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Virulence-associated Gene Profile and Multilocus Sequence Type Analysis of Human and Fomite-derived Methicillin-resistant *Staphylococcus aureus*

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of healthcare-associated (HA) and community-acquired (CA) infections. In the Philippines, isolation of MRSA has been reported from human and fomite sources, but very few studies have examined the relationship between the profiles of their virulence-associated genes (VAG) and multilocus sequence types (MLST), which is important to establish so that effective prevention strategies can be designed and implemented. In this study, 20 MRSA isolates from human and fomite sources were analyzed to determine possible associations between their VAG profiles and sequence types (ST). Results show that the *hla* and *hld* genes that code for hemolysins had prevalence levels of 95 and 100%, respectively. The *pvl* gene coding for Panton-Valentine leucocidin (PVL) had a prevalence of 30%, which puts into question its utility as a marker of CA-MRSA. MLST revealed six new ST that belonged to six clonal complexes (CC), which included three new singletons that are being reported for the first time. No significant association was observed between the VAG profiles and ST, primarily because of the high prevalence rate of the VAG analyzed in the study.

Keywords: methicillin-resistant *Staphylococcus aureus,* MLST, VAG

INTRODUCTION

The genus *Staphylococcus* consists of Gram-positive bacteria that colonize the mucosal membranes of humans and animals. Most are commensals, while some are known pathogens. *Staphylococcus aureus* is the most invasive species, colonizing about 30% of the human population. It causes a wide spectrum of diseases ranging from simple skin infections to life-threatening illnesses such as sepsis and endocarditis (McGavin and Heinrichs 2012).

Penicillin and its derivatives have been used to treat *S. aureus* infections. Due to misuse of antibiotics, however, some strains have developed resistance to commonly used antibiotics such as erythromycin, clindamycin, tetracycline, and methicillin (Han *et al*. 2007). MRSA carries the *mecA* gene that encodes for the production of a mutated penicillin-binding protein (PBP2A) and other regulatory sequences, which makes it resistant to β-lactam antibiotics (Kwon *et al*. 2005). MRSA infections can be classified as HA-MRSA, CA-MRSA, or LA-MRSA (livestock-associated). CA-MRSA infections are generally more common, and outbreaks have been documented in various community settings, including correctional facilities. In 2010, Cabrera *et al*. reported a high prevalence rate of MRSA among inmates of Manila City Jail, which suggested the ease of transmission of diseases within the confined spaces of correctional institutions.

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Staphylococcus aureus produces a number of virulence factors that are encoded by VAG. These include enterotoxins, exfoliative toxins, the toxic shock syndrome toxin, and PVL. The first three virulence factors are known as superantigens that overactivate T cells, resulting in massive cytokine production. In addition, the alpha-hemolysin and delta-hemolysin – encoded by the *hla* and *hld* genes, respectively – are virulence factors associated with clinical symptoms in humans (Motamedi *et al*. 2018).

There is a growing interest in characterizing MRSA isolates through molecular methods to correctly identify and manage infections. Genotyping tools such as MLST have helped in the investigation of outbreaks caused by MRSA. The high level of discrimination achieved through MLST analysis has been crucial in the study of different loci and uncharacterized regions of the genome, which are often highly variable within the MRSA population (Enright *et al*. 2000). This study was conducted to determine the profile of selected VAG in MRSA isolates from human and fomite samples, and possible association with the MLST of the isolates.

MATERIALS AND METHODS

Study Design

The MRSA isolates used in the study were from two sources as indicated in the next subsection. The isolates were revived from stock cultures using standard culture methods, followed by genomic DNA extraction. Polymerase chain reaction (PCR) amplification was used to determine the VAG profiles and to amplify seven housekeeping genes for MLST. The PCR products for MLST were sequenced and the data analyzed using the goeBURST algorithm. The MLST dataset was deposited in the *S. aureus* PubMLST database with ID numbers 33463–33471, 33473, and 33488–33497. Test for the association between the VAG profiles and ST was performed using Fisher's exact test and *z*-test.

Sources of Isolates

The study used 20 MRSA isolates from the culture collection of the Medical Microbiology Laboratory, Institute of Biology, University of the Philippines (UP) Diliman, Quezon City, Philippines. Ten (10) human isolates came from the Master of Science graduate thesis of Ayala *et al*. (2018), which were collected from the nasal swabs of residents of the Golden Reception and Action Center for the Elderly and other Special Cases (GRACES) in Quezon City. The other 10 isolates were collected from shirts and slippers worn by inmates at the Quezon City Jail. Sample collection from GRACES was performed with consent from the participants and bioethics approval and clearance from the UP Manila Research Ethics Board. For the fomite isolates, research protocol and clearance were approved by the review committee of the Bureau of Jail Management and Penology. All of the MRSA isolates were identified using morphological and biochemical tests, and PCR amplification of the *nuc* and *mecA* genes.

