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Performance of CHROMagar *Staph aureus* and CHROMagar MRSA for Detection of Airborne Methicillin-Resistant and Methicillin-Sensitive *Staphylococcus aureus*

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In recent decades, the rise of methicillin-sensitive *Staphylococcus aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) has become one of the most significant problems in public health. The CHROMagar medium, which allows for direct color-based identification of target pathogens, could potentially be used to rapidly monitor airborne *S. aureus* and MRSA. In this study, the recovery efficiency of CHROMagar Staph aureus (CSA) for collecting airborne MSSA was evaluated in a chamber study. Subsequently, the colony identification performance of bioaerosol samplers combined with CSA and CHROMagar MRSA (C-MRSA) was evaluated in a hospital setting. Our results demonstrated that the agar medium, the type of bioaerosol sampler used, and the relative humidity all affected the recovery of the collected MSSA sample. The sampling stress that influenced the recovery of MSSA on CSA did not differ from that used when measuring its recovery on the commonly used tryptic soy agar. The average sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the colony identification were 24%, 90%, 13%, and 95%, respectively, for CSA and 71%, 65%, 16%, and 96%, respectively, for C-MRSA. Both CSA and C-MRSA had low PPV and high NPV, suggesting that a nonmauve-colored colony that is recovered on CSA or C-MRSA by air sampling can be reported as negative; however, any mauve-colored colonies should be subjected to further identification processes. On the basis of these findings, and depending upon the methods used, using CHROMagar to detect airborne MSSA or MRSA may shorten the detection time from 24 to 48 h.

INTRODUCTION

Staphylococcus aureus is a common pathogen found in human mucosa and skin. This microorganism is an opportunistic pathogen that causes a wide spectrum of clinical manifestations, including scalded skin syndrome, pneumonia, food poisoning, and toxic shock syndrome (Francis et al. 2005). Since the introduction of methicillin into clinical use, methicillin-resistant *S. aureus* (MRSA) has emerged worldwide as an important nosocomial pathogen and has become known as a "SUPER BACTERIA" (Hulten et al. 2010; Sacar et al. 2010). Although MRSA is known to be transmitted by close contact with infected persons or with contaminated hands or clothing (Cooper et al. 2004), some evidence suggests that airborne dispersal and transmission may also be important (Shiomori et al. 2002; Wilson et al. 2004; Negrini Tde et al. 2009). In Taiwan, the incidence of nosocomial MRSA infections increased remarkably in the 1990s. MRSA has accounted for as many as 80% of all *S. aureus* isolates causing nosocomial infections in hospitals since 1998 (Ho et al. 1999). Therefore, rapid monitoring, followed by accurate and timely identification of airborne *S. aureus* and MRSA in hospitals, is critical for early awareness and preventative measures.

To date, very limited research has been conducted regarding the presence of MRSA in the air. It has been demonstrated that people can inhale antibiotic-resistant *S. aureus* in health care units (Wilson et al. 2004), swine feeding facilities (Gibbs et al. 2006), and residential environments (Gandara et al. 2006). Because the culture-based method remains the dominant method for taking bioaerosol measurements, loading an Andersen impactor with tryptic soy agar (TSA) is the most commonly used method for detecting airborne *S. aureus*. Although TSA theoretically has the ability to culture a variety of bacterial aerosols, the impaction stress of TSA on *S. aureus* colony recovery has not been fully characterized. Moreover, TSA may not be the most time-efficient method for extensively monitoring *S. aureus* bacterial species or for testing their antibiotic resistance.

