

Research Article: Analysis of Antimicrobial Resistance in Bacteria Found at Various Sites on Surfaces in an Urban University

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Analysis of antimicrobial resistance in bacteria found at various sites on surfaces in an urban university

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Abstract. The purpose of this study was to determine the extent of microbial contamination and antimicrobial resistance throughout a typical urban university environment. Determining the numbers of antibiotic resistant bacteria may provide insight into the continued development of antimicrobial resistance, and may lead to changes in university inhabitants' hygiene behaviors. In this study, samples of microorganisms were obtained from 35 surfaces at Pace University-NYC on three separate days. Samples were grown on plates of tryptic soy agar (TSA) and replicated onto control-plates, and plates containing either 100 µg/mL ampicillin, 1.0 µg/mL ciprofloxacin, or 1.0 µg/mL triclosan. The presence of bacterial growth on the drug containing plates after 24 hours indicated antimicrobial resistance. The restroom floors, toilet seats, computers, and cafeteria displayed the highest number of bacterial growth, 171, 301, 87, 143 colony forming units (CFUs) respectively. These sites also contained microorganisms displaying antimicrobial resistance to two or more of the antimicrobial agents. Resistance to triclosan, ciprofloxacin, and ampicillin were observed in 100% (± 0), 97% (± 0.16), and 68% (± 0.47) of sample sites respectively. Finally, an additional study of hygiene behavior of 100 university inhabitants was conducted to observe a possible mechanism in the high level of distribution of drug resistant microorganisms throughout the university. The results of this study have shown that while 88% (± 0.32) of inhabitants wash their hands, the time spent washing on average was 4.87 (± 3.97) seconds - well below the 20 seconds recommended to sufficiently remove microorganisms from the hands.

Introduction

A university is a microcosm, containing multiple students, faculty, and staff existing together for many hours, if not days, in one location-along with all of the microorgan-

isms that travel with them. Pace University-NYC presents an exemplary model of a closed environment with a large population. At the University, a single high rise building contains an academic section for classes and laboratories, a first-year-student residence hall, a cafeteria, a gym, a student center, and a library. For some, the University serves as a home, for others it is only a place of employment for several hours each day. There is a constant flow of individuals into and out of the University. Thus, the Pace University-NYC environment provides a good

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site for observation of antimicrobial resistance within a population of both healthy and susceptible individuals.

The transmission of antibiotic resistance genes between pathogenic bacteria has recently become an area of interest in scientific research, because of its close relation to the occurrence and severity of infection affecting human health. The recent rise in antimicrobial resistance has been attributed to the high levels of antimicrobial use in the clinical setting as well as their over-use in agriculture (Martinez and Baquero, 2002; Wassmer et al., 2006). When initially introduced, antimicrobial agents were only used for the treatment of bacterial infections. Antimicrobial agents such as penicillin and erythromycin are still used for the treatment of bacterial infections, however, when used improperly they contribute to the development of resistance in pathogenic strains (Gilbert and McBain, 2003; Wassmer et al., 2006). Widespread inappropriate prescription of antimicrobials, as well as non-adherence to prescription instructions (both improper uses of antimicrobials) contribute to antibiotic resistance (Wassmer et al., 2006). Antimicrobials are also distributed within an agricultural setting, including crop sprays and feed for livestock. Livestock are often fed antimicrobials primarily for their growth promoting properties and disease prevention. While most antimicrobials used in agriculture are unlike those used in human treatment, there are some antimicrobials (i.e. bacitracin, tetracyclines, and sulfonamides) that are used both in agriculture and for the treatment of human disease. Some examples of antimicrobial resistant strains found in agriculture that affected humans include: *Escherichia coli*, *Salmonella*, *Campylobacter*, and *Enterococcus* species (Conly, 2002). Recently, a new use for antimicrobial agents was ascertained: the agents have been used in multiple consumer cleaning products including, hand soaps, toothpastes, cosmetics, clothing to kill micro-organisms. (Gilbert and McBain, 2003) At first glance, the development of consumer products designed to kill large amounts of bacteria would seem ideal but, there are important factors to be considered when attempting to kill bacteria. If a bacterial population is not en-

tirely killed by an antimicrobial agent, subsequent exposure to the same antimicrobial agent will only promote antimicrobial resistance by natural selection (Martinez and Baquero, 2002; Depardieu et al., 2007).

