Nasal carriage of Staphylococcus aureus among healthcare workers in relation to patient contact

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Abstract

Background : Methicillin-resistant Staphylococcus aureus (MRSA) is a major public health concern worldwide. Healthcare workers (HCWs) are an important source of transmission of MRSA. We conducted a prospective study to define the frequency of S. aureus nasal colonization and emphasize on the carriage of MRSA in HCWs in relation to the intensity of patient contact. Methods: To evaluate the prevalence of S. aureus carriage and the relevance of transmission from community to the hospital, MRSA and methicillin-susceptible S. aureus (MSSA) nasal colonization in selected HCWs was compared. These included an emergency department, intensive care unit, out-of-hospital care emergency medical technicians and students, and a long-term care facility (LTCF). The MRSA isolates were further identified by their microbiological and molecular characteristics. Results: S. aureus was isolated from 63 of 248 HCWs (25.4%). The overall MRSA nasal carriage rate was 15/248, 6%. Most MRSA carriers were female (14/15, 93.3%), and HCWs who had worked for ≥5 years (11/15, 73.3%). LTCFs had the highest prevalence (3/25, 12%). In contrast, the overall carriage of MSSA was 48/248, 19.4%, and most carriers worked for ≥5 years (25/48, 52.1%). Hospital nurses had the highest rate of MSSA carriage (22/103, 21.4%). Most of the MRSA isolates were SCC mec IV/ST59 or ST45 (60%), and were resistant to erythromycin and clindamycin (53%). Only one MRSA was chlorhexidine resistant. All produced low level of biofilms. Conclusions: This study demonstrates hospital nurses have highest S. aureus nasal carriage, whereas HCWs worked in the LTCFs have the highest prevalence of nasal MRSA colonization. The differences in the characteristics of MRSA and MSSA nasal carriage among HCWs highlights the importance on inclusion of all strains of S. aureus in surveillance and infection control programs.

Background

Staphylococcus aureus is an important human pathogen. It has the ability to cause a wide variety of infections ranging from local invasion of skin and soft tissues to life-threatening sepsis. The emergence and spread of methicillin-resistant *S. aureus* (MRSA) is particularly troublesome because of its association with increased morbidity and mortality[1, 2] and the need to select the most appropriate therapy. Biofilm-forming variants are often difficult to treat even when susceptible to otherwise effective antibiotics.

MRSA infections are usually divided into healthcare-associated (HA-MRSA) or community-associated (CA-MRSA) because of differences in epidemiology, risk factors and choice of drug. HA-MRSA infections are more likely to occur in individuals with underlying diseases, the elderly, recent hospitalization, invasive procedures and residence in long term health care facilities[3]. CA-MRSA infections usually occur in otherwise healthy people and with minor trauma. HA-MRSA strains usually possess SCC*mec* types I, II or III, and tend to be multiple drug resistant. CA-MRSA strains usually possess SCC*mec* types IV or V and are strongly associated with the Panton-Valentin leucocidin (*pvl*) gene. CA-MRSA strains are considered to be more virulent, transmittable, and persistent than HA-MRSA[4, 5]. CA-MRSA strains can also be transmitted in healthcare facilities and mistaken for HA-MRSA[5].

The ability of *S. aureus* to colonize the anterior nares and other body sites is a significant predisposing risk factor for infection[5, 6]. Elimination of carriage decreases the incidence of *S. aureus* infection[7, 8]. The prevalence of MRSA nasal colonization in worldwide surveys of general populations ranges from 0.7 to 3%[9, 10]. The rate is somewhat higher in Taiwan, 3.8%[11, 12]. Hospitalized patients and those in long-term care facilities (LTCF) are at highest risk for MRSA carriage[13, 14].

Healthcare workers (HCWs), situated at interface between the hospital and community, are an important reservoir of *S. aureus* for both HA-MRSA and CA-MRSA[15]. Colonized HCWs can transmit MRSA to patients, their families, and other HCWs and have been implicated as a source of transmission in outbreaks[16, 17]. Identification of colonized HCWs combined with hand hygiene and other precautions have been shown to reduce the transmission and control the spread of MRSA[18].