Revival and Maintenance of MRSA Isolates

The MRSA isolates were revived from glycerol stocks by subculture on Brain Heart Infusion (BHI) slant and incubation at 37 °C for 24–72 h. Actively growing cultures were prepared by subculture on NA slants and incubation at 37 °C for 18–24 h.

DNA Extraction and Purification

Isolates were grown in 3 mL of BHI broth at 37 °C for 18–24 h. Cells were harvested by centrifugation at 10,000 x g at room temperature for 5 min. The supernatant was discarded and the cell pellet was washed with 1 mL of sterile distilled water. The pellet was re-suspended in 0.2 mL of sterile distilled water, and 24 µL of 1N NaOH was added to the suspension and incubated at room temperature for 10 min. The cell suspension was boiled for 10 min, cooled, and centrifuged at 10,000 x g for 5 min. The supernatant was collected and 150 µL of crude DNA sample was transferred to a 1.5-mL microcentrifuge tube. Aliquots of DNA were prepared by transferring 20 µL of DNA sample into several 1.5-mL microcentrifuge tubes and adding 180 µL of sterile distilled water. The purity and concentration of the DNA samples were determined using a NanoDrop™ 2000c Spectrophotometer (Thermo Scientific™). DNA samples with 260/280 nm absorbance ratios between 1.7–2.0 were used. The DNA concentrations of the samples were standardized to approximately 240 ng/µL, using sterile distilled water (pH 7), and stored at –20 °C until use.

PCR Amplification of the *nuc* and *mecA* Genes

The identity of the isolates as MRSA was verified using multiplex PCR amplification of the *nuc* and *mecA* genes. Shown in Table 1 are the primers used. PCR amplification was performed in a 25-µL reaction mixture using a MyCycler™ Thermal Cycler (Bio-Rad, USA). The primer sequences and PCR conditions used for the amplification of the *nuc*, *mecA*, and *16S rDNA* genes were based on the studies of Murakami *et al*. (1991), Brakstad *et al*. (1992), and Amit-Romach *et al*. (2004). The final PCR conditions for the amplification of the *nuc* and *mecA* genes were optimized by Ayala *et al*. (2018). All PCR assays included positive (MRSA BIOTECH 10378) and negative controls (*Staphylococcus epidermidis* BIOTECH 10098), and amplification of the *16S rDNA* gene as an internal control to indicate DNA sample quality. Amplicons were stored at –20 °C until further use.

Table 1. Primers used for MRSA identification, VAG profiling, and MLST.

Primers	Target genes	Sequences (5'-3')	Amplicon sizes (bp)	References
		MRSA identification		
Unibac-F	*16S rDNA*	CGTGCCAGCCGCGGTAATACG	611	Amit-Romach *et al.* (2004)
Unibac-R		GGGTTGCGCTCGTTGCGGGACTTAACCAACAT		
SA-F	*nuc*	GCGATTGATGGTGATACGGTT	267	Brakstad *et al.* (1992)
SA-R		AGC CAAGCCTTGACGAACTAAAGC		
mecA-F	*mecA*	AAAATCGATGGTAAAGGTTGGC	533	Murakami *et al.* (1991)
mecA-R		AGTTCTGCAGTACCGGATTTGC		
		VAG profiling		
lukPV-F	*pvl*	ATCATTAGGTAAAATGTCTGGACATGATCCA	433	Shrestha *et al*. (2014)
lukPV-R		GCATCAACTGTATTGGATAGCAAAAGC		
hla-F	*hla*	CTGATTACTATCCAAGAAATTCGATTG	209	Mehrotra *et al.* (2000)
hla-R		CTTTCCAGCCTACTTTTTTATCAGT		
hld-F	*hld*	AAGAATTTTTATCTTAATTAAGGAAGGAGTG	111	
hld-R		TTAGTGAATTTGTTCACTGTGTCGA		
		MLST		
arcC-F	*arcC*	TTGATTCACCAGCGCGTATTGTC	456	Enright *et al.* (2000)
arcC-R		AGGTATCTGCTTCAATCAGCG		
aroE-F	*aroE*	ATCGGAAATCCTATTTCACATTC	456	
aroE-R		GGTGTTGTATTAATAACGATATC		
glpF-F	*glpF*	CTAGGAACTGCAATCTTAATCC	465	
glpF-R		TGGTAAAATCGCATGTCCAATTC		
gmk-F	*gmk*	ATCGTTTTATCGGGACCATC	417	
gmk-R		TCATTAACTACAACGTAATCGTA		
pta-F	*pta*	GTTAAAATCGTATTACCTGAAGG	474	
pta-R		GACCCTTTTGTTGAAAAGCTTAA		
tpi-F	*tpi*	TCGTTCATTCTGAACGTCGTGAA	402	
tpi-R		TTTGCACCTTCTAACAATTGTAC		
yqiL-F	*yqiL*	CAGCATACAGGACACCTATTGGC	516	
yqiL-R		CGTTGAGGAATCGATACTGGAAC		