The mechanisms utilized by bacteria to evade the bactericidal effects of antimicrobial agents vary greatly between each bacterium. Resistance mechanisms acquired by bacteria typically occur at the genetic level. Chromosomal mutations resulting in an alteration in the target of an antimicrobial drug can lead to drug resistance. For example, resistance to erythromycin, a ribosome inhibitor, develops when an adenosine in the bacterium's rRNA is methylated. This results in an alteration of the binding site for erythromycin on the bacteria's ribosome (Depardieu et al., 2007). Bacteria can also acquire genes encoding enzymes that are capable of dismantling an antimicrobial agent such as the β -lactamase gene. Many antibiotics including penicillins and cephalosporins contain a β -lactam ring as their backbone structure. β -lactamase is an enzyme that hydrolyzes the ring structure of β -lactam antibiotics- thereby rendering the antibiotics inactive (Depardieu et al., 2007). Other bacteria acquire genes encoding drug efflux pumps. Drug efflux pumps are membrane proteins that pump antimicrobial agents and other toxic substances out of the bacterial cell (Fan et al., 2002; Ono et al., 2005).

The development of antimicrobial resistance extends beyond individual microorganisms. Microorganisms are able to share genetic information to become more resistant to antimicrobials. Recent studies have demonstrated that bacteria have the ability to transmit resistance genes through a process called conjugation. In conjugation, bacterial cells transfer genetic information through direct cell to cell contact via hollow tubes called pili (Courvain, 1994; Brunsima et al., 2003; Depardieu et al., 2007). Bacteria also utilize a mechanism called transformation, in which bacteria uptake and integrate foreign DNA into their own genome, to propagate resistance (Courvain, 1994). Transduction is an additional mechanism that contributes to the development of resistance in bacteria. In transduction, genetic material is transferred from bacteria to bacteria via a viral vector (Courvain,

1994). Thus, one method to reduce levels of resistance may be to prevent unnecessary exposure of bacteria to antimicrobial agents that would provide them the opportunity to develop and, sometimes, subsequently transmit resistance genes.

The development and spread of antimicrobial resistant microorganisms is of great importance to public health. Microorganisms that have become resistant to antimicrobial agents, play an important role in the development and persistence of disease. Once antimicrobials considered second or third line drugs are no longer able to inhibit or kill their intended targets, pathogens will gain an advantage and will be able to infect multiple hosts free of inhibition. The development of resistance is a danger to public health especially in the case of immune-compromised individuals, whose only defense against pathogens is provided by antimicrobials. While the majority of the public are not continuously immune-compromised, when experiencing increased stress or illness, (which is often the case in closed settings such as a university), individuals can easily become immune compromised and susceptible to opportunistic infection. Thus, informing the public of how resistant organisms develop and are transmitted from person to person is vital to lowering the amount of antimicrobial resistance observed.

An action as simple as hand washing plays a pivotal role in reducing the transmission of disease. The use of soap and water with vigorous rubbing of the hands aids in the removal of organisms from the hands. Many companies advertise the necessity of antimicrobial agents in soaps to completely remove microorganisms from the hands (Kampf and Kramer, 2003; Sickbert-Bennett et al., 2005), however it has been demonstrated that plain soap and water alone are effective in the removal of pathogens. Water and soap alone are favorable because they do not include the added risk of the development of bacterial antimicrobial resistance. Rather than killing micro-organisms, plain soap reduces the surface tension of any transient micro-organisms that might be present on the hands, enabling water to effectively rinse them away. It has been demonstrated that hand wash-

ing for a length of 15 seconds or more is an effective practice to disrupt the transmission of most pathogens (Martinez and Baquero, 2002).

In this study, the extent of antimicrobial resistance was examined within a typical urban university setting. Antimicrobial resistance to ciprofloxacin, triclosan, and ampicillin were monitored by plating samples taken from 35 surfaces throughout the university. Replica plating was utilized to transfer the initial surface sample growth to plates containing the three antimicrobial agents. The occurrence and time-length of hand washing was also observed to determine a possible mechanism behind the high rate of antimicrobial resistance determined within the University. Due to the high levels of antimicrobial resistance that was detected in this study, individuals were expected to have, and did demonstrate, below average hand washing times.

These studies demonstrate that the spread of microorganisms and occurrence of antimicrobial resistance in a typical urban university setting is of concern. The hand washing results suggest that improper hand washing might contribute to the spread of the microorganisms throughout the campus building.

