Fall 4-29-2020

Bacterial Load in Virtual Reality Headsets

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A thesis submitted to the faculty of The University of Mississippi in partial fulfillment of the requirements of the Sally McDonnell Barksdale Honors College.

Oxford
May 2020

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ABSTRACT
Bacterial Load in Virtual Reality Headsets
(Under the direction of Colin Jackson, Ph.D)

Virtual reality technology is a rapidly growing field of computer science. Virtual reality utilizes headsets which cover the user's eyes, nose, and forehead. In this study, I analyzed the potential for these headsets to become contaminated with bacteria. The nosepieces and foreheads of two HTC Vive VR headsets of the Department of Computer Science of the University of Mississippi were sampled over the course of a seven-week Immersive Media (CSCI 447) course. Serial dilutions were performed, and samples were plated on various culture media. Following incubation, counts of bacteria were determined. DNA was extracted from bacterial growth on plates from weeks 4, 5, 6, and 7 and the 16S rRNA gene was sequenced to identify bacterial contaminates present on the headsets. Chief among these contaminates was *Staphylococcus aureus*. Presumed *Staphylococcus aureus* colonies from mannitol salt agar plates were tested for resistance to the antibiotics penicillin, erythromycin, gentamycin, and tetracycline. The results of these tests indicated that the *Staphylococcus aureus* strains isolated from the headsets possessed high levels of antibiotic resistance. Other notable bacterial isolates included *Moraxella osloensis*, the bacteria responsible for foul odors in laundry and *Micrococcus luteus*, a communalistic bacterial species capable of causing opportunistic infections. Other bacterial isolates were detected in variable amounts throughout the trial. Results indicate that headsets pose a significant health hazard to users, especially those who are immunocompromised. Increased sterilization techniques are necessary to ensure the health and safety of users.

TABLE OF CONTENTS

LIST OF FIGURES AND TABLES..... v

INTRODUCTION 1

METHODS……..... 4

RESULTS ……..... 7

DISCUSSION……..... 15

BIBLIOGRAPHY 21

LIST OF FIGURES AND TABLES

Figure 1 Bacterial counts on nose and forehead sections of VR headsets (TSA) 8

Figure 2 Bacterial counts on nose and forehead sections of VR headsets (MSA) 9

Figure 3 Antibiotic Resistance of presumed *Staphylococcus aureus* colonies..……...11

Table 1 Bacterial phyla detected in mixed cultures of bacteria samples…..... 12

Table 2 Bacterial OTUs detected in mixed cultures of bacteria samples.…..…… 14

Introduction:

Objects undergoing constant human interaction are colonized by many different strains of bacteria (Reynolds et al. 2007, Al-Ghamdi et al. 2011, Messima et al. 2011, Zakai et al. 2016). These bacteria can include potential pathogens or indicator bacteria such as fecal coliforms, which have been detected in 7% of objects sampled from locations such as shopping centers, daycares, offices, playground, and movie theatres (Reynolds et al. 2007). Items that are touched by humans are even more contaminated, for example 97% of elevator buttons in residential and commercial buildings were found to be contaminated with mixed bacterial growth (Al-Ghamdi et al. 2011). Chief among the bacterial isolates obtained were coagulase negative staphylococci and Gram-positive bacilli, and pathogens included *Staphylococcus aureus, Pseudomonas* spp. and various Gram-negative bacteria (Al-Ghamdi et al. 2011). Bacteria from objects can be transferred to people's hands and detected in their home or on personal belongings hours later (Reynolds et al. 2007). Improper sanitation of these objects can lead to the spread of bacterial diseases and other infections (Shukla et al. 2019), and 80% of all infections are spread through contact with other peoples' hands or everyday objects (Reynolds et al. 2007). Thus, it is clear that objects undergoing human interaction can become hosts for bacteria and sites of potential contamination.

In recent years, technology has become ubiquitous in everyday life for people in developed countries, with items such as cell phones, tablets, and laptop computers being in near constant use. Technological items host bacteria, for example over 90% of cell phones

have been reported as being contaminated by bacteria, including potentially pathogenic *Staphylococcus* (Zakai et al. 2016). More specifically, 17% of cell phones have even been found to harbor *Staphylococcus aureus* (Zakai et al. 2016). Even non-mobile technology that is not exposed to many different public settings has been found to be rife with bacteria. Of 30 computers sampled in a study by Messina et al. (2011), 15 publicly shared and 15 non-shared, bacteria could be cultured from every keyboard, with counts ranging as high as 430 colony forming units (CFUs) per key of a keyboard. All but one keyboard harbored staphylococci, including *S. aureus*, which was much more commonly found on shared keyboards than on non-shared keyboards (Messina et al. 2011). Al-Ghamdi et al. (2011) found that all keyboards and computer mice in an internet café were contaminated with mixed bacterial growth and shared technology, such as the keyboards of university libraries, typically harbor a far greater number of bacteria as compared to private technology (Anderson and Palombo 2009). The reason for high rates of contamination in keyboards is two-fold: first, they are one of the most commonly touched public surfaces, and second, there is a stigma against cleaning keyboards, stemming from a fear of damaging the electronics (Marsden 2009). Thus, technology in general is often highly contaminated with bacteria.