Most studies of the transmission of MRSA have focused on isolates obtained from patients. Less is known about the frequency of carriage in HCWs, their genetic and clonal diversity, virulence gene determinants, and microbiological characteristics. The current study was designed to fill in some of these gaps.

In order to further identify the epidemiologic characteristics of MRSA strains and to clarify the spreading of epidemic clones, we employed molecular methods with sufficient discriminative power for studying clonal distribution. The objectives were to determine the frequency of MRSA compared to methicillin-susceptible *S. aureus* (MSSA) nasal colonization in different HCWs in relation to the intensity and duration of exposure to patients and characterize the molecular characteristics, antimicrobial resistant profiles, and biofilm-forming abilities of the isolates. The subjects included HCWs in an emergency department (ED), intensive care unit (ICU), and out-of-hospital emergency medical technicians (EMTs), and LTCFs.

Methods

Study design

This one-year prospective study was conducted from January to December 2015 at the National Cheng Kung University Hospital (NCKUH), Tainan, Taiwan, local fire department and long-term facilities. Healthcare providers who met the study criteria were offered the opportunity to participate in the study. The target population included out-of-hospital and in-hospital healthcare providers. Out-of-hospital providers were EMTs, student EMTs and staffs of LTCF, and in-hospital care providers included nurses and physicians working in the ED and ICU.

Selection of participants

Study samples were collected from student EMTs, EMTs, physicians and nurses in different departments of the healthcare facilities. The inclusion criteria for participants included HCWs such as EMTs, physicians, and nurses who have had more than 6 months of working experience at the acute and

chronic healthcare facilities. Student EMTs, regarded as representatives of the general population with limited exposure to patients, were enrolled from the annual routine new EMT training program; paramedics and private ambulance EMTs with short term urgent care, transport to hospitals, or transfer of patients between healthcare facilities were selected from two local private ambulance groups. Physicians and nurses who worked in the adult medical ICU (MICU) and the medical ED (MED) were asked to participate. The exclusion criteria included HCWs who had active infections such as fever and known respiratory tract infections, urinary tract infections or other occult illnesses, and HCWs who have taken an antibiotic during the prior 21-days. The protocol and consent forms were approved by the Ethical Review Committee of NCKUH (B-ER-104-029). Written informed consents were obtained from participants prior to taking nasal swabs. Participants were asked to fill out an anonymous questionnaire regarding their place of work (current and previous), wearing adequate personal protective equipment and washing hands before and after patient care.

Microbiologic methods

One sample was taken from each participant. A sterile cotton swab was used to circle the anterior 1 cm of the nasal vestibule both nares. The swabs were immediately placed into transport medium (Venturi Transystem, Copan Innovation Ltd.) and brought to the microbiology laboratory. Swab samples were inoculated by the streak plate method on Trypticase soy agar with 5% sheep blood plates and incubated overnight at 37°C. *S. aureus* was identified by colony morphology, gram stain and the coagulase test. The presumptive *S. aureus* isolates were confirmed by coagulase (*coa*) gene-based PCR [19]. MRSA were identified by the cefoxitin disk-diffusion method according to the recommendations of Clinical and Laboratory Standard Institutes[20].

PFGE

MRSA isolates were identified further by PFGE analysis with chromosomal DNA using the enzyme *Sma*I. The relatedness of strains was determined by comparison of restriction fragment-length polymorphism in accordance with the guidelines published by Tenover *et al*.[21] PFGE patterns resulting in 2-3 band differences were considered to be closely related, those with 4-6 band differences were considered to be possibly related, and those with ≥7 band differences were considered to be unrelated.

Susceptibility testing

The antimicrobial susceptibility of MRSA isolates to 10 antibiotics, including oxacillin, trimethoprim/sulfamethoxazole, penicillin, teicoplanin, linezolid, clindamycin, doxycycline, fusidic acid, vancomycin, and erythromycin, was determined in accordance with the guideline of Clinical Laboratory Standards Institute[20].