Materials and Methods

Study design

The data for the present study were obtained during a three day analysis for the presence of bacteria in public facilities at a typical urban undergraduate institution. To do this, each site was swabbed with sterile swabs in triplicate. Thirty-five sites were sampled within nine different areas of the University: a computer lab, a frequently used lecture hall, a cafeteria, a science laboratory, the front and side entrances to the university and three high traffic restrooms. The sample sites were selected based upon their location within the University and the amount of traffic they receive during a typical day of classes. Other sites such as the cafeteria, science laboratories, and science floor restroom were selected due to their proximity to either food serving facilities (in the case of the cafeteria) or to aide in the analysis of the spread of laboratory

organisms (in the case of science laboratories). At each location, specific surfaces were sampled. For the restrooms, samples were taken from the floor near the toilet, toilet seat, faucet, soap dispenser, door at the entrance of restroom, knob on the stall, and the button on the hand dryers. In the computer lab, samples were taken from a keyboard, mouse, computer user tag, and a stapler. Lecture hall samples were taken from the door, a desk, and a podium. In the cafeteria, samples were taken from an eating area table, self-serve utensils, and a coffee dispenser. In the lab area, samples were taken from a lab table where lab technicians often break between preparations (this site is sometimes used by the technicians to eat). At the side entrance of the university, samples were taken from the door, elevator buttons, and stair rail. At the front entrance, samples were taken from a computer and revolving door. All door samples were taken from the interior side of the university i.e. door handles facing the inside of the university.

Sample collection

Samples were obtained through the use of sterile cotton swabs (Fischer scientific). The dry sterile cotton swabs were passed over the sample site surface with a rotating motion to ensure complete coverage of the swab. Once the sample was obtained, the swab was placed in a sterile 15 ml centrifuge tube (Fischer scientific) and placed in a 4°C refrigerator until further analysis.

Each sample swab was struck across a tryptic soy agar (TSA) plate (Sigma) to create a lawn. The plates were then placed in an incubator at 37°C for 18-24 hours. Following incubation, the plates were removed from the incubator and examined for colony number and colony morphology. All plates were stored at 4°C for subsequent analyses.

Determination of antibiotic resistance profiles using replica plating

The TSA plates representing each collected sample were replica plated in triplicate onto antimicrobial agent containing plates. The antimicrobial agents used were ampicillin (100 µg/mL), ciprofloxacin (1.0 µg/mL), and triclosan

(1.0 µg/mL). The concentrations used for this study were chosen because of their definition as minimal inhibitory/bactericidal concentrations in the literature, (Gilbert and McBain, 2003). Additional plates without antibiotic were used as controls. The replica plates were incubated for 18-24 hours at 37°C. The number of antibiotic resistant colonies present was recorded for each plate.

Hand washing behavior

Individuals within the university were monitored for hand washing behavior. The study was conducted according to the methods described in a study by Harris Interactive (as described in *Microbe*, American Society for Microbiology News Magazine. Study finds decline in hand washing behavior. 2007. 12:610-612). Observation of hand washing behavior was conducted in the restrooms used in the above described surface analyses. Restrooms were monitored for hand washing behavior for intervals of 15 minutes. Hand washing and hair and clothing adjustment was simulated in order to evade subject knowledge of the observation. Hand washing behavior was defined as the placement of hands in water with soap. Hand washing time varied from zero seconds, equivalent to no hand washing, to a total washing time of approximately 15 seconds using a watch with a second hand.

Results

Surface analysis

Multiple surfaces throughout the university were sampled in order to analyze the extent of microbial growth within an urban university. Determining the extent of microbial growth provided a fundamental view of the presence of microorganisms within the university. The initial step in this study was to sample surfaces for the presence of microorganisms. As described in materials and methods, triplicate swab samples from each site were plated on TSA. Following an 18-24 hour incubation at 37°C, colony number and morphology were evaluated. Table 1 depicts the colony counts for each sample site. Of the 35 surfaces sampled throughout the university, the

Table 1. Surface sample initial colony growth on TSA (n = 3).