PCR Amplification of VAG

Three VAG (*pvl*, *hla*, and *hld*) were chosen based on their role in MRSA pathogenicity and targeted by PCR amplification using gene-specific primers that were adopted from literature. The primer sequences for the detection of *lukS-PV* and *lukF-PV* genes were adopted from Shrestha *et al*. (2014), while the primer sequences for the detection of the *hla* and *hld* genes were adopted from Mehrotra *et al*. (2000). The sequences of the primers are summarized in Table 1. PCR amplification was carried out in a 25-µL reaction mixture using a MyCycler™ Thermal Cycler (Bio-Rad, USA). The PCR conditions used were based on the studies of Mehrotra *et al*. (2000), Amit-Romach *et al*. (2004), and Shrestha *et al*. (2014).

Agarose Gel Electrophoresis

Ten microliters (10 µL) of PCR product from each sample and a 100 bp molecular ladder (Vivantis Technologies, USA) were loaded onto a 1.5% (w/v) agarose gel containing 0.5 µg/mL of ethidium bromide. Samples were electrophoresed at 100 V for 30 min, and the PCR products were visualized using a UV (ultraviolet) transilluminator.

MLST Analysis

Seven housekeeping genes (*arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi*, and *yqiL*) were used in the MLST analysis and were amplified using the primers shown in Table 1 (Enright *et al*. 2000). PCR amplification was carried out in a 25-µL reaction mixture, using a MyCycler™ Thermal Cycler

(Bio-Rad, USA) and PCR conditions indicated by Enright *et al*. (2000). Amplicons were stored at –20 °C until use.

PCR product purification and direct sequencing were outsourced to Macrogen (South Korea). The sequences were processed using the Staden Package v2.0.0 (http://staden.sourceforge.net) and trimmed to the same length in BioEdit v7.2.5 (http://www.mbio.ncsu.edu/BioEdit/bioedit.html) to generate the consensus sequences from the forward and reverse sequences. The consensus sequences were compared to reference sequences in the GenBank database using the BLASTN program of the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov). The consensus sequences were then used to generate the STs in the *S. aureus* PubMLST database (https://pubmlst.org/organisms/staphylococcus-aureus/), and the MLST data generated was deposited in the database under accession numbers: 33463–33471, 33473, and 33488–33497. ST that shared at least six identical alleles of seven MLST loci were grouped in a CC and the allelic profiles of isolates were analyzed using the goeBURST 1.2.1 algorithm in PHYLOViZ 2.0 (https://www.phyloviz.net/goeburst/). The input files consisted of the 20 allelic profiles of the 20 isolates and 6,766 allelic profiles of ST that were obtained from the *S. aureus* PubMLST database. The data were analyzed using an expanded version of the goeBURST rules or goeBURST Full MST, where default parameters were used to allow groups to include single-locus variants (SLV).

Data Analysis

To test the association of the VAG and ST, the Fisher's exact test and *z*-test were performed using the SPSS® v17.0 (https://www.ibm.com/analytics/spss-statistics-software). A *p*- and *z*-value of ≤ 0.05 was considered statistically significant

RESULTS AND DISCUSSION

Prevalence of Selected VAG in the MRSA Isolates

Twenty (20) *S. aureus* isolates were revived and confirmed as MRSA through the amplification of the *nuc* and *mecA* genes. The hemolysin genes, *hld* and *hla*, had a prevalence of 100% (20/20) and 95%, (19/20) respectively, while the *pvl* gene had a prevalence of 15% (3/20). The *hla* gene was found in 100% of the human isolates and 90% (9/10) of the fomite isolates, while the *pvl* gene was only detected in 30% (3/10) of the fomite isolates (Appendix Figures I–III).

