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Staphylococcus aureus and Methicillin-resistant *S. aureus* (MRSA) carriage in Public Computer Service Providers and Utility Jeepneys in UP Diliman

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ABSTRACT

Staphylococcus aureus is a Gram-positive bacterium that causes minor skin infections to life-threatening diseases. It is transmitted through direct contact with fomites, such as computer peripherals and handrails. Treatment of *S. aureus* infections is generally straightforward, but is complicated by drug-resistant strains, particularly methicillin-resistant *S. aureus* (MRSA). The University of the Philippines Diliman (UP Diliman) has hundreds of computer service providers (CSPs) and public utility jeepneys (PUJs) regularly used by faculty, students, staff, and visitors. While no outbreaks of *S. aureus* and MRSA have been reported, the possibility of infection with this pathogen through CSPs and PUJs is very likely. The objectives of this study are to determine the carriage rates of *S. aureus* and MRSA in CSPs, computer peripherals, and handrails of PUJs inside UP Diliman, and to identify the risk factors associated with *S. aureus* and MRSA contamination. A total of 162 computer peripherals from 27 CSPs and 196 PUJ handrails were swabbed. *S. aureus* isolates were identified using colony morphology, biochemical tests, and amplification of the *nuc* gene, whereas MRSA isolates were identified using the cefoxitin challenge and amplification of the *mecA* gene. *S. aureus* was identified in 92.6% of CSPs, 36.4% of computer peripherals, and

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7.1% of PUJs, while MRSA carriage was 3.1% in CSPs and 2% in PUJs. No significant associations between *S. aureus*/MRSA and the assessed risk factors were observed ($p > 0.05$). Results indicate that while *S. aureus* prevalence is relatively high, MRSA carriage is low in CSPs and PUJs in UP Diliman.

Keywords: Staphylococcus aureus, MRSA, computer peripherals, handrails

INTRODUCTION

Staphylococcus aureus is a Gram-positive, coccus-shaped bacterium belonging to the phylum Firmicutes and the family Staphylococcaceae. It is a facultative anaerobe which can ferment mannitol and produce enzymes, such as catalase, coagulase, and deoxyribonuclease (Plata et al. 2009; Kateete et al. 2010). It asymptomatically colonizes the skin and nose in humans, and is able to survive in fomites like plastic and steel surfaces (Chiller et al. 2001; Kusumaningrum et al. 2003).

S. aureus is the causative agent of several diseases, ranging from mild skin infections like impetigo and folliculitis, to toxin-mediated conditions like food poisoning and toxic shock syndrome, and severe infections like staphylococcal bacteremia and endocarditis (Lowy 1998; Chiller et al. 2001). Staphylococcal infections are common and can generally be treated without complications. However, methicillin-resistant strains of *S. aureus* (MRSA), mediated by the *mecA* gene, are recently becoming more frequent in the community. In the Philippines, 30% of community-acquired *S. aureus* (CASA) and 38% of hospital-acquired *S. aureus* (HASA) infections are caused by MRSA (Song et al. 2011). According to the Antimicrobial Resistance Surveillance of the Philippines (ARSP), the prevalence of MRSA in the country is 31%. Dicloxacillin remains as the antibiotic of choice for methicillin-susceptible *S. aureus* (MSSA) infections, whereas MRSA infections are treated with vancomycin or teicoplanin (Rayner and Munckhof 2005).

Transmission of *S. aureus* commonly occurs through hand contact with fomites like computer peripherals and handrails contaminated by *S. aureus* (Alkhezali and Taha 2013). According to Yahoo-Nielsen (2009), 20% of Filipinos in urban areas access the internet using public computer service providers (CSPs). The Land Transportation Franchising and Regulatory Board listed 210,840 public utility jeepneys (PUJs) nationwide in 2012, with 58,000 operating in Metro Manila (Ronda 2012). CSPs and PUJs are the dominant access points and mode of transportation, respectively,

for users from lower socio-economic classes. Despite this, there is very little published data on the prevalence of *S. aureus* and MRSA in CSPs and PUJs in the country.