In recent years, the use of chromogenic media has become a key method for rapid microorganism identification in clinical samples (Merlino et al. 2000; Wendt et al. 2010). These

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media exploit enzyme substrates that release colored dyes upon hydrolysis through a specific bacterial enzyme causing target pathogens to form colored colonies (Perry and Freydiere 2007). Consequently, chromogenic media allow for direct color-based identification and can be used to target pathogens with high specificity. Among the various chromogenic media, CHROMagar has been demonstrated to rapidly distinguish *S. aureus* and MRSA from clinical nasal swab and blood samples with high sensitivity (from 89.7% to 100%) and specificity (from 87.2% to 100%; Pape et al. 2006; Chihara et al. 2009; Goodwin and Pobuda 2009). However, a recent study indicated that the sensitivity and specificity of CHROMagar for identification of *S. aureus* in seawater did not perform as well for identification in clinical samples (Goodwin and Pobuda 2009). The difference in the application of CHROMagar for clinical and environmental samples may be related to the presence of some coagulase-negative *Staphylococcus* spp. that yielded a faint mauve color on CHROMagar (Chihara et al. 2009). Additionally, *S. aureus* growth on the CHROMagar may decrease its sensitivity due to the competing effects of other environmental bacteria species (Perez et al. 2003; Diederen et al. 2005). For clinical samples, a qualitative result, either positive or negative, is primarily looked for. However, the desired application of CHROMagar when used for environmental samples is to detect and quantify target colonies. If CHROMagar's sensitivity and specificity for detecting environmental samples are lower than for detecting clinical samples, its efficacy for quantifying environmental samples could present a particular challenge.

To date, CHROMagar's performance for identifying bioaerosols has not been characterized. Because CHROMagar can isolate bacteria and detect antibiotic resistance in one step, one would expect that using CHROMagar to detect antibiotic-resistant bioaerosols should result in high sensitivity and specificity. It may also reduce detection time from over 3 days to approximately 16–24 h. Nevertheless, the performance of CHROMagar when used in bioaerosol sampling still needs further investigation. In addition to questions about its relative sensitivity and specificity, another concern is the recovery of bacteria on CHROMagar. Microbial collection and survival in bioaerosol samplers strongly depends on the type of sampler, the sampling time, the sampling flow rate, and the collection medium (Macher and Willeke 1992; Lin and Li 1998; Li and Lin 1999a; Tseng and Li 2005). In an Andersen impactor at a constant operating flow rate, sampling time, and relative humidity (RH), the impact stress on bacterial recovery is directly related to the components of the loaded agar (Juozaitis et al. 1994; Stewart et al. 1995). Injury of collected bacteria due to impaction can be divided into structural and metabolic injury. Bacteria with metabolic or structural injury are able to multiply and form colonies on a nonselective agar medium but not on selective media that contain inhibiting agents or inorganic salts (Stewart et al. 1995). In contrast to other nonselective agar, some components of the chromogenic and selective mix in CHROMagar are still proprietary. Therefore, the resulting impaction stress influencing the recovery and injury of collected *S. aureus* on CHROMagar could differ from that resulting from nonselective agar.

The purpose of this study was to evaluate the recovery efficiency of CHROMagar for collecting airborne methicillin-sensitive *S. aureus* (MSSA) in a chamber study and to demonstrate the colony identification performance of CHROMagar for identifying airborne *S. aureus* and MRSA in a hospital environment. To evaluate the recovery efficiency of CHROMagar compared with other nonselective agar, three agar media, blood agar plate (BAP), Luria-Bertani agar (LB), and TSA, were also used in the chamber study. The recovery efficiency of an agar medium for collecting a bioaerosol is directly related to the type of bioaerosol sampler used. Therefore, two different bioaerosol samplers, an Andersen one-stage impactor and a Nuclepore polycarbonate filter, were combined with the four types of agar medium and investigated. An environmental monitoring program for detecting airborne *S. aureus* and MRSA with an Andersen impactor and a Nuclepore filter was conducted in the hospital to test the colony identification performance of CHROMagar. After sampling, all colonies were recovered using CHROMagar that demonstrated positive (mauve) or negative results that were verified using conventional laboratory tests and real-time PCR.

MATERIALS AND METHODS

Chamber Study

Test Microorganism

In the chamber study, the single strain of MSSA (ATCC 29213) was used as a surrogate for MRSA because of safety concerns. An active MSSA culture was inoculated in LB broth and incubated for 24 h at 37°C. After cultivation, the microbe pellets were aseptically washed with sterile phosphate-buffered saline (PBS) into a 15-mL sterile conical centrifuge tube, which was capped and centrifuged at 2500 rpm (model 2010, Kubota, Japan) for 5 min. Finally, the supernatant was discarded and the pellets were resuspended in sterile distilled water for preparation of spray suspensions.