Biofilm formation assay

Four microliters of a bacterial overnight culture were inoculated into 1 ml of tryptic soy broth containing 0.25% glucose. An aliquot (200 μl) of the sample was poured into each of a 96-well polystyrene

microplate (167008, Thermo Fisher Scientific, Waltham, MA, USA), and incubated for 3 days at 37°C. The fluid was removed and the plate was stained with 0.1% safranin solution. The OD_{490} was measured using a microplate reader (µQuant, BioTek Instruments, Winooski, VT, USA).

Molecular characterization

Genomic DNA was obtained from the MRSA isolates by a Qiamp DNA mini kit protocol (Qiagen, Hilden, Germany) for molecular characterization. The presence of Panton-Valentine leucocidin (*pvl*) gene and genes for fibronectin binding protein A and B (*fnbA, fnbB*) were determined by PCR as previous described[22, 23]. The *S. aureus* multilocus sequence typing (MLST) scheme uses internal fragments of the following seven house-keeping genes: *arc*, *aro*, *glp*, *gmk*, *pta*, *tpi* and *yqi*. PCR amplification was carried out on chromosomal DNA using an extension time of 30 seconds, and an annealing temperature of 55°C, with Taq polymerase. The PCR products were then sequenced and the data were uploaded to the MLST website (http://www.mlst.net) for further analysis[24]. Typing of the staphylococcal chromosomal cassette *mec* (SCC*mec*) was done by PCR with primers and by the methods published previously[25]. PCR for *mecA, mupA, and qacA/B* were performed by the methods described previously[24, 26].

Methods of measurements

Primary outcome

Positive nasal swabs for MSSA and MRSA were reported. Further nasal carriage of the MSSA and MRSA prevalence were calculated by descriptive statistics and cross tabulations to determine the frequency distribution of the MRSA nasal carriage among the different groups of healthcare professionals. Pearson's chi-square test, Fisher's exact test Cochran–Mantel–Haenszel test, logistic regression, and generalized linear models were used to compare MRSA colonization between groups. Odd ratios (ORs) were calculated with 95% confidence intervals (CIs). Student t test or Mann–Whitney U test were used to compared continuous variables. SAS software version 9.4 (SAS, Inc., Cary, North Carolina, USA) was applied for data entry, processing and statistical analysis.

Secondary outcome analysis

Positive MRSA samples were enrolled for further pathogenicity by molecular analysis. The drug susceptibility, basic molecular typing, virulence factors such as *pvl* gene and genes for fibronectin binding protein and the biofilm formation assay were conducted.

Results

Prevalence of MRSA and MSSA

Two hundred and forty-eight healthcare providers were enrolled in the study. The distribution of the HCWs by site of work is shown in Figure 1. The frequency of isolation of MSSA and MRSA according to the characteristics of the study population is shown in Table 1.

S. aureus was isolated from 63 of the 248 HCWs (25.4%). Fifteen (23.8%) of isolates were MRSA. The overall MRSA nasal carriage rate was 6%. Most MRSA carriers were female (93.3%), and HCWs who had worked in a facility for ≥5 years (73.3%). LTCFs had the highest prevalence (12%), followed by hospital nurses and EMTs. In contrast, the overall carriage of MSSA was 48/248, (19.4%); female (50%); and worked in a facility for ≥5 years (52.1%). Hospital nurses had the highest rate of MSSA nasal carriage (21.4%), followed by EMTs and LTCFs. None of 10 physicians and student EMTs was colonized by MRSA.

Characterization of MRSA isolates

MRSA strains can be identified using various typing methods, such as PFGE, MLST, and SCC*mec* typing. The information can be epidemiologically useful for identifying the likely source of colonization, tracing outbreaks, and distinguishing between community and healthcare-associated strains. In this study we performed various typing methods and antimicrobial resistance tests to study the molecular epidemiology of MRSA.

All of the 15 MRSA isolates were positive for *mecA* by PCR. Most were SCC*mec* IV (9, 60%) and belonged to two endemic CA-MRSA genotypes, ST59 (6, 67%) and ST45 (3, 33%). All the isolates could be divided into 6 major clones by PFGE pattern analysis (Fig. 2). The most predominant pulsotype contained 5 isolates carrying SCC*mec* IV or V/PVL$^-$/ST59. One isolate, belonging to ST398 and carrying SCC*mec* V, was isolated from a MICU nurse who had traveled to Europe within 12 months.