One of the newest advancements in technology is virtual reality (VR). It is projected that by 2021, 57.1 million people in the United States will use VR headsets at least once a month, representing an over 100% increase in usage since 2017 (Petrock 2019). VR allows users to immerse themselves in a computerized universe through the use of a headset that covers the eyes and most of the nose. Most VR headsets follow a standard design, with a cloth or foam strip that contacts the forehead and cheeks and a plastic nosepiece that sits on the nose. The internal component of the headset consists of two computer screens that, when

in use, are about 5 cm from the wearer's eyes. Given the proximity of VR headsets to the user's skin and the near ubiquitous presence of bacteria on objects subject to human use, it is reasonable to expect that VR headsets could be hosts for bacteria, although no studies have examined that concern. The close proximity of VR headsets to the nose also suggests that they could harbor *S. aureus*, as the nasal cavity is one the main locations colonized by such bacteria with 14% of people reported as persistent carriers of *S. aureus*, and another 69% as intermittent or occasional carriers (Eriksen et al. 1995).

In this study, I set out to assess whether VR headsets, like other forms of technology, harbor bacteria. I sampled headsets in a shared VR computer lab at the University of Mississippi and assessed whether there were increases in the amount of bacteria present following increased student use.

Methods:

Two HTC Vive VR headsets of the Department of Computer Science of the University of Mississippi were sampled over the course of a seven-week Immersive Media (CSCI 447) course. The headsets were sterilized at the start of the study with a 70% ethanol solution. The users of the headsets were instructed to maintain their current usage and sanitation processes. Their procedures included cleaning the headsets with specialized wipes, but they admitted that this was rarely done. VR headset usage for each week was estimated based on the workload of the students and the observations of the Computer Science graduate students

Once a week for seven weeks, 10 cm^2 of the forehead and the nosepiece were sampled by swiping with a sterile cotton swab. These swabs were placed in a 0.9% sterile saline solution and returned to the laboratory approximately 30 minutes after collection. There, samples were vortexed and hundred-fold serial dilutions performed to 10^{-6}. 100 µL of each dilution was plated onto each of Tryptic-Soy Agar (TSA), Mannitol Salt Agar (MSA), and Eosin Methylene Blue Agar (EMB). Plates were incubated at 36 °C for 48 hours, after which the number of colonies on each plate was counted. After three weeks showing no growth of colonies on plates beyond the first dilution, the dilution procedure was adjusted and limited to 10^{-2}. Counts were expressed as number of colony forming units (CFU) per cm^2.

Colonies on MSA plates that resulted in a change of agar color from red to yellow were presumed to be *Staphylococcus aureus*. Such colonies from weeks 4, 6, and 7 were transferred to fresh MSA plates and isolated. Those that continued to result in a color change on MSA were then assessed for antibiotic resistance using the disc diffusion approach. Colonies were re-plated to Mueller-Hinton Agar (MHA) and tested for resistance to tetracycline, penicillin, gentamycin, and erythromycin using BBL Sensi-Disc Antimicrobial Susceptibility Test Discs. After incubation for 48 h at 36 °C , the diameter of growth inhibition around each disc was measured and compared to the known values for resistance. For erythromycin, a zone of 13 mm or less indicated resistance. For gentamycin, the zone was 6 mm or less. For penicillin the zone was 28 mm or less, and for tetracycline the zone was 14mm or less.