The objectives of this study are the following: to determine the prevalence of *S. aureus* and MRSA in computer peripherals in CSPs and handrails of PUJs in the University of the Philippines Diliman (UP Diliman), and to analyze the risk factors associated with carriage. Findings from this study may be used to influence university policies regarding the regular sanitation in CSPs and PUJs to prevent or reduce further contamination.

MATERIALS AND METHODS

Sample Size

The number of CSPs in UP Diliman was obtained from two sources: the Business Permit Licensing Office of the Quezon City Hall for Department of Trade and Industry-accredited internet cafés, and the website www.mainlib.upd.edu.ph (University Library Diliman 2010) for libraries affiliated with UP Diliman. The number of PUJs was obtained from the UP Diliman Police through the Office of the Vice-Chancellor for Community Affairs. Only CSPs with at least three computers units, signed the consent forms, and allowed unannounced sampling dates were included in the study. The PUJs per route were randomly sampled. The sample size was determined from each population with a 95% confidence level and a confidence interval of 10 (Creative Research Systems 2014). A total of 162 computer peripherals from 27 CSPs and 196 PUJ handrails were sampled.

Consent and Survey Forms

An introductory letter and consent form explaining the purpose of the study and rights regarding participation were provided to the participants. Head librarians and owners of internet cafés were given survey forms for the assessment of the following risk factors: years in service, service hours, comfort room availability, number of computer units, number of clients per day, usual gender of clients, duration of computer use, consumption of food and drink, frequency of cleaning the facility, frequency of cleaning the peripherals, and availability of hand sanitizers.

Sample Collection

Three computers were selected from each CSPs: the computers nearest to the door, furthest to the door, and in the middle of the facility. Sample collection in CSPs was performed on weekdays between 1:00 PM and 4:00 PM. Sterile cotton swabs dipped into 0.9% sterile saline were swabbed on the entire surface of keyboards and mice. The number of PUJs sampled per route is indicated in Table 1. Sampling of PUJs was performed every Thursday from 2:30 PM to 5:30 PM. For each handrail, a 10-cm length was swabbed with 10 sweeps of consistent length. The plate number of each PUJ was recorded to prevent duplication. The location and distribution of the CSPs are shown in Figure 1. The cotton swabs were placed into 15-mL tubes of mannitol salt broth (MSB) and delivered to the Medical Microbiology Laboratory of the Institute of Biology, UP Diliman within 4 hours of collection for incubation at 37°C for 18-24 hours.

Table 1. Prevalence of *S. aureus* and MRSA in PUJs

Route	*S. aureus* Prevalence	MRSA Prevalence
Ikot	2.9% (1/35)	ND
Katipunan	11.6% (5/43)	2.3% (1/43)
Pantranco	6.4% (3/47)	4.3% (2/47)
Philcoa	3.3% (1/30)	ND
SM North EDSA	ND (0/28)	ND
Toki	30.8% (4/13)	7.7% (1/13)
Total	**7.1% (14/196)**	**2% (4/196)**

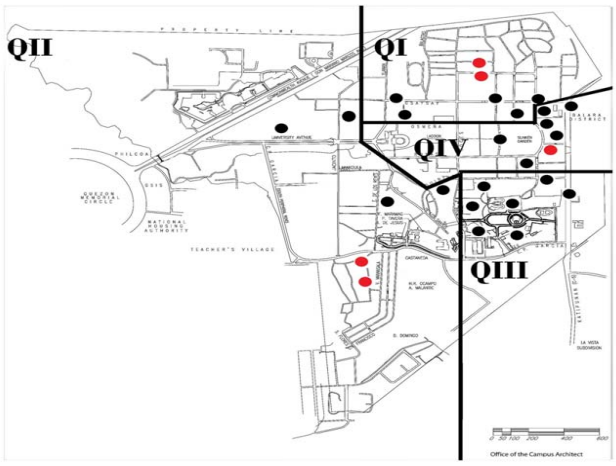

Figure 1. Location and distribution of CSPs that participated in the study. Red dots indicate CSPs where MRSA isolates were obtained.