The MRSA isolates exhibited high rates of resistance to erythromycin (53%) and clindamycin (53%). Only one isolate was resistant to fusidic acid. One isolate from a MICU nurse was detected as *qacA/B*-positive, conferring resistance to chlorhexidine in *S. aureus*. The MIC to chlorhexidine was 4mg/L. None of the MRSA isolates were resistant by phenotypic or genetic tests to mupirocin, linezolid or glycopeptide antibiotics (vancomycin or teicoplanin).

All of the MRSA nasal colonizing isolates, regardless of the SCC*mec* type, formed low levels of biofilm that were indistinguishable from the CA-MRSA V/PVL$^+$/ST59 clinical isolates (Fig. 3). In contrast, the traditional HA-MRSA III/PVL$^-$/ST239 clinical isolates had significantly higher levels of biofilm formation. Additionally, all the MRSA nasal colonizing isolates had *fnbA* gene but none possessed the *fnbB* gene.

Discussion

This study aimed to determine the frequency of MRSA compared to MSSA nasal colonization in different HCWs in relation to the intensity and duration of exposure to patients and characterize the molecular characteristics, antimicrobial resistant profiles, and biofilm-forming abilities of the isolates. We found that 25.4% of HCWs were nasal carriers of *S. aureus*. About a quarter (23.8%) of the population was colonized by MRSA. The overall frequency of MRSA was 6%. The prevalence of MRSA was highest among LTCF staffs, followed by ICU nurses, ED nurses and EMTs. SCC*mec* typing revealed that the majority of strains were type IV and V. These are the most common types of CA-MRSA in Taiwan [27].

The prevalence of MRSA (6%) in our study are consistent with the frequency of HCW MRSA nasal carriage in previous studies in Taiwan (5.0-7.8%)[27], but higher than in other countries[15]. They are also consistent with other studies that found the highest prevalence of MRSA nasal carriage among HCWs with close contact with patients, poor attention to infection control policy, and high work-load[15]. Direct patient contact is considered to be the main transmission route for MRSA[28]. In the current study the greatest risk of MRSA nasal carriage was in HCWs working for 5 to 10 years.

Most of the MRSA isolates in our study carried either SCC*mec* type IV or V, suggesting a community origin. Only one MRSA isolate with SCC*mec* type II, the traditional HA-MRSA type [29], was isolated from an ICU nurse. This finding indicates that the CA- MRSA types (type IV and V) were more common among healthcare providers and the HA-MRSA strain still remained in the hospital. This may be due to strict infection control measures in our hospital. Another possibility is clonal replacement in the hospital setting. In the past decade, CA-MRSA has been increasingly identified as a cause of hospital-onset and healthcare associated infections. This suggests that certain clones have the ability to cross the barriers between hospitals and the community[30, 31].

Several unusual MRSA strains were isolated from the current study population. An isolate from a nurse working in the MICU was SCC*mec* II ST5. This HA-MRSA strain carried chlorhexidine resistant genes (*qacA/B*). Chlorhexidine is widely used as antiseptic for central venous catheter care bundle and patient bathing to prevent nosocomial infection in out hospital. Chlorhexidine based soaps and mupirocin ointment are commonly used for cleaning and decontamination of MRSA. Prior investigators have also described MRSA strains carrying chlorhexidine and mupirocin resistant genes[32, 33], but these are uncommon in Taiwan.

Another unusual MRSA strain, ST398, was isolated from an MICU nurse in our study. This is an important emerging strain associated with the livestock mainly in Europe and North America[34, 35]. MRSA ST398 is usually associated with pigs and veal calves but can colonize other host species. These include cows, sheep, poultry and farmers who are in frequent contact with MRSA-colonized animals, and can cause infections in humans[36]. The nurse who acquired this strain had traveled to Europe within 12 months and had contact to animals. The role of livestock as a potential source of MRSA infection is a growing public health problem. The risk and impact of HCWs carrying this clone need to be closely monitored.