Sample Sites	Avg	StDev
Lecture Hall		
Desk	14.6	±8.62
Door	7.66	±4.04
Podium	8.33	±8.13
Lab Bench		
	9.00	±7.81
Entrances		
SE stair rail	11.6	±13.4
SE door	9.66	±9.02
SE button	11.6	±7.23
FE door	24.7	±19.3
FE computer	21.0	±6.24
Computer Lab		
Keyboard	58.6	±31.9
Stapler	26.0	±24.4
Mouse	28.0	±29.5
Tag	3.33	±3.51
Cafeteria		
Coffee dispenser	39.6	±32.9
Eating area table	52.3	±30.9
Self-service utensil	59.0	±73.9
Restrooms		
<i>1st Floor</i>		
Floor	135	±33.7
Faucet	30.6	±33.7
Soap	1.00	±.707
Door	8.33	±7.60
Seat	84.0	±35.5
Knobs	3.66	±1.53
<i>3rd Floor</i>		
Floor	79.3	±63.9
Faucet	35.6	±33.3
Soap	7.33	±1.15
Door	6.33	±8.38
Seat	126	±152.3
Knobs	8.33	±8.08
Dryer	104	±97.4
<i>5th Floor</i>		
Floor	122	±24.5
Faucet	11.0	±6.93
Soap	12.0	±14.2
Door	1.33	±1.53
Seat	40.6	±33.5
Knobs	3.33	±.577

restroom floor (135 ± 33.7), toilet seat (126 ± 152.3), and dryer button (104 ± 97.4) contained the largest average colony counts, respectively. In the lecture hall, the desks contained the highest average number of microorganisms (14.6 ± 8.62). Of the University entrances, the front entrance contained the highest average number of microorganisms (24.7 ± 19.3). Within the computer lab,

the most colonies were obtained from the keyboards (58.6 ± 31.9). In the cafeteria, the most microorganisms were isolated from the self-service utensils (59 ± 73.9). All of the sample sites produced CFUs that were pin point circular, opaque, and convex. The only sites that contained CFUs with differing morphology were the coffee dispenser in the cafeteria, the front entrance door, and the restroom floors. Both sites contained large scaly growths as well as some transparent CFUs interspersed between the more commonly observed CFUs.

Analysis of antimicrobial resistance

Once the initial plates were observed for colony growth and morphology each sample plate was replicated on triplicate plates containing either, ampicillin (100 µg/mL), ciprofloxacin (1.0 µg/mL), or triclosan (1.0 µg/mL). This step of the study was used to determine the extent of antimicrobial resistance within the university. The extent of antimicrobial resistance was demonstrated by the growth of microorganisms on plates containing the three antimicrobial agents. Table 2 depicts the results of this study. The front entrance door, front entrance information computer, and the restroom floor were the only surfaces within the University that contained resistant strains to all three antimicrobial agents used in the experiment on each of the three sampling days. Surfaces that showed resistant growth to all three antimicrobial agents on at least one of the sampling days included the toilet seat, computer lab keyboard, soap dispenser, faucet, cafeteria table, and coffee dispenser. Of the three antimicrobial agents used in this study to determine resistance, triclosan resistance was the most common, occurring in all of the sample sites on at least one of the sample days. Triclosan resistance was followed by resistance to ciprofloxacin, which appeared in 97% of the samples collected. Ampicillin resistance was the least common, appearing in 68% of the samples collected.

Observation of hand washing behavior

Observation of hygiene behavior was conducted in order to monitor a possible mechanism for the transmission of microorganisms throughout

Table 2. Occurrence of ciprofloxacin, triclosan, and ampicillin resistance. Amp = ampicillin, Cipro = ciprofloxacin, and Tric = triclosan. Superscript numbers indicate total samples out of the three individual samples obtained that produced resistance for the specified agent (maximum = 3).