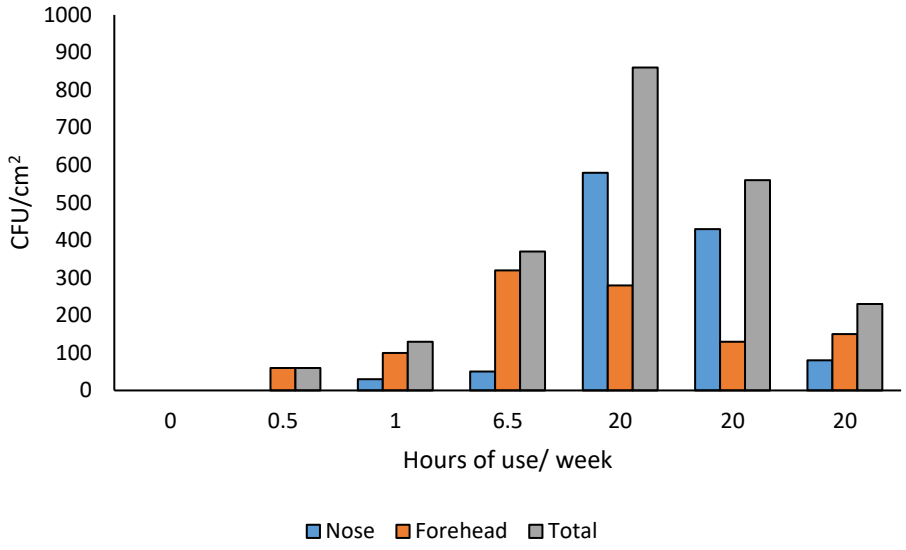
To get an overall assessment of the types of bacteria present, bacterial growth on the most general medium used (TSA) was processed for 16S rRNA gene sequencing. Colonies on TSA plates from weeks 4, 5, 6, and 7 were washed with 750 µL of sterile saline and transferred into 2 mL sterile tubes. Tubes were centrifuged for 10 minutes at 8000xg to pellet the cells, and DNA extracted from each pellet using a MoBio Ultra Clean Microbial DNA Isolation Kit, following the manufacturer's instructions. Agarose gel electrophoresis was used to confirm the presence of DNA. Bacterial DNA was amplified targeting the V4 region of the 16S rRNA gene using dual-indexed barcoding and the primers and procedures of Kozich et al [2013]. Amplified fragments were sequenced through the Molecular and Genomics Core Facility at the University of Mississippi Medical Center. Sequence data was processed in the bioinformatics software mothur and major bacterial phyla identified by comparisons to the Ribosomal Database Project (RDP) database. For each sample, the

number of sequences in a phylum was divided by the total number of sequences and presented as a percentage. For percentages of the total, each phylum's sequences were added together across all the samples, and the result was divided by the total number of sequences in all the samples. Sequences were grouped into operational taxonomic units (OTUs) based on 97% sequence similarity and representative DNA sequences of dominant OTUs used to identify that OTU by BLAST searches against the NCBI nucleotide database. For each sample, the number of sequences recorded for an OTU was divided by the total number of sequences in that sample, and the result was presented as a percentage. For the total percentages, the sum of a particular OTU's sequences across all samples was divided by the total number of sequences for all OTUs. OTU data was also presented as percentages of total samples from the nose and from the forehead to determine differences in sample composition vs. location.

Results:

Bacterial load increased over the course of the study, coinciding with increased usage of the VR headsets. Weeks with increased levels of use showed increased counts of CFUs per cm^2. Weeks 5, 6, and 7 each had approximately 20 hours of student headset use, and displayed the highest number of CFUs/cm^2 on both TSA and MSA plates (Figures 1, 2). Total bacterial load on TSA plates (Figure 1) peaked at 860 CFUs/cm^2 on Headset 1 in week 5, following 20 hours of use that week. Bacterial counts on Headset 2 peaked at 1190 CFUs/cm^2 in week 6, also following 20 hours of use. Headset 1 accumulated CFUs/cm^2 more quickly than headset 2, showing much higher levels in weeks 1-5; however, CFUs/cm^2 in Headset 1 decreased after week 5, although the headset was still used for 20 hours in weeks 6 and 7. Headset 2 collected CFUs/cm^2 more slowly than Headset 1 did, but its count held steady after the peak in week 6, remaining high in week 7. The peak for Headset 2 was much higher than that of Headset 1 (1190 vs 860 CFUs/cm^2). There was no difference between samples taken from the nose or forehead part of the headset, in terms of CFU counts on TSA or MSA. Trends in growth on MSA plates (Figure 2) followed those seen on TSA plates, but counts were generally lower. There was no growth on EMB plates for all sample dates.

A.



B.