Some of the MRSA strains in our study are able to produce biofilm on both mucosal and inanimate surfaces, making them difficult to eradicate[37]. Biofilm formation is considered to have a role in *S. aureus* colonization[38, 39]. Recently fibronectin-binding proteins (Fnb A and Fnb B) have also been reported to play a role in biofilm formation. The association of the expression of Fnb A and Fnb B with increased bacterial aggregation suggests that fibronectin-binding proteins can promote the accumulation phase of biofilms[40]. In the current study, all the MRSA nasal colonization isolates carried the *fnbA* but not the *fnbB* gene and only exhibited low levels of biofilm formation. This is consistent with the recent concept that a dispersed mode of growth in the vestibulum nasi is preferable to a biofilm mode during *S. aureus* nasal colonization[41].

MSSA strains were more abundant than MRSA in nasal carriers (19.4%) and were differently distributed in our study. In contrast to MRSA, MSSA exhibited an equal sex distribution and was much more likely to be isolated from EMTs. We believe this is important because all *S. aureus* have the potential to produce invasive disease and need to be included in surveillance studies and control measures.

This study has several limitations. First, it was conducted in a large metropolitan region in southern Taiwan and the findings may not be generalizable to other localities. Second, single cross-sectional sampling did not allow us to differentiate between transient and persistent carriers of MRSA and MSSA. Third, samples were collected only from the nares. It has been estimated that 15-50% of MRSA carriers are non-nasal[42]. Therefore it is likely that we underestimated the overall prevalence of MRSA and MSSA. Forth, nasal swab samples were not analyzed using pre-enriched culture in the study. Pre-enriched culture was found to be more sensitive than direct culture in the detection of nasal *S. aureus* [43]. The use of pre-plating enrichment of swabs in TSB to improve nasal *S. aureus* detection levels is warranted in the future studies. Finally, whole-genome sequencing is more sensitive than molecular analysis to classify MRSA strains as community or hospital-associated. It has the added advantage of establishing genetic relatedness and recent transmission.

The strengths of this study include its prospective design, observation over a full year, adequate sample size of a diverse representative population of HCWs with major differences in their exposure to patients, comprehensive molecular characterization of the MRSA, biofilm-forming ability and antibiotic susceptibility testing.

Conclusions

This study demonstrates that nasal colonization by *S. aureus* differs among healthcare professionals in relation to the extent and duration of exposure to patients. Hospital nurses have highest *S. aureus* nasal carriage, whereas HCWs worked in the LTCFs have the highest prevalence of nasal MRSA colonization. Most of the MRSA isolates belong to CA-MRSA strains, exhibited high rates of resistance to erythromycin and clindamycin, and produced low level of biofilms. An unusual MRSA strain, ST398, was isolated from an MICU nurse who had exposure to livestock in Europe. Another MICU nurse was colonized a *qacA/B*-positive strain conferring resistance to chlorhexidine. MRSA represents only the tip of the iceberg of nasal colonization by *S. aureus*. MSSA strains were 3.2 times more common and much more frequent in hospital nurses and EMTs who had limited exposure to patients. This supports the inclusion of all strains of *S. aureus* in surveillance and infection control programs.

Abbreviations

MRSA: Methicillin-resistant *Staphylococcus aureus*; CA-MRSA: community-associated Methicillin-resistant *Staphylococcus aureus*; ED: emergency department; EMTs: emergency medical technicians; HA-MRSA: healthcare-associated Methicillin-resistant *Staphylococcus aureus*; HCWs: Healthcare workers; ICU: intensive care unit; LTCF: long-term care facilities.

Declarations

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Availability of data and materials

Data and materials are available upon request to the corresponding author.

Authors' contributions

HIS, CYC did the study design; HIS, HCH and CHW collected the study materials; HIS, HCH, FCS, YJL, CMC and CYC analyzed and interpreted the data; HIS, HCH and CYC wrote the article.

Ethics approval and consent to participate

This study was conducted in accordance with the Helsinki Declaration. The study protocol and the study data were approved by the Institutional Review Board (IRB), National Cheng Kung University Hospital (B-ER-104-029). Written informed consent was obtained from each participant. Participants' information was kept anonymous and de-identified prior to the analysis.

Consent for publication

Not applicable.

Conflicts of interest

The authors declare that they have no competing interests.