Isolation

Samples positive for mannitol fermentation were streaked on mannitol salt agar (MSA) plates and incubated at 37°C for 18-24 hours. Medium-sized yellow colonies with smooth surfaces were purified in MSA, and the resulting isolated colonies were subcultured on nutrient agar (NA) slants for maintenance.

S. aureus Identification

Gram staining, KOH test, catalase test, coagulase test, and DNase test were used to identify *S. aureus*. Identification was confirmed through the PCR amplification of the *nuc* gene. Genomic DNA was extracted using the microwave lysis method (Ahmed et al. 2014). The cell pellets were briefly washed and resuspended in 100 µL TE buffer. Fifty (50) µL of 10% SDS was added to the mixture for incubation at 65°C for 30 minutes. The lysates were centrifuged at 10,000 x g for 10 minutes. Supernatants were discarded and the cell pellets were heated three times for 1 minute at the high setting of a microwave oven (3D, model no. WP-70B17-65, input: 230V ~60 Hz 1200 W, output: 700 W 2450 MHz). The pellets were dissolved in 200 µL TE buffer. An equal volume of phenol/chloroform/isoamyl alcohol (25:24:1) was added to extract the DNA, followed by overnight absolute ethanol precipitation at -20°C. DNA was recovered by centrifugation at 10,000 x g for 10 minutes, air-dried, and resuspended in 30 µL TE buffer. DNA yield and purity were determined using the Nanodrop™ 2000c Spectrophotometer (Thermo Scientific, USA).

PCR was performed as previously reported with slight modifications (Brakstad et al. 1992) using the forward primer SA-01F 5'-GCGATTGATGGTGATACGGTT-3' and the reverse primer SA-02R 5'-AGCCAAGCCTTGACGAACTAAAGC-3'. Each 25 µL reaction mixture was composed of 5 µL DNA (48 to 50 ng), 3.5 µL sterile water, 2.0 µL of each primer (0.8 µM), and 12.5 µL 2X GoTaq® master mix (Promega, USA). PCR amplifications were performed in a MyCycler™ Thermal Cycler (Bio-Rad, USA) using the following conditions: 94°C for a 2-minute initial denaturation; 37 cycles of 94°C for 1 minute, 42°C for 30 seconds, 72°C for 1 minute; and 72°C for a 7-minute final extension. *S. aureus* BIOTECH 1350 and *S. epidermidis* BIOTECH 10098 were used as controls. PCR products were electrophoresed in 1.3% agarose gel pre-stained with 0.5 µg/mL ethidium bromide using the PowerPac Basic electrophoresis system (Bio-Rad, USA) at 100 V for 22 minutes. The gel was viewed using a White/2UV transilluminator (Thermo Scientific, USA).

PCR amplification of the 16S rDNA was performed as internal control, in order to rule out false-negative results (Amit-Romach et al. 2004). Amplification of the 16S rDNA was performed using the forward primer Unibac-F 5'-CGTGCCAGCCGCGGTAATACG-3' and the reverse primer Unibac-R 5'-GGGTTGCGCTCGTTGCGGGACTTAACCCAACAT-3' under the following conditions: 94°C for 3-minute initial denaturation; 37 cycles of 94°C for 30 seconds, 60°C for 1 minute, 68°C for 2 minutes; and 68°C for a 7-minute final extension. PCR products were electrophoresed and visualized as previously described.