Aerosol Generation and Test System

The MSSA sampling test chamber was 29 cm in diameter and 32 cm in height. A Collison three-jet nebulizer (BGI, Inc., Waltham, MA, USA) was used to nebulize the MSSA stock in deionized water at 20 psi with dry, filtered, compressed laboratory air, and the suspension was then passed through a Kr-85 particle-charge neutralizer (model 3077, TSI Inc., St. Paul, MN, USA). The aerosolized suspension was then diluted with filtered, compressed air at 50 L/min. Because the concentration of airborne *S. aureus* rarely exceeds 1000 colony-forming units (CFU)/m^3 in the field (Gandara et al. 2006), MSSA at 1000 CFU/m^3 was generated to simulate field environments. On the basis of our preliminary analyses (data not shown), nearly 1000 CFU/m^3 of airborne MSSA

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can be generated by an MSSA suspension in a nebulizer at 1000 CFU/mL. Therefore, the MSSA concentrations in the nebulizer ranged from 2×10^3 to 7×10^3 CFU/mL in all of the experiments. Because the RH strongly affects bacterial culturability, collection was done at three RH levels (30%, 55%, and 85%). To evaluate the effect of RH, the humidified gas stream was heated by adding a dry gas stream to produce dry (RH 30%), moderate (RH 55%), or humid conditions (RH 85%) at 25–28°C. A humidified gas stream was generated by passing pure compressed air through a humidity saturator. The water vapor content (i.e., RH) in the gas stream was adjusted by changing the ratio of the humidified gas stream flow rate to the dry gas stream flow rate and was measured using a hygrometer (Sekunden-Hygrometer 601, Testo, Lenzkirch, Germany) that was placed in the sampling chamber.

Test Samplers and Sample Processing

To evaluate the recovery efficiency of different agar media, an Andersen one-stage viable impactor (Andersen Samplers, Inc., Atlanta, GA) and a Nuclepore filter were used to collect MSSA aerosols. An Andersen impactor has four hundred 0.25-mm holes, a sampling flow rate of 28.3 L/min (corresponding to a velocity of 24 m/s), and a sampling time of 7 min. The Nuclepore filter (Costar, Cambridge, MA) consisted of a polycarbonate membrane supported by cellulose pads with a 0.4-μm pore size and a 37-mm diameter. Before the filter was loaded into a closed-face, two-piece plastic cassette, both the filter and support pads were autoclaved, and the plastic cassette was sterilized with ethylene oxide. The sampling flow rate of the Nuclepore filter was 20 L/min and the sampling time was 60 min.

Currently, there are commercially available media for selective and differential growth of *S. aureus* and MRSA by colony color-based identification; such media include CHROMagar Staph aureus (CSA; CHROMagar Microbiology, Paris, France) and CHROMagar MRSA (C-MRSA; CHROMagar Microbiology), respectively. Because MSSA was used as a surrogate for MRSA in the chamber study, only CSA (not C-MRSA) was evaluated for collecting airborne MSSA in a chamber. In addition to the CSA, BAP, LB, and TSA agar were also used as complete, nonselective media in a comparison of recovery efficiencies. After sampling, the plate with the collection medium (CSA, BAP, LB, or TSA) from the Andersen impactor was placed in an incubator at 37°C for 24 h. For the Nuclepore sample, the plate was eluted after sampling by rinsing in 1 mL sterile deionized water, and the suspension was slowly vortexed in a rotator (Vortex-2Genie, G-560, Scientific Industries, Inc., New York, USA) for 30 s. After transferring MSSA particles from the filter to the eluate, the eluate was spiked separately on the different agars and placed in an incubator at 37°C for 24 h. Finally, the number of CFU (CFU/m^3) was calculated on the basis of the dilution ratio, plated volume, sampling time, and flow rate.