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Tables

For technical reasons the tables needed to be attached as a supplemental file and can be found below.

Figures

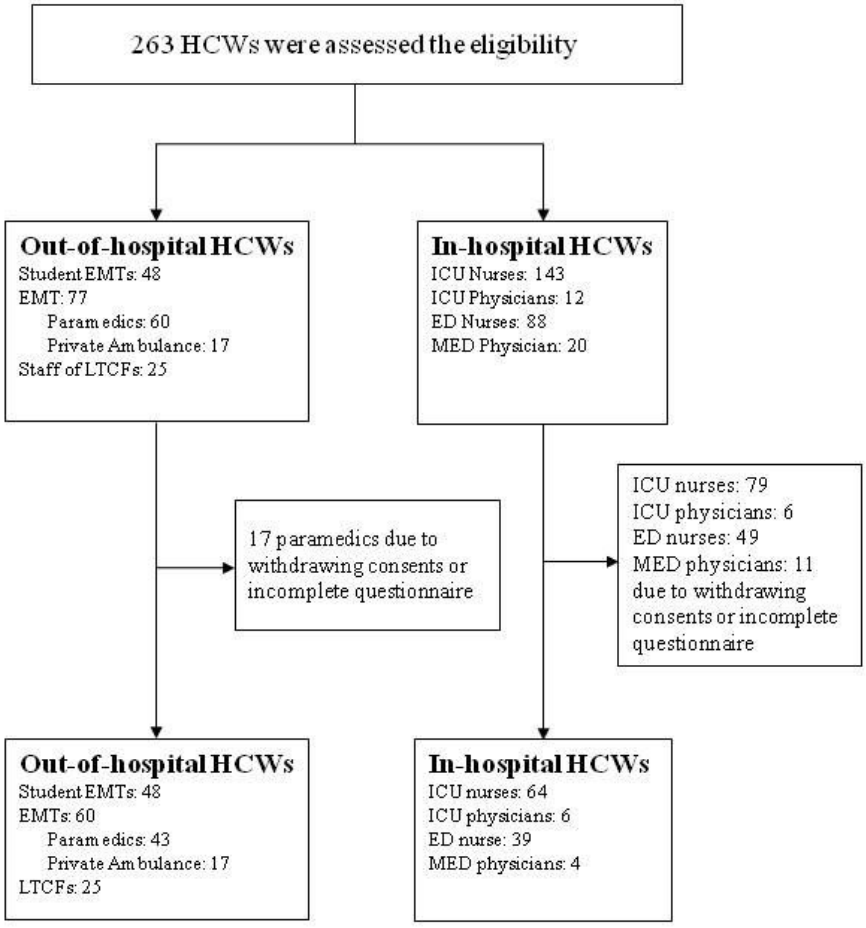
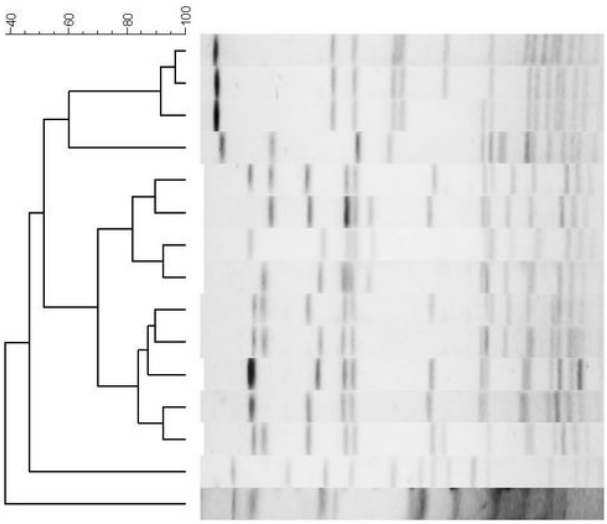


Figure 1

Figure 1

Enrollment of healthcare workers for MRSA and MSSA Nasal Carriage Study. ED, emergency department; EMT, emergency medical technician; ICU, intensive care unit; LTCF, long term care facility; MED, medical emergency department.