Sample Sites		Observed Resistance	
Lecture Hall			
Desk		Cipro ²	Tric ²
Door	Amp ¹	Cipro ²	Tric ¹
Podium	Amp ¹	Cipro ³	Tric ³
Lab Bench	Amp ²	Cipro ³	Tric ²
Entrances			
SE stair rail		Cipro ¹	Tric ²
SE door		Cipro ²	Tric ²
SE button		Cipro ³	Tric ³
FE door	Amp ³	Cipro ³	Tric ³
FE computer	Amp ³	Cipro ³	Tric ³
Computer Lab			
Keyboard	Amp ³	Cipro ²	Tric ³
Stapler	Amp ²	Cipro ²	Tric ¹
Mouse		Cipro ³	Tric ³
Tag	Amp ¹	Cipro ³	Tric ²
Cafeteria			
Coffee dispenser	Amp ²	Cipro ³	Tric ³
Eating area table	Amp ¹	Cipro ³	Tric ³
Self-service utensil		Cipro ³	Tric ³
Restrooms			
<i>1st Floor</i>			
Floor	Amp ³	Cipro ³	Tric ³
Faucet	Amp ²	Cipro ³	Tric ²
Soap	Amp ¹	Cipro ²	Tric ¹
Door		Cipro ¹	Tric ²
Seat	Amp ²	Cipro ³	Tric ³
Knobs			Tric ¹
<i>3rd Floor</i>			
Floor	Amp ³	Cipro ³	Tric ³
Faucet	Amp ²	Cipro ²	Tric ²
Soap	Amp ¹	Cipro ²	Tric ²
Door	Amp ¹	Cipro ²	Tric ²
Seat	Amp ²	Cipro ³	Tric ³
Knobs	Amp ¹	Cipro ³	Tric ³
Dryer		Cipro ²	Tric ¹
<i>5th Floor</i>			
Floor	Amp ³	Cipro ³	Tric ³
Faucet	Amp ²	Cipro ²	Tric ²
Soap	Amp ¹	Cipro ²	Tric ¹
Door		Cipro ²	Tric ¹
Seat	Amp ²	Cipro ²	Tric ¹
Knobs		Cipro ³	Tric ¹

the university. Poor hand washing skills can lead to widespread pathogen dispersion, especially within a closed environment; microorganisms on the hand can spread to all surfaces that the individual contacts (Stefkovicova et al.,

2006). In addition, our laboratory has demonstrated that improper hand washing using soaps containing triclosan can lead to the development of antimicrobial resistance (Bello and Peteroy-Kelly, 2007). We defined hand washing behavior as the placement of hands in water with soap. Hand washing was timed from the placement of the hands into water until the subject removed their hands from the water and turned off the faucet. Hand washing times varied from zero seconds, equivalent to no hand washing, to a total washing time of approximately 15 seconds. 12% ±.33 of the individuals observed did not wash their hands at all. The average hand washing time of those individuals who washed their hands, was 4.87 (±3.97) seconds.

Discussion

The development and spread of antimicrobial resistant pathogens has recently become of great concern to the public (ASM, 2007; Gilbert and McBain, 2003; Wassmer et al., 2006). The evolution of pathogens that are resistant to multiple antimicrobial agents makes it difficult to treat many illnesses that were caused by agents that were once easily treated with a single antimicrobial agent (Thomson, 1999; Depardieu et al., 2002; Martinez and Baquero, 2002; Wassmer et al., 2006). In recent years, multiple drug resistance has been observed in *Mycobacterium tuberculosis*, *Staphylococcus aureus*, organisms that cause sexually transmitted diseases, as well as organisms that cause pneumonia (Suller and Russell, 2000; Martinez and Baquero, 2002). Thus, it is important to inform the public of the pathogens that surround them, the significance of antimicrobial resistance, and how to prevent the development and transmission of resistant pathogens. It was the goal of this study to determine the prevalence of bacteria and antimicrobial resistance at different sites in Pace University, NYC.

Our study has demonstrated that there is a high level of antimicrobial resistance within the University. Pace University-NYC is a self contained building, providing students with all of the typical university amenities within an 18-story building. The University is located in

downtown Manhattan, serves a student population of at least 8,000 and is also contains a privately owned theatre of 600 seats, that is accessible to the public. Thus, Pace University's large student population, faculty, staff, and outside visitors provide multiple opportunities for the transmission of pathogens. Antimicrobial resistant bacteria were prevalent in areas of high traffic and entrances; in a computer lab, a cafeteria, and in several restrooms. Table 1 depicts the population density of microorganisms within the university. Large colony counts were observed in locations where the outcome hoped for would have been exceptional cleanliness—such as any area near food. During the study, methods of pathogen transmission became apparent. The transmission of pathogens within a closed environment is dependent upon colony population growth and contact. When hand washing is not conducted for a sufficient amount of time to remove multiple microorganisms from the hands, microorganisms are free to transiently populate the hands. Pathogen transmission occurs all throughout the university, whether through direct contact via a hand shake, or through fomites such as pencils and door knobs. Any subsequent surface contact allows for the transfer of microorganism populations from an individual's hands to another's.

The ease of transmission of antimicrobial resistant pathogens as observed by the presence of resistant bacteria in multiple locations throughout the university is of great concern. Table 2 depicts the presence of antimicrobial resistance upon surfaces within the university. While resistance to triclosan was expected, as previous studies have demonstrated its prevalence due to the use of antimicrobial containing consumer products (Martinez and Baquero, 2002; Gilbert and McBain, 2003), resistance to ampicillin and ciprofloxacin was a great cause for concern.