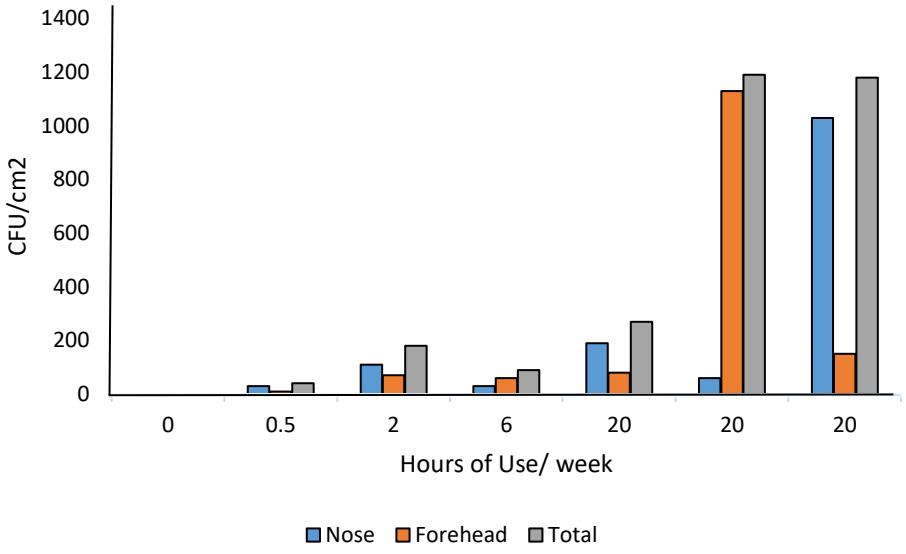
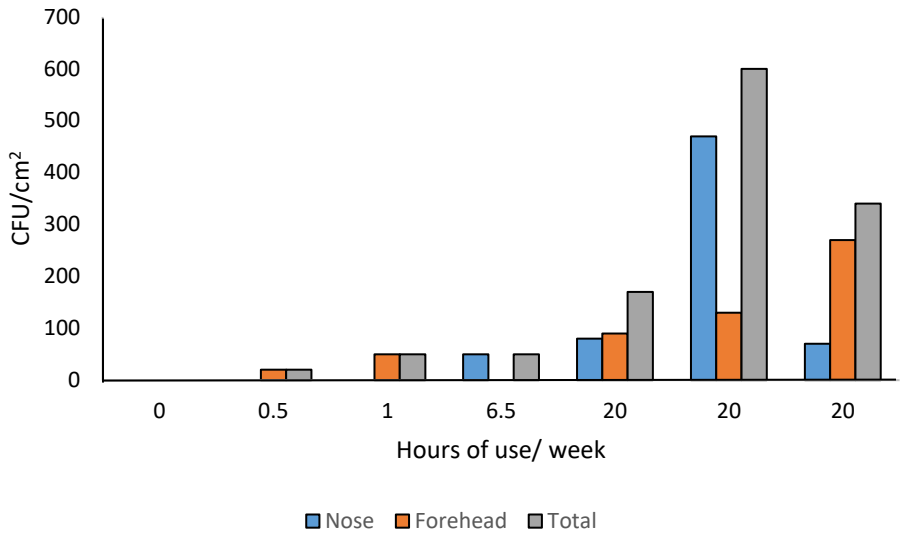


Figure 1. Bacterial counts (CFU/cm^2) on nose and forehead sections of Virtual Reality headsets as determined from growth on TSA plates over seven weeks of increased usage. Weeks are in chronological order with numbers indicating estimated hours of use for that week. Panels represent counts on VR Headset 1 (A) and 2 (B).

A.



B.

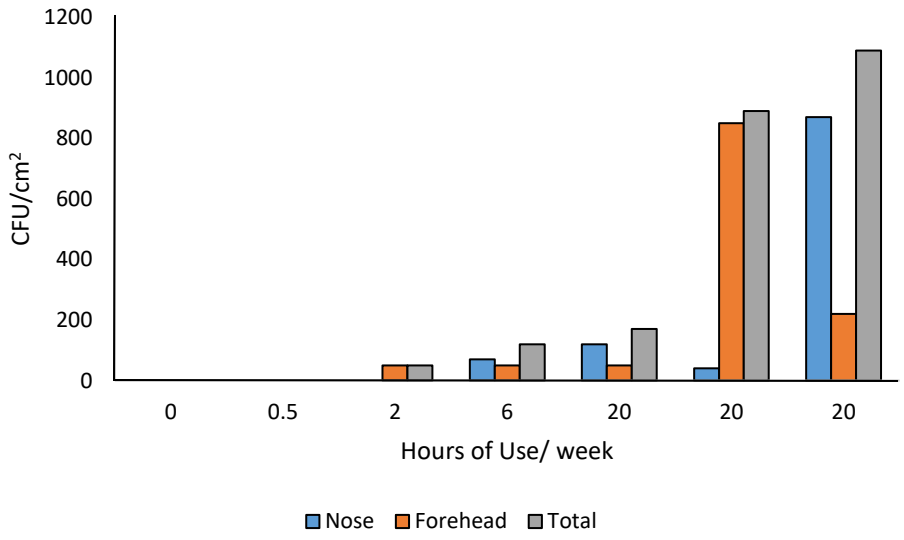
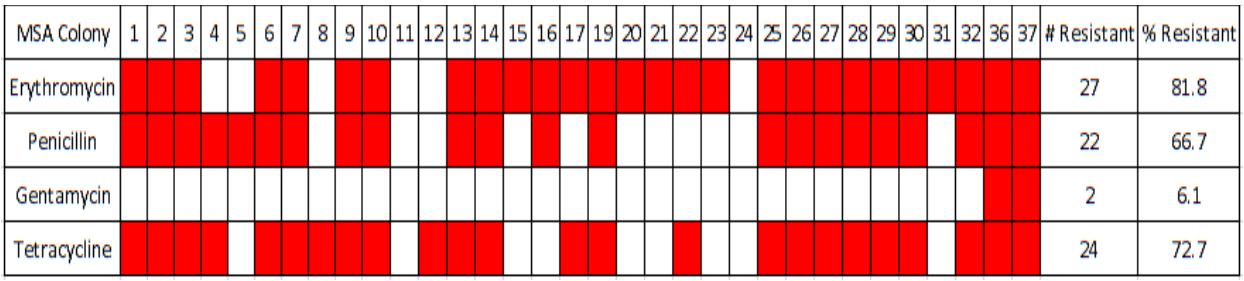


