand to produce a lengthy sequence. The nucleotide sequences were then entered into a portal in the NCBI website and the bacterium in which the sequences were most similar were computed. The hockey locker room drinking fountain culture sequence was determined to be 99.48% identical to Brevundimonas Vesicularis. Likewise, the baseball locker room shower culture was shown to be 98.49% identical to Pseudomonas Synxantha.

Conclusion

It was concluded that the bacteria found inhabiting the hockey locker room drinking fountain was likely Brevundimonas Vesicularis. Research of case studies involving this bacteria further helps implore the possibility that this bacteria was certainly found on the drinking fountain. For example, it is first defined as follows, "*B. vesicularis* is an aerobic, nonsporulating and glucose non-fermenting Gram-negative bacillus (GNB) with distinct nutritional requirements and biochemical characteristics.The organism produces slow-growing and yellow-pigmented colonies". Additionally, case studies displayed that *B. vesicularis* was resistant to clinical doses of ampicillin (AM) and ciprofloxacin (CF) yet susceptible to trimethoprim/sulfamethoxazole (CXT) (6). This information provides some evidence that the bacteria has been correctly identified because the culture in the experiment also displayed a yellow hue, was resistant AM and CF yet susceptible to CXT, in addition to having an RNA sequence that is 99.48% identical. Furthermore, *B. vesicularis* is not known to inhibit any particular region and the factors predisposing patients to this bacteria remain unknown (6). Collectively, the possibility of *B. vesicularis* inhabiting a locker room setting has been maintained.

Conversely, it was determined that baseball locker room showers hosted Pseudomonas Synxantha. *P. Synxantha* is defined as a bacterium that can produce, "a bioactive compound which is effective against several strains of Mycobacteria. Extensive biophysical and biochemical analysis has revealed that the bioactive compound is a long chain aliphatic hydrocarbon with a terminal double bond and intermediate electronegative atom with activity similar to bio-surfactant molecule" (3). Once background of the surfactant bacteria was more understood, additional research was done which determined *P. Synxantha* is an, "amphiphilic surfactant molecule that has a tendency of aggregation when exposed to a polar solvent like water". Due to the water-based environment like a shower room, this explanation further evidences that *P. Synxantha* was likely correctly identified as this location's bacteria. Furthermore, "*P. synxantha* showed antimicrobial activity against a wide range of bacteria ranging from Gram positive to Gram negative ones. Looking at the mode of action of surface active compounds like biosurfactant, it can be assumed that this compound may also accumulate in cell membrane and thus kill the organism by disrupting cellular homeostasis (3). This definition fits with the experiment because when the shower culture was exposed to a cell wall inhibitor like CTX, bacterial growth was inhibited. Therefore, *P. Synxantha* is shown to possibly provide a useful purpose in the showers of the locker room due to its ability to inhibit additional shower fungal growth via cell wall inhibition. Overall, the antimicrobial nature of this bacteria as well as its cell membrane inhibition method of action help peg *P. Synxantha* as a microbe populating the baseball locker room showers.

Research shows that treatment methods of *B. vesicularis* and *P. Synxantha* are variable. However, based on the results from our experiment a few conclusions can be made about treating athletes who are infected by these microbes. A combination of ampicillin and ciprofloxacin were observed to inhibit further growth of *B. vesicularis* taken from the hockey locker room drinking fountain (Figure 4, Culture 4). The cumulative effect of the DNA inhibition and cell wall disruption from CIP and AM, respectively, provided an effective method of preventing further microbial growth (Table 3). In contrast, grouping CF and AM as well as

grouping CTX and AM proved to inhibit further growth of *P. Synxantha* in showers of the baseball locker room. All three of these antibiotics use a cell wall inhibition method of action to prevent bacterial growth (Table 3). Therefore, it can be concluded that CTX was shown to always inhibit *P. Synxantha* growth in our experiment. Additionally, CF and AM individually did not prevent bacterial growth, however, in combination they did stop the spread of *P. Synxantha*.

Future Research

The methods of this experiment relied heavily on determining the correct combination of drugs to inhibit bacterial growth. Future research could focus instead on manipulating the dosage of the five antibiotics used. Due to limitations, dosage studies were not able to be carried out, and these tests would likely be valueable in assessing health risk. These drugs could be administered individually and, similarly, the effects on inhibition of bacterial growth could be observed. This testing could be done in solution, using powder antibiotics administered into the solution, as to control for the concentration of antibiotic.

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