Several VAG have been associated with the emergence of MRSA, which contribute to their colonization and disease manifestation in the host. These VAG include those that encode for capsules, hemolysins, adhesins, toxic shock syndrome toxins, enterotoxins, and exfoliative toxins. In the study of Kateete *et al*. (2011), the frequency of the *hld* gene among HA-MRSA isolates was reported to be 100%. The production of the hld toxin is the main mechanism used by *S. aureus* to target host phagocytes. It triggers mast cell degranulation and is a significant key factor in the pathogenesis of atopic dermatitis. The frequency of *hld* gene is higher than the two other genes examined in this study. Similar to our results, Bantel *et al*. (2001) reported the detection of the *hld* gene in MRSA strains isolated from asymptomatic *S. aureus* carriers.

Hla is one of the major virulence determinants implicated in the pathogenesis of *S. aureus*. It is associated with severe skin infection, sepsis, septic arthritis, and brain abscess (Mehrotra *et al*. 2000). Our results show that 95% (19/20) of the MRSA isolates were positive for the *hla* gene, and there was no significant difference in the occurrence of the gene between human and fomite isolates (*z*-value > 0.05). Similar findings were reported by Liu *et al*. (2015), where 81% of their CA-MRSA isolates – from the skin and soft tissue infections – harbored the *hla* gene. It has been observed that hemolysins are the most abundant virulence determinant in *S. aureus*, and Tavares *et al*. (2014) suggested the *hla* gene is likely to be expressed more in CA-MRSA compared to HA-MRSA.

The PVL is the most studied virulence factor in *S. aureus*. It is associated with soft tissue and deep dermal infections. The emergence of *PVL*-positive MRSA in communities, particularly those with poor sanitary conditions, is a public health concern worldwide. In this study, 15% (3/20) of the MRSA isolates were positive for the *pvl* gene, and this prevalence rate is lower compared to other studies. Interestingly, the isolates that were positive for the *pvl* gene all came from fomites. Based on our statistical analysis, however, there was no significant difference between the number of fomite and human isolates that tested positive for the *pvl* gene (*z*-value > 0.05).

In Ireland, the *pvl* gene is not considered a reliable marker for CA-MRSA, since most of their isolates are asymptomatic carriers of *S. aureus* and negative for the PVL toxin (Rossney *et al*. 2007). It should be noted, however, that the prevalence of the *pvl* gene in MRSA isolates varies among countries. In the Philippines, Cabrera *et al*. (2010) reported a prevalence of 83% among MRSA isolated from a correctional institution, while Holmes *et al*. (2005) reported low prevalence rates in the United Kingdom (4.9%) and France (5%). While it is tempting to correlate the prevalence rates with geographical location, it is likely that risk factors such as crowding and poor hygiene play more important roles. Since the occurrence of *pvl*-negative MRSA has been documented in the Philippines and by other researchers, it is clear that CA-MRSA cannot be identified or

differentiated based solely on the presence of the *pvl* gene. We, therefore, share the position of other researchers that questions the utility of the PVL toxin as a reliable marker for CA-MRSA (Rossney *et al*. 2007).

MLST Profiles of the MRSA Isolates Reveal the Presence of New ST in the Philippines

MLST of the 20 isolates revealed 11 ST (Table 2). ST45 was the most prevalent followed by ST789 and ST4743. The 11 ST were assigned to six CC, including three singletons. Six new ST with unique sequences (ST4226, ST4743, ST4762, ST4763, ST4764, and ST4767) were submitted as new registrations to the MLST database (https://pubmlst.org/organisms/staphylococcus-aureus). The isolates were obtained from the nasal swab of elderly residents in a healthcare unit and the personal effects of prisoners.

In general, the human and fomite MRSA isolates had different ST, although one ST was detected in both human and fomite isolates. The human isolates were distributed in three ST (ST45, ST 4226, and ST4743), while the fomite isolates were distributed in nine ST (ST789, ST1852, ST4762, ST4763, ST4764, ST45, ST5, ST4767, and ST50). The greater number of ST in the fomite isolates suggests that the genetic diversity of MRSA is greater in the correctional facility than in the residential care facility. This could be due to a difference in the number of occupants between facilities. While both facilities are overcrowded, the level of overcrowding in the correctional facility is greater. In addition, the transfer or movement of inmates between correctional facilities (https://www.bucor.gov.ph/images/Revised%20IRR%202016%20RA10757.pdf) may also introduce MRSA isolates with a different ST from those already present.

The most prevalent ST in this study is ST45, which is also predominant in North America, Israel, and Eastern Asia (Egyir *et al*. 2014). Most of the ST45 strains in the study were isolated from nasal swabs in Ghana, suggesting ST45 is associated with CA infections from nasal carriage

Table 2. Distribution of VAG, CC, and ST in the MRSA isolates.