Figure 2. Bacterial counts (CFU/cm^2) on nose and forehead sections of Virtual Reality headsets as determined from growth on MSA plates over seven weeks of increased usage. Weeks are in chronological order with numbers indicating estimated hours of use for that week. Panels represent counts on VR Headset 1 (A) and 2 (B).

37 colonies of presumptive *Staphylococcus aureus* were transferred from MSA plates and assessed for antibiotic resistance (Figure 3). Many of these colonies showed high levels of resistance to erythromycin (27 or 81.8% of colonies resistant), penicillin (22 or 66.7% of colonies resistant), and tetracycline (24 or 72.7% of colonies resistant). Only two colonies (6.1% of those tested) showed resistance to gentamycin (Figure 3). These two colonies (colonies 36 and 37) were also resistant to the other three antibiotics. Only two colonies (colonies 11 and 24) were susceptible to all four antibiotics.

16S rRNA gene sequencing revealed that the most common phylum of bacteria that was cultured on TSA plates was the Firmicutes, representing 85% of the total sequences obtained. Proteobacteria was the next most common phylum at 9 % of the total, followed by Actinobacteria (6% of the total). Sequences identified as 11 other bacterial phyla were also detected, although these represented very small percentages of the total (Table 1). Though Firmicutes was the most common phylum in all samples, it ranged from as low as 61% in the sample from Headset 1, week 4, nose (1, 4, N). This sample saw a large rise in Proteobacteria, which made up 39% of this sample. Proteobacteria was found in higher levels on samples from the nosepieces, peaking at the aforementioned sample. The highest incidence of Firmicutes occurred in the sample from 2, 7, N (99.96%). Actinobacteria was much more common in samples taken from the foreheads of the headsets than in those from the nosepieces, spiking at 28% in sample 2, 4, F.



MSA Colony	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	19	20	21	22	23	24	25	26	27	28	29	30	31	32	36	37	# Resistant	% Resistant
Erythromycin	■	■	■			■	■		■	■			■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	27	81.8
Penicillin	■	■	■	■	■	■	■		■	■			■	■		■		■						■	■	■	■	■	■		■	■	■	22	66.7
Gentamycin																																■	■	2	6.1
Tetracycline	■	■	■	■		■	■	■	■	■		■	■	■			■	■			■			■	■	■	■	■	■		■	■	■	24	72.7

Figure 3: Antibiotic resistance as determined by disk diffusion on MHA plates with BBL Sensi-Disc Antimicrobial Susceptibility Test Discs of erythromycin, penicillin, gentamycin, and tetracycline. Colonies were presumed *Staphylococcus aureus* from MSA plates from weeks 4, 6, and 7. Filled squares indicate resistance.

Phylum	Total %	1, 4, F	1, 4, N	1, 5, F	1, 5, N	1, 6, F	1, 7, F	1, 7, N
Firmicutes	85.464	73.055	60.754	86.209	93.380	70.180	85.382	79.915
Proteobacteria	8.678	7.690	38.892	0.078	5.848	20.257	0.025	19.760
Actinobacteria	5.740	19.083	0.224	13.624	0.703	9.433	14.476	0.215
Bacteria_unclassified	0.071	0.127	0.094	0.050	0.035	0.058	0.092	0.103
Acidobacteria	0.015	0.024	0.012	0.017	0.000	0.019	0.005	0.003
Verrucomicrobia	0.009	0.000	0.000	0.011	0.009	0.014	0.015	0.000
Bacteroidetes	0.006	0.005	0.008	0.000	0.009	0.000	0.005	0.000
Planctomycetes	0.006	0.005	0.004	0.006	0.009	0.000	0.000	0.003
Armatimonadetes	0.003	0.005	0.008	0.006	0.000	0.000	0.000	0.000
Deinococcus-Thermus	0.003	0.000	0.000	0.000	0.000	0.038	0.000	0.000
Chloroflexi	0.003	0.005	0.004	0.000	0.009	0.000	0.000	0.000
Ignavibacteriae	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Gemmatimonadetes	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nitrospirae	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Spirochaetes	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Phylum	2, 4, F	2, 4, N	2, 5, F	2, 5, N	2, 6, F	2, 6, N	2, 7, F	2, 7, N
Firmicutes	71.832	99.794	90.128	99.763	99.204	99.948	99.535	99.963
Proteobacteria	0.158	0.117	0.120	0.124	0.049	0.031	0.199	0.019
Actinobacteria	27.557	0.039	9.556	0.012	0.670	0.016	0.000	0.006
Bacteria_unclassified	0.284	0.022	0.077	0.036	0.014	0.000	0.114	0.000
Acidobacteria	0.063	0.011	0.077	0.006	0.007	0.000	0.047	0.000
Verrucomicrobia	0.053	0.000	0.026	0.006	0.014	0.000	0.038	0.006
Bacteroidetes	0.021	0.011	0.000	0.041	0.000	0.000	0.000	0.000
Planctomycetes	0.011	0.000	0.009	0.012	0.021	0.005	0.009	0.006
Armatimonadetes	0.011	0.006	0.000	0.000	0.014	0.000	0.000	0.000
Deinococcus-Thermus	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Chloroflexi	0.000	0.000	0.000	0.000	0.007	0.000	0.028	0.000
Ignavibacteriae	0.011	0.000	0.000	0.000	0.000	0.000	0.009	0.000
Gemmatimonadetes	0.000	0.000	0.000	0.000	0.000	0.000	0.009	0.000
Nitrospirae	0.000	0.000	0.009	0.000	0.000	0.000	0.000	0.000
Spirochaetes	0.000	0.000	0.000	0.000	0.000	0.000	0.009	0.000