Calculation of Recovery Efficiency

The culturability and viability of aerosolized bacteria depend on the culture preparation process (Lin and Li 1998, 1999; Chen and Li 2005; Tseng and Li 2005) and strongly affect the recovery efficiency of a collection medium. Therefore, to better understand the recovery efficiency of each evaluated agar medium for MSSA aerosols, the culturability of microorganisms in the liquid suspension used as the source of the bioaerosols should be evaluated. Therefore, we used a parameter called the relative recovery (RR) as an indicator of the recovery efficiency of the agar medium.

$$RR = \frac{C_{\text{test}}}{C_{\text{susp}}},$$

where

C_{test} is the CFU/m^3 determined for CSA, BAP, LB, and TSA agar (i.e., the number of culturable bacterial particles per cubic meter of air that passed through the sampler),

C_{susp} is the CFU/mL in the suspension determined for CSA, BAP, LB, and TSA agar (i.e., the number of culturable bacterial particles in the suspension).

Sampling Stress

The stress of sampling the MSSA by the Nuclepore filter was also evaluated in the chamber. The MSSA concentrations were determined prior to the stress sampling experiment based on optical density readings at 600 nm (OD_{600}). The bacteria were cultured overnight and then transferred to a flask and incubated at 37°C until an OD_{600} of 0.3–0.6 (corresponding to 1×10^8 CFU/mL) was achieved. To determine the effect of sampling stress on MSSA, bacterial cultures were serially diluted to 1×10^5 CFU/mL. Next, 50 μL of bacterial culture was spiked on the Nuclepore filter (approximately 5×10^3 CFU on each filter). All of the filters loaded in the cassettes were simultaneously evaluated by passing clean air through the filters at 20 L/min and over sampling times of 0, 30, 60, 90, 120, and 240 min. Immediately after sampling, the filters were removed from each cassette and eluted by rinsing in 1 mL sterile deionized water to be used for bacterial culture on CSA, BAP, LB, and TSA agar. The sampling stress on MSSA was defined by the ratio C_t/C_0, where C_t and C_0 are the bacterial concentrations in the simultaneously collected samples subjected to airflow for t h and 0 h, respectively.

Statistical Analysis

For each experiment, a comparison of the recovery efficiency, or sampling stress, of MSSA on the evaluated medium was performed using one-way analysis of variance (ANOVA) followed by Tukey's test to evaluate statistically significant differences ($p < .05$).

Field Study

Sampling Location

From November 27 to December 26, 2009, we collected aerosol samples twice weekly from the waiting areas of the outpatient hall (H), pediatrics department (P), and respiratory

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care department (R) at a medical center in Taiwan. The central air conditioning of the hospital was supplied by air-handling units. The air exchange rates at the sampling locations were found to be in the range of 8/h to 10/h, within the range (8/h to 12/h) suggested by the American Society of Heating, Refrigerating and Air-Conditioning Engineers (ASHRAE). Physical factors such as temperature, RH, and carbon dioxide (CO_2) concentration at each sampling site were also recorded.

Aerosol Sampling

At each sampling site, parallel aerosol samples were collected using an Andersen impactor and a Nuclepore filter for 86 samples at H, 74 samples at P, and 73 samples at R. Therefore, we collected a total of 233 aerosol samples, including 157 impactor samples and 76 filter samples (as shown in Figure 1). The pump and sampling apparatus were placed about 1 m from patient seating in the sampling location. In addition, the sampling locations were 3 m from square ceiling diffusers to prevent wind interference. The sampling heights were 0.8–1 m above the floor, which was within the breathing zone of the seated patients. The sampling flow rates and sampling times of the apparatus were the same as those used in the chamber experiments. The Andersen impactor had a flow rate of 28.3 L/min and was operated for 7 min, and the Nuclepore filter was operated at 20 L/min for 60 min. From our preliminary test (data not shown), the sampling flow rates and sampling times for the impactor and filter would result in counts of 30–300 CFU from the agar media, a suitable level for determining the concentration of airborne bacteria in the hospital. The agar media CSA and C-MRSA were used in conjunction with each sampler to identify airborne *S. aureus* and MRSA, respectively. To compare the concentrations of total bacterial aerosol, nonselective media such as LB and TSA agar were also investigated. The sample processing was the same as that used in the chamber study. All evaluated agar media (CSA, C-MRSA, TSA, and LB) in the field were placed in an incubator at 37°C for 24 h.