Figure 2



Isolate ID	Ward/Staff	SCC*mec*	MLST	*pvl*	*fnbB*	*qacA/B*	ERY	CC	SXT	FA
162	EMTP	IV	45	-	-	-	R	S	S	S
210	SICU/N	IV	45	-	-	-	S	S	S	S
177	MICU/N	IV	45	-	-	-	S	S	S	S
146	LTCF/N	V	45	-	-	-	S	S	S	R
5	ED/N	IV	59	-	-	-	R	R	S	S
80	ED/N	IV	59	+	-	-	R	R	S	S
1	ED/N	V	59	+	-	-	S	S	S	S
193	LTCF/SW	V	59	+	-	-	S	S	S	S
194	LTCF/N	IV	59	-	-	-	R	R	S	S
198	MICU/N	V	59	-	-	-	S	S	S	S
172	MICU/N	IV	59	-	-	-	S	S	S	S
235	SICU/N	IV	59	-	-	-	R	R	S	S
78	ED/N	IV	59	-	-	-	R	R	S	S
168	MICU/N	II	5	-	-	+	R	R	S	S
164	MICU/N	V	398	-	-	-	R	I	S	S

Figure 2

Molecular characterization, antibiogram of nasal carriage isolates and the PFGE dendrogram compares fingerprint patterns of the methicillin-resistant Staphylococcus aureus (MRSA) isolates from 15 healthcare workers. SCCmec and MLST indicate the results for MRSA type. Columns marked “pvl”, “fnbB”, and qacA/B” are the results for genetic tests performed to detect the PVL, fibronectin B and chlorhexidine resistance genes. ERY, erythromycin; CC, clindamycin; SXT, trimethoprim-sufamethoxazole; FA, fucidic acid; S, susceptible; R, resistant. ED, Emergency department; EMTP, Emergency Medical Technician Professional (Paramedic); MICU, Medical ICU; N, nurse; SW, Social Worker; SICU, Surgical ICU; LTCF, Long term care facility.

Figure 3

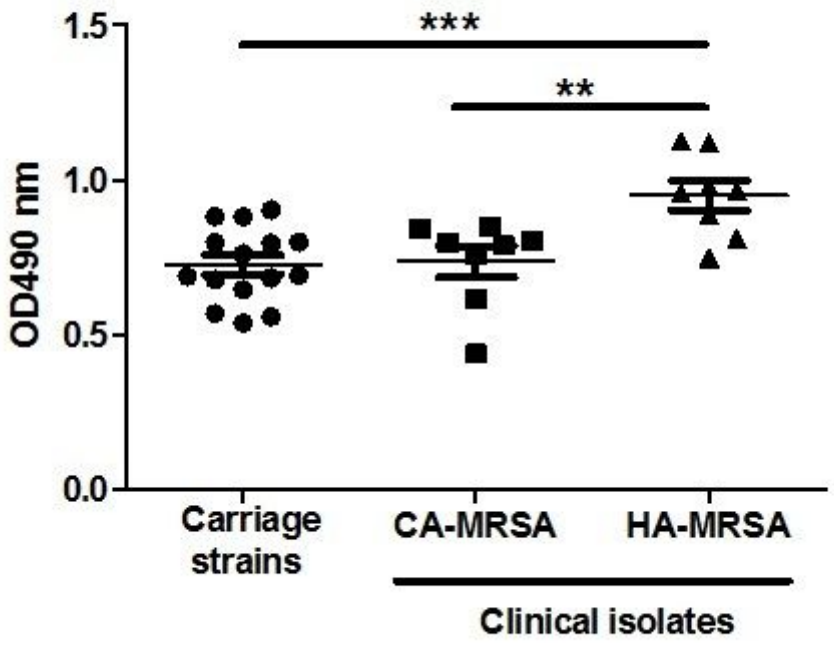


Figure 3

Biofilm formation ability. Biofilm formation of the MRSA nasal colonization isolates (carriage), CA-MRSA V/PVL+/ST59 clinical isolates (CA-MRSA), and the HA-MRSA III/PVL-/ST239 (HA-MRSA) onto polystyrene microplates were measured. ** $p<0.01$, *** $p<0.001$ for significant differences based on two-sided unpaired t test.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TaiwanMRSANasalColonizationTable.docx