Table 1: Bacterial phyla detected in mixed cultures of bacteria samples from Virtual Reality (VR) headsets and plated on to TSA. Column labels are in the format of Headset number (1, 2), Week number (4, 5, 6, 7), and Forehead or Nose portion of the headset sampled (e.g. 1, 4, F is Headset 1, Week 4, Forehead). Numbers indicates the percent of total 16S rRNA gene sequences recovered from cultures that grouped with that bacterial phylum.

Analysis of the 16S rRNA sequences of ten most abundant OTUs using NCBI Blast revealed that the most common representative sequence was *Staphylococcus aureus* (Firmicutes; accounting for 87% of the sequences of the ten most common OTU's; Table 2). Other abundant OTUs were identified as *Moraxella osloensis* (Proteobacteria) and *Micrococcus luteus* (Actinobacteria), the second (7% of sequences) and third (3% of sequences) most abundant sequences respectively. *Rothia kristinae* (Actinobacteria) was found in high abundance (26% of sequences in the sample) on the week 4, forehead sample from Headset 2, accounting for the increased percentage of Actinobacteria in that sample's bacterial taxonomy. This was the only sample in which this bacterium was found in any large amount. Spikes of *Kocuria rosea* (Actinobacteria) were seen in headset 1, week 6, forehead (8%) and headset 1, week 7, forehead (6%). Other bacteria were found in small amounts (Figure 5). Nose samples had higher percentages of *Moraxella osloensis* (Proteobacteria, 12% of total sequences in nosepiece samples vs 1% of total sequences in forehead samples). Forehead samples showed higher levels of *Micrococcus luteus* (Actinobacteria, 7% of total sequences in forehead samples vs. 0.06% of total sequences in nose samples).

Sample	*Staphylococcus aureus*	*Moraxella osloensis*	*Micrococcus luteus*	*Kocuria rosea*	*Rothia kristinae*	*Dermacoccus nishinomiyaensis*	*Moraxella osloensis2*	*Corynebacterium ihumii*	*Staphylococcus argensis*	*Moraxella osloensis3*
1, 4, F	72.940	7.219	14.932	0.240	0.000	3.153	0.268	0.000	0.000	0.020
1, 4, N	60.470	37.444	0.000	0.000	0.000	0.220	0.000	0.000	0.000	0.193
2, 4, F	71.909	0.042	0.032	0.000	26.580	0.000	0.000	0.000	0.000	0.000
2, 4, N	99.850	0.000	0.006	0.000	0.000	0.000	0.268	0.000	0.000	0.000
1, 5, F	85.275	0.006	13.133	0.000	0.000	0.006	0.000	0.000	0.924	0.000
1, 5, N	93.385	5.660	0.694	0.000	0.000	0.000	0.000	0.000	0.000	0.061
2, 5, F	90.256	0.026	9.239	0.000	0.000	0.000	0.268	0.000	0.000	0.000
2, 5, N	99.834	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1, 6, F	70.004	19.633	0.000	8.197	0.000	1.016	0.000	0.024	0.000	0.063
2, 6, F	99.267	0.007	0.000	0.000	0.007	0.000	0.268	0.021	0.000	0.000
2, 6, N	99.958	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.000
1, 7, F	85.316	0.000	4.937	6.476	0.000	0.000	0.000	2.548	0.005	0.000
1, 7, N	79.862	18.997	0.015	0.003	0.000	0.000	0.268	0.000	0.000	0.134
2, 7, F	99.753	0.057	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2, 7, N	100.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Forehead	83.609	4.495	6.021	2.441	2.032	0.691	0.152	0.410	0.135	0.014
Nose	87.684	11.768	0.062	0.001	0.000	0.042	0.372	0.000	0.000	0.072
Total	85.769	8.349	2.863	1.148	0.955	0.347	0.268	0.193	0.063	0.044