Bacterial Identification by Conventional Laboratory Tests

CSA and C-MRSA are selective and differential media for *S. aureus* and MRSA, respectively. In both cases, the target colonies are mauve in color. After 20–24 h of cultivation, colonies that appeared mauve positive or negative were identified using conventional laboratory tests in sequence (including the Gram stain, hemolysis testing, catalase testing, and slide coagulase testing) to verify the results (Figure 1). A total of 6015 colonies on CSA or C-MRSA were subjected to conventional laboratory tests. *S. aureus* is recognized to be Gram positive and can reveal β-hemolysis on sheep blood agar. In addition, it can also be differentiated from other aerobic Gram-positive cocci and other staphylococci by a positive catalase test and by the coagulase test, respectively. The colonies recovered from CSA and C-MRSA were analyzed using Baso Rapid Gram Stain analysis (BASO Biotech CO., Ltd, New Taipei City, Taiwan). When the cocci demonstrated a Gram-positive response, the

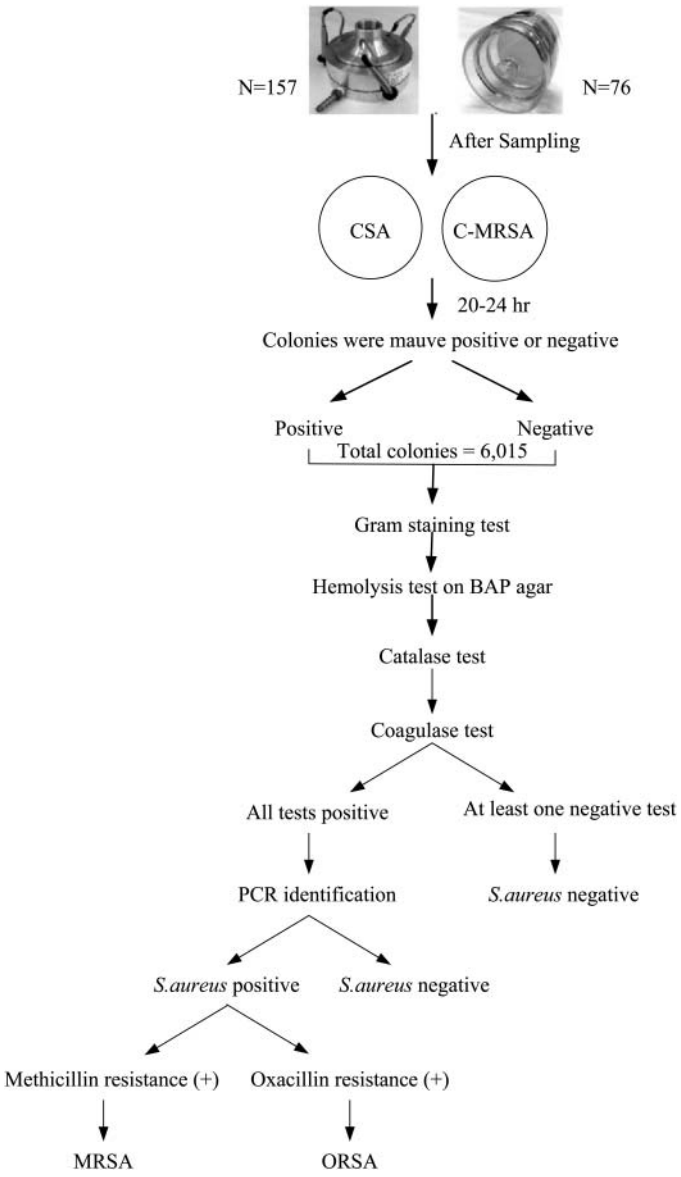

FIG. 1. Screening of airborne field samples for *S. aureus* identification.

isolates were tested for hemolysis after overnight incubation at 37°C on sheep blood agar (Creative Microbiologicals, Ltd, Taipei, Taiwan). Following incubation, Gram-positive cocci with β-hemolysis were placed on a microscope slide, a drop of 3% hydrogen peroxide was added, and the generation of bubbles was examined. Finally, a coagulase test was performed to identify *S. aureus* with rabbit plasma following the recommendations of the manufacturer (BBL coagulase plasma, rabbit with EDTA; BD Bioscience, San Jose, CA, USA).