Isolates[a]	VAG[b]			CC	ST
	pvl	*hla*	*hld*		
MRSA.MGBA1	–	+	+	3	45
MRSA.MGBA2	–	+	+	3	4226
MRSA.MGBA3	–	+	+	3	45
MRSA.MGBA4	–	+	+	S	4743
MRSA.MGBA5	–	+	+	3	45
MRSA.MGBA6	–	+	+	3	45
MRSA.MGBA7	–	+	+	S	4743
MRSA.MGBA8	–	+	+	3	45
MRSA.MGBA9	–	+	+	3	45
MRSA.MGBA10	–	+	+	3	45
MRSA.JRPC13	–	+	+	14	789
MRSA.JRPC27	–	+	+	14	789
MRSA.JRPC35	–	+	+	81	4762
MRSA.JRPC38	+	+	+	81	1852
MRSA.JRPC61A	–	+	+	18	4763
MRSA.JRPC61B	–	+	+	S	4764
MRSA.JRPC66	+	–	+	3	45
MRSA.JRPC79	–	+	+	1	5
MRSA.JRPC85	+	+	+	19	4767
MRSA.JRPC87	–	+	+	S	4767

[a]MRSA isolates with MBGA and JRPC in their codes indicate the isolates were obtained from the nasal swabs of elderly residents in GRACES and personal effects of inmates in the Quezon City Jail, Philippines, respectively.
[b]VAG examined: *pvl* – Panton-Valentine leucocidin gene, *hla* – hemolysin-alpha gene, and *hld* – hemolysin-delta gene

strains. In this study, ST45 was detected in both human and fomite isolates, which is not surprising, considering that MRSA is mainly transmitted *via* direct contact between humans and fomites. Similar to the study of David *et al*. (2014), which reported a *pvl*-positive ST45 from the detainees of Dallas County Jail in Texas, we determined that ST45 was also present in the isolates from Quezon City Jail, Philippines. Based on our goeBURST analysis, ST45 was predicted to be a primary founder – or an ST that has a high number of SLV – and our analysis showed that strain ST4226 is an SLV of ST45 (Figure 1).

The second most common ST in this study was ST789, which has been previously identified in Angola and China (Yu *et al*. 2015). Using goeBURST, ST789 was predicted to be a sub-group founder of ST80 (Figure 1). Subgroup founders can be described as SLV or double-locus variants (DLVs) of a founder of a CC that has become prevalent in a population that may subsequently have diversified to produce its own set of SLV and DLV (Enright *et al*. 2000). In the current study, ST789 was only detected from fomites isolates. This is the first report of ST789 detected from fomite isolates. Most ST789 strains are usually isolated from skin and soft tissue infections in healthcare institutions and bovine isolates (Egyir *et al*. 2014). This finding may have implications on the transmission of MRSA associated with these infections.

Among the strains of MRSA, ST5 is one of the most globally disseminated lineages. ST5-MRSA is currently widespread and is the major clone distributed in Asia and was previously referred to as the New York/Japan clone. An increasing prevalence rate of ST5 was observed in inmates of the San Francisco County Jail (Pan *et al*. 2003). Similar to the present study, ST5 was detected on the personal effects of inmates. Using goeBURST, ST5 was the predicted ancestral genotype with 382 SLVs and 121 DLVs, including ST4767 (Figure 1).