MRSA Identification

S. aureus isolates were challenged with 30 µg cefoxitin using the Kirby-Bauer disk diffusion assay as described in the Clinical and Laboratory Standards Institute (2014). Isolates were identified as MRSA if the zone of inhibition was less than or equal to 21 millimeters. MRSA identification was confirmed by PCR amplification of the *mecA* gene. PCR was performed as described above using the forward primer mecA1F 5'-AAAATCGATGGTAAAGGTTGGC-3' and the reverse primer mecA2R 5'-AGTTCTGCAGTACCGGATTTGC-3'. PCR was performed using the following conditions: 94°C for a 4-minute initial denaturation; 30 cycles of 94°C for 30 seconds, 53°C for 30 seconds, 72°C for 1 minute; and 72°C for a 7-minute final extension (Murakami et al. 1991). MRSA BIOTECH 10378 and *S. aureus* BIOTECH 1350 were used as controls. PCR products were electrophoresed and visualized as previously described.

Statistical Analysis

The prevalence of *S. aureus* and MRSA was determined using the data from samples that yielded positive results. The association of potential risk factors in *S. aureus* and MRSA contamination was performed using the Chi-square test in the IBM SPSS software (IBM Corporation, 2013). P-values less than 0.05 were considered to be statistically significant. Otherwise, the null hypothesis was accepted, and the risk factor in question was concluded to play no role in contamination.

Waste Disposal

All materials that were contaminated with *S. aureus,* MRSA, and reference microorganisms were decontaminated using an autoclave prior to disposal (CDC 2009).

RESULTS

Prevalence of *S. aureus* and MRSA among CSPs

A total of 162 samples (81 keyboards and 81 mice) were collected from 27 CSPs, which were artificially categorized into four quadrants based on their locations (Figure 1). The prevalence of *S. aureus* among CSPs was 92.6% (Table 2). *S. aureus* was detected in all CSPs located in Quadrants II and III, while only 85.7% of the CSPs in Quadrants I and IV were contaminated. The prevalence of *S. aureus* among keyboards was 40.7%, with more than half (56%) of the keyboards in Quadrant II contaminated with *S. aureus*. Keyboards of the CSPs in Quadrant IV were the least contaminated (24%). The prevalence of *S. aureus* among mice was 32.1%, which is lower compared to the keyboards. Mice of the CSPs in Quadrant II were the least contaminated. The difference in contamination between quadrants was not statistically significant ($p > 0.05$). MRSA had a prevalence of 3.1% and was isolated from one keyboard in Quadrant I, 2 keyboards in Quadrant IV, and one keyboard and one mouse in Quadrant III.

Table 2. Prevalence of *S. aureus* and MRSA in CSPs

Quadrant	*S. aureus* Prevalence in CSPs	*S. aureus* Prevalence in Keyboards	*S. aureus* Prevalence in Mice	Total	MRSA Prevalence
I	85.7% (6/7)	38.1% (8/21)	33.3% (7/21)	35.7% (15/42)	ND
II	100% (6/6)	55.6% (10/18)	27.8% (5/18)	41.7% (15/36)	2.8% (1/36)
III	100% (7/7)	47.6% (10/21)	38.1% (8/21)	42.9% (18/42)	4.8% (2/42)
IV	85.7% (6/7)	23.8% (5/21)	29.6% (6/21)	26.2% (11/42)	4.8% (2/42)
Total	**92.6% (25/27)**	**40.7% (33/81)**	**32.1% (26/81)**	**36.4% (59/162)**	**3.1% (5/162)**

Prevalence of *S. aureus* and MRSA among PUJs

Samples were collected from a total of 196 PUJ handrails designated in 6 different routes (Table 1). The prevalence of *S. aureus* among PUJs was 7.1%. *S. aureus* was detected in all routes except for PUJs traveling to SM North EDSA. PUJs traveling to Katipunan had the highest prevalence of 11.6%. The difference in contamination between routes was not statistically significant ($p > 0.05$). MRSA had a prevalence of 2% and was isolated from PUJs traveling along the Katipunan, Pantranco, and Toki routes.

Risk factor analysis

None of the risk factors assessed in this study was found to have a significant effect on the contamination of *S. aureus* and MRSA on computer peripherals and handrails (Table 3).