Detection of the nuc *Gene from Aerosol Samples*

Any sample that passed all of the conventional laboratory tests was identified using the real-time PCR assay with a LightCycler (Roche Diagnostics, Mannheim, Germany; see

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Figure 1). For DNA extraction, an aliquot (10 μL) of bacterial broth from the airborne sample was mixed with 90 μL of sterile water and centrifuged at 14,000 rpm for 2 min. After centrifugation, the supernatant was removed and the pellet was suspended in 100 μL of sterile water. The suspension was then heated at 95°C for 15 min and subjected to 2 min at 14,000 rpm to sediment the debris. Finally, the clear supernatant was used as a DNA template in PCR analysis. For identification of *S. aureus*, the primer sequences of NUC1 (5′-GCG ATT GAT GGT GAT ACG GTT-3′) and NUC2 (5′-AGC CAA GCC TTG ACG AAC TAA AGC-3′) were used to detect the *S. aureus*-specific marker, the *nuc* gene as described by Fang and Hedin (2003). The SYBR green kit (LightCycler FastStart DNA Master SYBR Green I; Roche Diagnostics, Mannheim, Germany) and amplification conditions used for real-time PCR analysis were all followed by Fang and Hedin (2003), except that the primer-annealing temperature was changed from 55°C to 60°C. For the *S. aureus* that we tested, the target *nuc* gene from a cloning plasmid was synthesized by Mission Biotech (Taipei, Taiwan, R.O.C.). A calibration curve was created by serially diluting the external standard and the number of copies was calculated automatically by LightCycler software. For *S. aureus* identification, the known amounts of plasmid DNA yielded cycle threshold (C*t*) values ranging between 22.9 and 36.8 cycles. The calibration curves were linear for five orders of magnitude for *S. aureus*, and the lowest detection limit was 3.0 copies/μL (Figure 2a). The PCR product from a sample analyzed using SYBR green can be confirmed by a melting curve analysis. The samples with melting temperatures (T_m) within the range of the T_m of the plasmid DNA $\pm$ 0.5°C were therefore regarded as *S. aureus* positive. The T_m of our plasmid DNA (80.40°C) was close to previous findings (Fang and Hedin 2003) and demonstrated that the T_m of the *nuc* gene was in the range from 79.9°C to 80.6°C (Figure 2b).

Antibiotic Susceptibility Test

After the organism was verified to be *S. aureus*, room-temperature Mueller-Hinton agar (Difco 225250, BD, Sparks, MD, USA) plates were used to evaluate each of the microorganisms for antibiotic resistance. The methicillin and oxacillin susceptibilities were tested using the disk test according to the Clinical and Laboratory Standards Institute (CLSI 2005) guidelines. *S. aureus* with methicillin resistance was defined as MRSA, and *S. aureus* with oxacillin resistance was defined as oxacillin-resistant *S. aureus* (ORSA; Figure 1). By definition, MRSA is resistant to beta-lactam antibiotics, including methicillin, oxacillin, penicillin, and amoxicillin. Methicillin was the first and most reliable beta-lactam antibiotic for routine susceptibility testing (Brown et al. 2005). Oxacillin was later used in clinics as an alternative to methicillin susceptibility tests. To compare the concentrations of airborne *S. aureus* in the hospital, both MRSA and ORSA concentrations were demonstrated. For evaluating the performance of C-MRSA, we still use methicillin in susceptibility tests of MRSA.