Table 2: Bacterial OTU's detected in mixed cultures of bacteria samples from Virtual Reality (VR) headsets and plated on to TSA. Column labels are in the format of Headset number (1, 2), Week number (4, 5, 6, 7), and Forehead or Nose portion of the headset sampled (e.g. 1, 4, F is Headset 1, Week 4, Forehead). Numbers indicates the percent of total 16S rRNA gene sequences recovered from cultures that grouped with that bacterial OTU. Forehead and Nose refer to total percentages for samples from those locations.

Discussion:

In this study I examined the bacterial load of VR headsets in a Computer Science laboratory at the University of Mississippi. One goal was to see if the load of bacteria on headset would increase with more student usage. In addition, I set out to identify what species of bacteria were present on these headset, in order to determine whether they could pose a health hazard for users. Finally, I performed antibiotic resistance tests on any presumed *Staphylococcus aureus* colonies to demonstrate the potential health risks should a user become infected.

As expected, CFUs per cm^2 increased over the course of the study, which coincided with increased weekly usage of the VR headsets. During the first four weeks, usage of the two headsets was minimal. As a result, the headsets displayed relatively low CFUs/cm^2. Usage for both headsets increased to 20 hours in week 5, and the mixed bacterial growth on TSA plates subsequently increased. The higher levels of counts (100's of OTUs per cm^2) on VR headsets that were observed in these weeks, were at or above counts reported for individual keys of computer keyboards (Messina et al. 2011). However, there was no growth on EMB plates for all samples, which suggests an absence of fecal coliforms on the headsets.

MSA plates showed similar growth patterns to those of TSA plates. MSA plates are selective for bacteria which can survive at high salt concentrations, such as staphylococci and other skin-associated bacteria. The similarity of the growth patterns of samples on MSA and TSA plates (CFUs/cm^2) suggests that the majority of bacteria present in the mixed bacterial

growth on TSA plates were also able to survive on MSA plates. This suggestion is corroborated by 16S rRNA gene sequencing, which revealed that the most common phylum present in the TSA mixed bacterial growth from weeks 4, 5, 6, and 7 was Firmicutes. Further analysis of these sequences revealed that the most common OTU in all of these samples was *Staphylococcus aureus*, a member of the Firmicutes phylum, which comprised up to 99% of the sequences obtained from several samples. *Staphylococcus aureus* is a leading cause of soft tissue infection in humans (Naimi et al. 2003). Community acquired *Staphylococcus aureus* infections normally present as erythematous, pyogenic skin in a normally healthy individual (Boucher and Corey 2008). However, *Staphylococcus aureus* is also a major pathogen associated with conjunctivitis and keratitis. These infections can be quite serious and can lead to a loss of visual acuity or even blindness (O'Callaghan 2018).

In recent years, community acquired *Staphylococcus aureus* has become increasingly resistant to antibiotics, especially beta-lactams (Fowler et al. 2005). The results of antibiotic resistance tests performed in this study using disk diffusion with erythromycin, penicillin, gentamycin, and tetracycline, support this claim. Strains of *Staphylococcus aureus* isolated from the two VR headsets showed high levels of resistance to erythromycin, penicillin, and tetracycline. Only gentamycin was effective at stopping bacterial growth. Both the high incidence and the antibiotic resistance of *Staphylococcus aureus* isolated from the VR headsets in this study make these headsets a potential source of dangerous community acquired *Staphylococcus aureus* infections.

In addition, 16S rRNA sequencing detected *Moraxella osloensis* (Proteobacteria) in all samples, with a highest occurrence in the sample from headset 1, week 4, nose. Two other strains of *Moraxella osloensis* were also identified in the top ten dominant OTU's

(OTUs 7, 10). *Moraxella osloensis* was more commonly found on the nosepieces of the headsets than the foreheads (12% of total sequences in nosepiece samples vs 1% of total sequences in forehead samples). This Gram negative bacterium has been known to cause opportunistic infections but is most commonly known for causing malodorous smells in laundry (Kubota et al. 2012). The major component of this sweaty, dirty odor has been determined to be 4-methyl-3-hexenoic acid. Though the mechanism is unclear, *Moraxella osloensis* produces 4-methyl-3-hexenoic acid at high levels when in the presence of soil, sebum, or sweat. Thus, the prolonged presence of this bacteria on VR headsets could lead to the production of 4-methyl-3-hexenoic acid and result in foul-smelling odors. While a minimal health concern, the presence of this bacteria could lead to an unpleasant experience for future users.