Table 3. Statistical analysis of risk factors assessed in the study

Risk factor assessed	X^2	df	*P* value
Years in service	0.270	2	0.874
Service hours	0.909	2	0.635
Comfort room availability	0.173	1	0.678
Number of computer units	2.70	2	0.259
Number of clients per day	0.513	3	0.163
Usual gender of clients	0.376	1	0.540
Duration of use of computer	1.392	4	0.846
Food consumption in facility	0.376	1	0.540
Drinking inside the facility	0.003	1	0.957
Frequency of cleaning the facility	0.756	3	0.860
Frequency of cleaning the peripherals	1.121	4	0.891
Availability of hand sanitizers	0.270	1	0.603

DISCUSSION

The University of the Philippines Diliman has 59 CSPs and 324 PUJs, which cater to faculty, students, administrative staff, and visitors. Computer peripherals, such as keyboards and mice, and PUJ handrails can serve as fomites for the transmission of pathogenic bacteria like *S. aureus*. In the absence of clear sanitation guidelines and regular cleaning of computer peripherals and handrails, contaminated keyboards and mice in CSPs and handrails in PUJs pose a risk to the health of computer users and commuters, respectively.

The inclusion and exclusion criteria of the study specified that only CSPs with at least three computer units may participate, reducing the number of qualified CSPs in calculating our sample size. Some internet cafés also refused to sign the consent forms on grounds of possible bad publicity despite a clause on the confidentiality of the study. In order to have a representation of UP Diliman, the effective sampling sizes were 21 libraries and 5 internet cafés. In this study, 22 libraries and 5 internet cafés were sampled.

Among the 27 CSPs, 25 were positive for *S. aureus* contamination, resulting to a prevalence of 92.6%. No significant difference in the prevalence was observed among quadrants ($p > 0.05$) because most of the CSPs had at least one computer peripheral contaminated with *S. aureus*. Out of the 162 peripherals sampled, 36.4% were positive for *S. aureus* contamination. The high prevalence observed among the CSPs is likely due to the lack of disinfection policies before and after use of the computers. Library computers are high-traffic computer units with high-contact surfaces. Given the large number of students accessing these computers on a daily basis, contamination rates must be intuitively high. The lack of routine disinfection, coupled with the absence of hand sanitizers near the computer terminals, likely contribute to the high prevalence observed. *S. aureus*, including MRSA, has been reported to be a persistent pathogen because it can survive for months on dry surfaces. If no regular surface disinfection is performed, these dry surfaces can be a source of transmission (Kramer et al. 2006). *S. aureus* and other pathogenic microorganisms have also been demonstrated to persist on non-porous surfaces, such as keyboards and mice, even in the absence of enrichment. Unwashed moist or sweaty hands and a room temperature that favor the growth of *S. aureus* can also be factors in the high prevalence observed. *S. aureus* can survive in a salt environment, and sweat is a hospitable environment for the carriage and transfer of the bacterium onto various surfaces (Kahanov et al. 2015).

The prevalence of *S. aureus* was higher in keyboards (40.7%) compared to mice (32.1%), although the difference was not statistically significant ($p > 0.05$). The total surface area of a keyboard is larger than that of a mouse, allowing for the colonization by a greater number of microorganisms. Keyboards also have spaces between keys where dirt and food particles can accumulate. Moreover, both hands are in contact with the keyboard.

The prevalence of *S. aureus* in CSPs observed in this study is lower compared to a similar study conducted in Kogi State University in Nigeria, where *S. aureus* was isolated from all CSPs (Enemuor et al. 2012). It should be noted, however, that in their study, only 30 samples were collected from five sampling sites. By contrast, the prevalence of *S. aureus* among keyboards and mice in this study is higher compared to a study in Al-Mustansiriya University in Iraq, where *S. aureus* had a prevalence of 18.6% among computer peripherals (Alkhezali and Taha 2013). The difference is likely due to the larger number of samples and sampling sites used in this study (162 versus 50 samples). Understandably, a study conducted in Ebonyi State University in Nigeria observed a higher prevalence of 42.6% after sampling 250 keyboards and mice in three campuses (Chukwudi et al. 2013), because it only

included internet cafés, where food and drinks are generally allowed, unlike in school libraries.