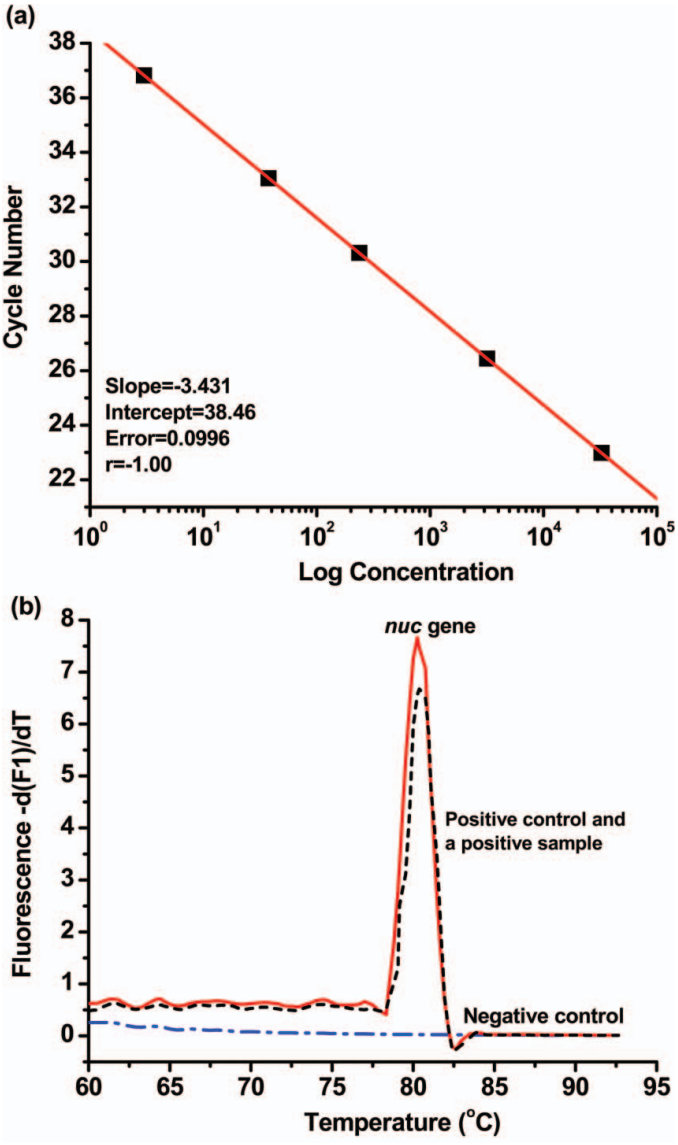

FIG. 2. Real-time PCR for *S. aureus* identification: (a) Standard curve of known DNA and C*t* values measured using real-time PCR and (b) T_m curves for detection of the *nuc* gene. (Color figure available online.)

Data Analysis

The performance of CSA or C-MRSA was arranged in a 2 $\times$ 2 contingency table format as shown in Table 1. The performance statistics reflect sensitivity [a/(a+b)] and specificity [d/(c+d)]. Sensitivity refers to the proportion of *S. aureus* or MRSA with a positive test result (mauve color) using CSA or C-MRSA. Specificity refers to the proportion of non-*S. aureus* or non-MRSA with a negative test result (nonmauve color) using CSA or C-MRSA. A sensitivity of 100% indicates that CSA or C-MRSA recognizes all *S. aureus* or MRSA colonies (mauve color). A specificity of 100% indicates that CSA or C-MRSA recognizes all non-*S. aureus* or non-MRSA colonies (nonmauve color). In addition to sensitivity and specificity,

TABLE 1
Air sampling performance of CSA or C-MRSA in a 2 × 2 contingency table format.

	S. aureus or MRSA	Non-*S. aureus* or non-MRSA	Total
Mauve	a	c	a + c
Nonmauve	b	d	b + d
Total	a + b	c + d	a + b + c + d

Note: *S. aureus* or MRSA = the collected colonies that were verified as *S. aureus* or MRSA, respectively, using conventional laboratory tests and real-time PCR. Non-*S. aureus* or non-MRSA = the collected colonies that were verified as not *S. aureus* or not MRSA, respectively, using conventional laboratory tests and real-time PCR. Mauve = the collected colonies that were mauve on CSA or C-MRSA. Nonmauve = the collected colonies that were not mauve on CSA or C-MRSA.

the positive predictive values [PPV; (a/(a+c)] and negative predictive values [NPV; d/(b+d)] were also calculated. PPV and NPV correspond to the probability that any particular positive (mauve color) or negative (nonmauve color) test result is a true positive or negative result, respectively.