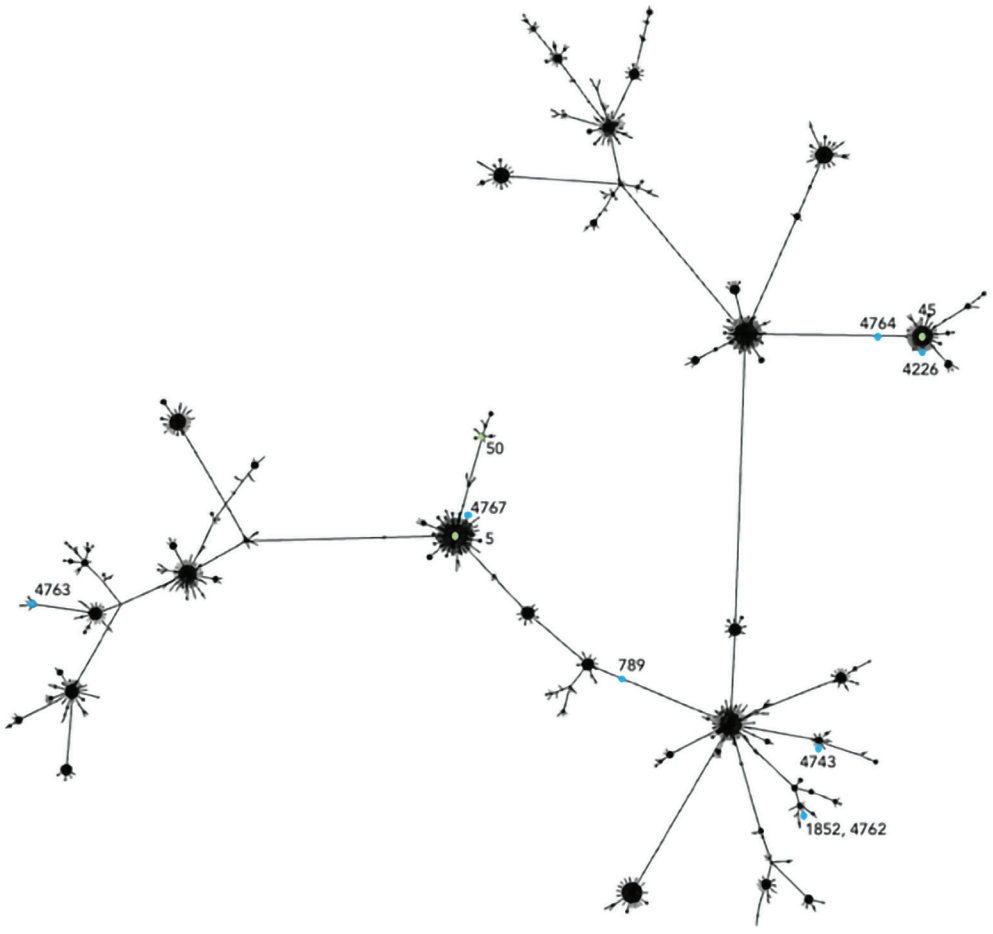

Figure 1. The global population distribution and phylogenetic positions of the ST of the 20 isolates relative to the 6,766 ST in the *S. aureus* PubMLST database are shown in circles of different colors. The minimum spanning tree of the isolates was constructed using the goeBURST 1.2.1 algorithm in PHYLOViZ 2.0 based on the sequences reported in the MLST database. The size of the clusters is proportional to the number of STs included in the cluster. Founder and subgroup ST are indicated by green and blue circles, respectively.

In communities in China, ST50 was identified as the dominant strain among children with asymptomatic *S. aureus* nasal carriage, and it was determined that ST50 carries a SCC*mec* IV, which is a characteristic feature of CA-MRSA strains (Takano *et al*. 2013). In the study of Mammina *et al*. (2012), ST50-MRSA-IVa were detected from clinical cases in a healthcare facility in Italy. In this study, ST50 was present only in fomite isolates. Using goeBURST, ST50 was predicted to be an ancestral genotype with only 14 SLV and five DLV (Figure 1).

While there are few studies on ST1852, it was found to be present in indigenous communities in the Northern Territory of Australia (Holt *et al*. 2011), while the identification of ST4762 in this study is the first report in the Philippines. ST1852 isolates clustered together with ST4762, which indicates relatedness between the isolates (Figure 1). This study highlights the discovery of ST4762, which is a new ST identified in the Philippines. Future studies on MRSA can be performed to determine the prevalence of ST4762 and confirm its association with fomite-derived MRSA.

Three singletons were found in the study (ST4743, ST4764, and ST4767) (Table 2). These singleton ST were each represented by a single isolate. In addition, the singletons did not belong to any CC and differed from all ST in the dataset in at least two MLST loci. Based on the study of Driebe *et al*. (2015), the emergence of different singletons across *S. aureus* lineage is likely due to homologous recombination. However, the impact and possibly differing levels of recombination across diverse ST in *S. aureus* remain undefined. All of the singletons present in the study were detected in both human and fomite MRSA isolates and were reported as new ST in the PubMLST database. At present, there are 6,766 STs of *S. aureus* globally (https://pubmlst.org), and this number continues to increase.

CC of the MRSA Isolates

The six CC determined were CC1, CC3, CC14, CC18, CC19, and CC81. In Asia, studies have shown that CC5, CC8, and CC22 are the most prevalent clones (Li *et al*. 2013). The most common CC identified in this study, however, was CC3, where nine MRSA isolates clustered together (Table 2). This is the first time that CC3 is being reported as a major CC in Asia and in the Philippines. It should be noted, however, that the MRSA isolates under the CC were CA-MRSA from asymptomatic *S. aureus* carriers.

CC1 and CC19 are known as common CA-MRSA lineages. In this study, both CC were represented by isolates coming from fomite samples. According to Nitschke *et al*. (2014), CC1 accounts for 32% of bovine isolates, while CC19 accounts for 75% of human skin isolates from Germany and 50% of human skin isolates from Trinidad and Tobago. In this study, CC14 and CC81 were identified in fomite isolates. In contrast, Sato'o *et al*. (2014) reported that CC14 is mostly found in indigenous communities residing in South West Pacific and that CC81 is primarily found in food, and is the cause of staphylococcal food poisoning outbreaks in Japan with a prevalence rate of 2.7%.