16S rRNA sequencing also detected *Micrococcus luteus*. This bacterium was more commonly found on the foreheads of the headsets than the nosepieces. *Micrococcus luteus* is a Gram positive bacteria commonly found in a commensal relationship with humans and are known to colonize the skin and mucosa. Usually harmless, this bacterium occasionally causes opportunistic infections such as pneumonia, meningitis, or septic arthritis in immunocompromised individuals (Hetem et al. 2017). Although this bacterial species poses a minor risk, it is still potentially dangerous should such an individual use a VR headset.

While *Staphylococcus aureus* remained the dominant OTU in each sample, other bacterial contaminants showed a fair amount of variability. Notable are the spikes of bacteria such as *Kocuria rosea* and *Rothia kristinae* which appeared in some samples. These spikes could be attributed to the fact that the users between the weeks were not always consistent. Students worked in teams in the lab, taking turns using the headsets and programming.

However, groups used the same headset each week, as the headsets were attached to computers to which their data was saved. Spikes of specific bacterial contaminants could be attributed to a specific student whose skin was colonized by the bacteria. For example, spikes of *Kocuria rosea* seen in the study could be attributed to one student whose forehead was colonized by this species. *Kocuria rosea* is a Gram positive, non-pathogenic commensal found on the skin and mucosal surfaces of many humans (Lee et al. 2013). However, *Kocuria rosea* has been known to cause infection in immunocompromised patients, such as those undergoing cancer treatment (Altuntas et al. 2004). It is important to note that the spikes of this bacteria appeared one week apart on the same location on headset 1. This lends credence to the idea that the contamination could be due to a single student.

Another irregularity in the overall makeup of bacterial contaminants was the large increase of *Rothia kristinae* detected in the forehead sample from headset 2 in week 4. This was an abnormally large spike, comprising 26% of the total bacterial sequences from that sample. *Rothia kristinae*, like *Kocuria rosea*, is part of the normal human flora of the human oropharynx and upper respiratory tract. *Rothia* species are gram positive bacteria commonly associated with dental caries and other dental diseases (Trivedi and Malhotra 2015). *Rothia kristinae* has also been found to cause serious infections, primarily in immunocompromised patients but also occasionally in healthy individuals. Risk factors for *Rothia* infection include hematological malignancies and neutropenia, as well as alcoholism, diabetes mellitus, and chronic hepatitis (Ramanan 2014). As this bacterial contaminant was seen so rarely in the other samples, it is likely that the large spike seen in this sample was also due to a single student.

The compositions of samples differed between the nosepieces and foreheads of the headsets. Nosepiece samples showed slightly elevated levels of *Staphylococcus aureus* and much higher levels of all three strains of *Moraxella osloensis*. Forehead samples showed greater levels of *Micrococcus luteus, Kocuria rosea, and Rothia kristinae*. These differences could be contributed to the difference in material between the two locations. The nosepiece of the headsets was made of a hard, non-porous plastic, while the forehead was a porous foam face cushion. *Staphylococcus aureus* and *Moraxella osloensis* are highly suited to surviving on most surfaces. *Staphylococcus aureus* can survive up to 7 months on dry surfaces (Kramer et al. 2006). *Moraxella osloensis* has a high tolerance to desiccation and UV exposure, allowing it to survive in environments unsuitable for other bacteria (Hetem et al. 2017). In general, Gram negative bacteria such as *Moraxella osleonsis*, survive longer on dry surfaces (i.e. the nosepiece of a VR headset) than Gram positive bacteria such as *Rothia kristinae* or *Kocuria rosea* (Kramer et al. 2006). This may explain why these Gram positive bacteria, excluding *Staphylococcus aureus* which has developed survival mechanisms, were found in low quantities on the nosepiece of the headsets. However, as the forehead of the headset is lined with a porous foam, it may remain damp with sweat after prolonged use. This would allow for the survival of Gram positive bacteria for an extended period of time. High levels of *Staphylococcus aureus* were seen both in the nosepiece samples and in the forehead samples, indicating that it is capable of surviving well in both environments.

In conclusion, VR headsets subjected to extended use are colonized by high levels of bacterial contaminants, equivalent or exceeding those on computer keyboards. The levels of these contaminants increase as usage increases. Chief among the isolates in this study was *Staphylococcus aureus*, which can cause serious infections, even in previously healthy

individuals. While the other bacteria isolated are known to be a part of the normal flora of most humans, they can still cause opportunistic infections in immunocompromised users or users with other risk factors. Without proper sterilization techniques, VR headsets pose a potential health hazard for their users. However, following sterilization with 70% ethanol in week 1, no bacteria were detected on the headsets. Thus, sterilization with ethanol is an effective way of reducing the contamination and infection risk of VR headsets and should be recommended as a standard procedure.

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