The prevalence of *S. aureus* in PUJs in UP Diliman is surprisingly low at 7.1%, considering the heavy traffic of commuters PUJs encounter during school days. However, previous studies on public transportation have reported prevalence values ranging from 8% in London (Otter and French 2009) to 68% in the United States (Lutz et al. 2014), or to even the absence of *S. aureus* (Yeh et al. 2011). The prevalence of *S. aureus* in PUJs is dependent on the nature of the fomite surveyed for the study. The handrails of PUJs are made of smooth steel. The lack of rough surface limits the amount of dirt or organic material that *S. aureus* may use for attachment or nutrient, unless the turnover of passengers using the handrails is high.

PUJs along the Toki route had the highest contamination of *S. aureus* at 30.8%, while PUJs along the SM North EDSA route had no contamination of *S. aureus*. The differences in the prevalence of *S. aureus* across PUJ routes is multifactorial and may be due to the following: passenger profile, personal hygiene of the passengers, bacterial contamination from paper bills and coins used as payment or change, and eating and drinking inside the PUJs. Different numbers and profiles of passengers (students versus non-students) per PUJ were observed during sample collection.

In this study, methicillin resistance was detected through the cefoxitin disk diffusion test and the PCR amplification of the *mec*A gene. The cefoxitin disk test was used as a surrogate test for oxacillin and methicillin test because cefoxitin can better detect heteroresistant strains or strains that carry the resistance gene but express different levels of resistance (CDC 2015). Furthermore, cefoxitin can better induce the *mec*A gene and produce more reproducible and accurate results than oxacillin and methicillin. Based on the results, the prevalence of MRSA in UP Diliman CSPs and PUJs were low at 3.1% and 2%, respectively. Different studies have varied reports on the prevalence of MRSA isolated from public transportation. Stepanoviæ (2008) reported the presence of methicillin-resistant coagulase-negative Staphylococci in the handrails of public buses in Belgrade, Serbia, but no MRSA was detected. A study in Portugal reported a prevalence of 36.2% for MRSA in public buses (Conceição et al. 2013). Based on our search in published literature, no points of comparison could be found for MRSA colonization in CSPs and PUJs in universities in other countries, but it would seem that MRSA prevalence is low in CSPs and PUJs in UP Diliman. However, the isolation of MRSA from these places indicates a potential risk for the transmission of these bacteria in an out-hospital environment.

Based on the risk factor analysis, no correlation was observed between any of the risk factors considered and the contamination of CSPs and PUJs by *S. aureus* and MRSA (Table 3). Previous studies showed similar results (Kassem et al. 2007; Oguzkaya et al. 2015). Such observation could be due to the ubiquitous nature of *S. aureus,* its easy mode of transmission by hand contact, and its status as a normal microflora of the body, allowing *S. aureus* contamination of fomites to be prevalent and unnoticed.

Understanding the spread of infectious diseases involves gaining insight into its complex spatial diffusion through a network of people. Individuals in a given population participate in various activities that may either be mobile or stationary. Mobile activities include commuting through the PUJs, while stationary activities take place at fixed locations such as CSPs. Tracking disease transmission not only involves the individual members of the population but also the physical environment, where these activities are carried out. The epidemiologic model of infectious disease propagation in the work by Perez and Dragicevic (2009) revealed that dynamic spatial interactions within a population lead to high numbers of exposed individuals who carried out stationary activities after moving between places within their environment. It was found that individuals at risk were concentrated in locations like universities. The findings presented in the work support the significance of public areas, such as PUJs and CSPs, in the transmission of microorganisms to commuters and computer users.

In conclusion, this study documents the prevalence of *S. aureus* and MRSA in CSPs and PUJs in UP Diliman, and emphasizes the potential of computer peripherals and handrails as environmental vehicles for the transmission of potentially pathogenic bacteria within the university. The isolation of MRSA, in particular, calls for a need to increase public awareness among computer users and commuters to disinfect hands after being in CSPs and PUJs.

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