Each of the antimicrobial agents used in this study differ in their mechanism of bacterial population control and primary usage. Triclosan, 2,4,4'-Trichloro-2'-hydroxydiphenyl ether, is an antimicrobial agent that is often found in consumer products. It disrupts fatty acid biosynthesis in bacteria by blocking the active site of enoyl-acyl carrier protein reductase. Bacte-

rial fatty acid synthesis is an essential process in bacteria. Interruption of fatty acid synthesis results in the breakdown of cell function and retraction of the bacterial cell membrane (Tierno, 1999; Glaser, 2004). Triclosan is a broad spectrum germicide that is used to prevent the spread of microorganisms including bacteria, fungi, and viruses (Tierno, 1999; Glaser, 2004). Ampicillin is an antibiotic that interferes with bacterial cell wall biosynthesis (Hujer et al., 2005). Finally, ciprofloxacin is a member of the quinolone family of antibiotics. Ciprofloxacin disrupts the bacterial life cycle by binding to DNA gyrase resulting in the disruption of DNA replication (Thomson, 1999). The disruption of DNA replication limits all processes throughout the cell including cell growth, protein production, and replication (Campion et al., 2005).

Although resistance to any antimicrobial agent is cause for alarm, ciprofloxacin is a significant, last resort, form of treatment for difficult infections in which other drugs have failed to treat (Thomson, 1999). Therefore, the observation of ciprofloxacin resistance throughout the university environment demonstrates a pivotal point: a common and efficient treatment method for multiple illnesses may soon become widely ineffective in the treatment of multiple bacterial pathogens. This has already been observed in the hospital setting (Campion et al., 2005). The high levels of resistance observed in the university demonstrate that the development of resistance and widespread transmission of resistant pathogens is already occurring.

Hand washing with soap free of antimicrobial agents is the most simple and effective method to prevent a pathogen's path of transmission from person to person (Kampf and Kramer, 2004; ASM, 2007). Pace University's soap dispensers contain detergents free of antimicrobials (GOJO skin cleansing lotion). Use of soap free of antimicrobials is of great significance in the prevention of the development of resistance. Using soaps free of products such as triclosan, limits the development and spread of resistant bacteria. This is because several studies have demonstrated that there is a connection between triclosan usage and the emergence of antibiotic resistance in bacteria. (Courvalin, P., 1994, Fan,

F., et al., 2002, Gilbert and McBain, 2003, Glaser, 2004, Tkachenko, 2007). However, simply washing hands with water and soap long enough to wet the hands lowers the effectiveness of hand washing in the prevention of pathogen transmission. The efficiency of hand washing in the prevention of pathogen transmission relies upon the removal of large amounts of microorganisms from the hands with every wash, such a removal can only occur through vigorous rubbing and rinsing with soap (to remove natural oils from the hands) and water for at least 20 seconds (Kampf and Kramer, 2004; ASM, 2007). In order to experience the optimal effects of hand washing, washers are instructed to wash their hands with soap and water vigorously for at least 20 seconds and to dry their hands thoroughly with air dryers or paper towels to remove harmful pathogens (Kampf and Kramer, 2004). Our results show that the Pace University students wash their hands for an average of 4.87 (± 3.97) seconds. They do not wash their hands long enough to remove harmful pathogens (ASM, 2007). The high levels of antimicrobial resistance observed on multiple surfaces throughout the university, especially in areas of high traffic (computer lab, cafeteria, and restrooms) may be due to the poor hand washing techniques of members of the university community. One area of high level microorganism growth was the restroom. The restrooms, excluding the floors, are cleaned five days a week during school hours with mild ammonia containing cleaning products. At least three nights a week the restrooms are cleaned with stronger cleaning chemicals at which time the floor is also cleaned. Thus, each day, the restrooms begin as areas of low levels of microorganism growth, at least until individuals use the restroom and begin to populate the area with microorganisms.

The results of this study suggest that improper hand washing behavior within a closed university environment paired with high levels antimicrobial resistance provides a "breeding-ground" for the growth and spread of multiple antimicrobial resistant microorganisms. Therefore, the public, universities especially, should increase their awareness of the significance of antimicrobial resistance and methods in the prevention of

pathogen transmission including: proper hand washing technique.

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