Based on the number and type of CC identified in the study, it seems that there are several different clones of MRSA in the Philippines, with the presence of some of the CC being reported for the first time in the country. Considering the small number of MRSA isolates examined in the study, it is likely that other CC are present and that the diversity of *S. aureus* clones circulating in the country is high. This indicates significant clonal dissemination of *S. aureus*, which is understandable, considering that its mode of transmission is through direct physical contact.

Lack of Association between VAG and ST in the MRSA Isolates

The profile of the VAG examined had no significant association with the ST of the MRSA isolates. In the case of the *hld* and *hla* genes, it was primarily due to the high prevalence of the genes, making any association of the genes with a particular ST difficult, because the genes would likely be detected in isolates with these ST. Previous studies have shown that genes for hemolysins were among the most abundant toxin genes among CA-MRSA strains (Tavares *et al*. 2014). In this study, 100 and 95% of the isolates harbored the *hld* and *hla* genes, respectively.

The *pvl* gene, on the other hand, was detected in isolates that belong to ST45, ST50, and ST1852. The association was determined to be not significant due to the small number of samples. Wannet *et al*. (2005) reported that the *pvl* gene can be detected in 40% of different ST among MRSA isolates in the Netherlands. Similarly, this study did not find any significant association between the prevalence of the *pvl* gene with any particular ST.

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STATEMENT ON CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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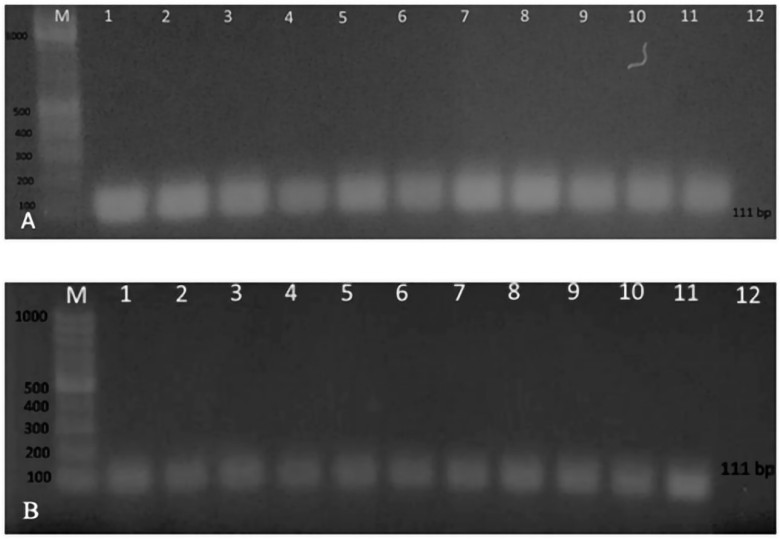

Figure I. AGE results for the PCR amplification of the *hld* gene. A) Results of the PCR amplification of the *hld* (111 bp) gene from fomite isolates obtained from the Quezon City Jail. Lane M contains the 100 bp ladder, Lane 1 contains the positive control (MRSA BIOTECH 10378), and Lane 12 contains the negative control (*S. epidermidis* BIOTECH 10098); Lanes 2–11 contain the fomite isolates that were all positive for the *hld* gene. B) Results of the PCR amplification of the *hld* (111 bp) gene from human isolates obtained from a residential care facility in Quezon City. Lane M contains the 100 bp ladder, Lane 1 contains the positive control (MRSA BIOTECH 10378), and Lane 12 contains the negative control (*S. epidermidis* BIOTECH 10098); Lanes 2–11 contain the human isolates, which were all positive for the *hld* gene.

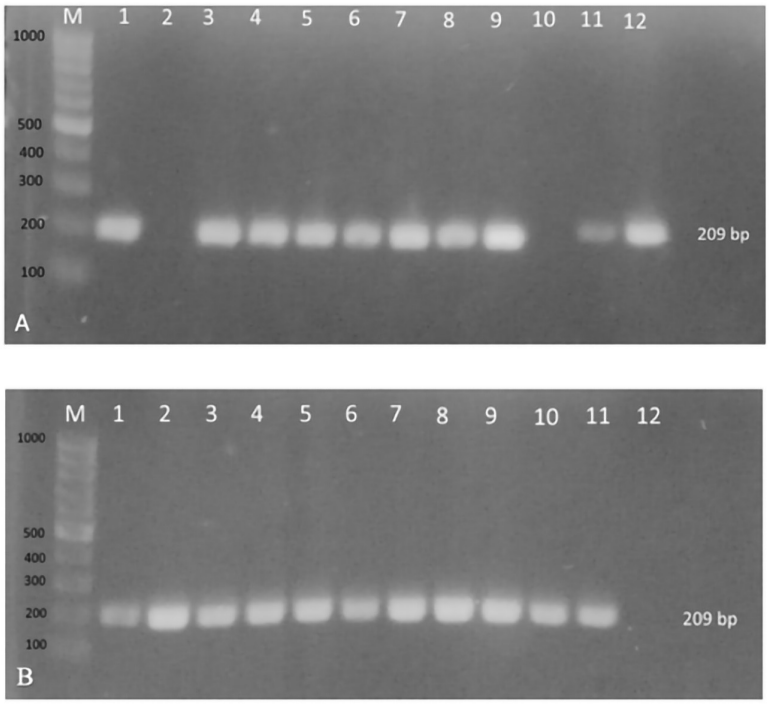

Figure II. AGE results for the PCR amplification of the *hla* gene. A) Results of the PCR amplification of the *hla* (209 bp) gene from fomite isolates obtained from the Quezon City Jail. Lane M contains the 100 bp ladder, Lane 1 contains the positive control (MRSA BIOTECH 10378), and Lane 2 contains the negative control (*S. epidermidis* BIOTECH 10098); Lanes 3–9 and 11–12 contain the fomite isolates, which were positive for the *hla* gene. B) Results of the PCR amplification of the *hla* (209 bp) gene from human isolates obtained from a residential care facility in Quezon City. Lane M contains the 100 bp ladder, Lane 1 contains the positive control (MRSA BIOTECH 10378), and Lane 12 contains the negative control (*S. epidermidis* BIOTECH 10098); Lanes 2–11 contain the human isolates, which were all positive for the *hla* gene.

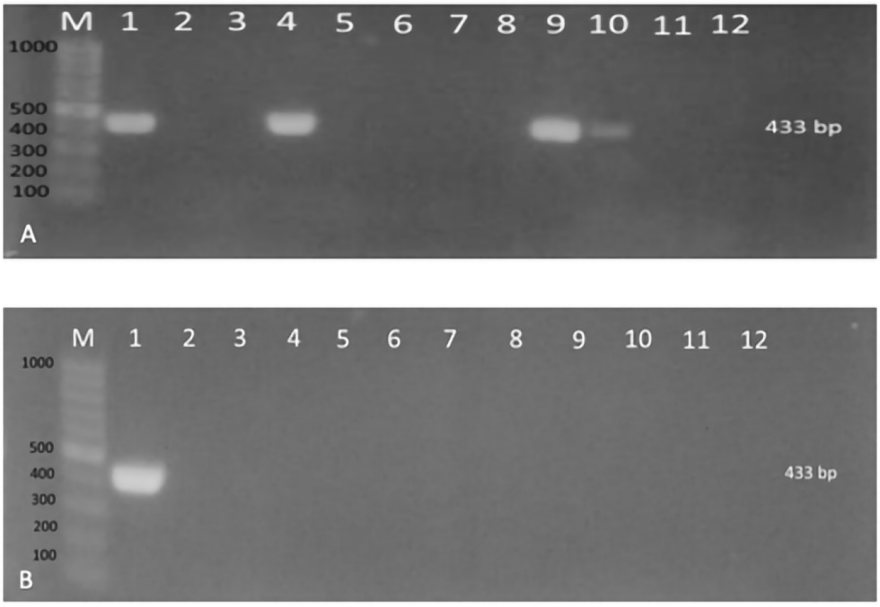

Figure III. AGE results for the PCR amplification of the *pvl* gene. A) Results of the PCR amplification of the *pvl* (433 bp) gene from fomite isolates obtained from the Quezon City Jail. Lane M contains the 100 bp ladder, Lane 1 contains the positive control (MRSA BIOTECH 10378), and Lane 12 contains the negative control (*S. epidermidis* BIOTECH 10098); Lanes 2–11 contain the fomite isolates, three of which were positive for the *pvl* gene. B) Results of the PCR amplification of the *pvl* (433 bp) gene from human isolates obtained from a residential care facility in Quezon City. Lane M contains the 100 bp ladder, Lane 1 contains the positive control (MRSA BIOTECH 10378), and Lane 12 contains the negative control (*S. epidermidis* BIOTECH 10098); Lanes 2–11 contain the human isolates, which were all negative for the *pvl* gene.