RESULTS AND DISCUSSION

In this study, the recovery efficiencies of four agar media (CSA, BAP, LB, and TSA) for collecting airborne MSSA using an Andersen one-stage impactor and a Nuclepore filter were evaluated in a chamber study. In addition, the sampling stress of closed-face filter cassettes on MSSA was also investigated. The bioaerosol samplers combined with CSA and C-MRSA were then used in a hospital to evaluate the colony identification performance of CHROMagar.

Chamber Study

Recovery Efficiency of Tested Agar Medium

Andersen One-Stage Impactor. The RRs of the Andersen one-stage impactor for MSSA on CSA, BAP, and TSA (Figure 3) were all within the 10^{-1} range at all three RH levels (30%, 55%, and 85%, respectively). The RH level did not affect the RR of MSSA on LB, but the average RR of LB was 1.1–2.8 times higher than the RR of the other three agar media ($p < .05$). A previous study had suggested that impaction onto an agar surface may cause injury to bacteria, especially when the bacteria are impacted onto a selective agar medium such as CSA (Stewart et al. 1995). Nonselective agar offers a more effective means of growth for preserving bacteria culturability, but the injured bacteria are usually difficult to grow on the selective medium because of limited nutrition and inhibiting agents. However, we observed that the RR of MSSA on CSA was only slightly lower than the RR on LB; the two other nonselective agar media, BAP and TSA, had similar RRs to CSA. For bioaerosol sampling, TSA is considered an excellent general agar to culture a variety of bacteria, but it did not perform better than CSA

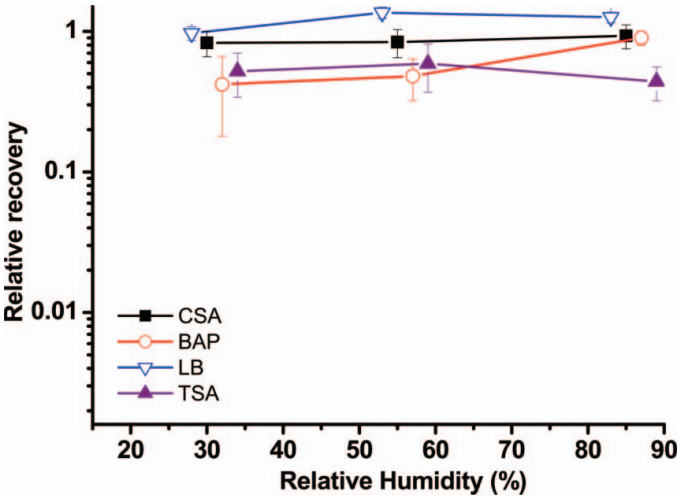

FIG. 3. The effects of relative humidity on the RR of CSA, BAP, LB, and TSA when collecting airborne MSSA using an Andersen impactor. ($RR = C_{test}/C_{susp}$; C_{test}: CFU/m^3 on the evaluated agar medium, the number of culturable bacteria particles per m^3 of air on the evaluated agar medium. C_{susp}: CFU/mL in the suspension, the number of culturable bacteria particles in the suspension on the evaluated agar medium). (Color figure available online.)

when sampling for airborne MSSA. Although CSA is considered a selective and differential agar, it was specifically used for the isolation and direct differentiation of *S. aureus.* Therefore, the components and selective mix that characterize CSA may help MSSA to recover and may enable a similar RR level as is achieved with TSA. Nevertheless, once non-*S. aureus* species are impacted onto the surface of CSA, the injured bacterial cells can still be suppressed.

The parameter RR used in this study represented the recovery efficiency of each agar medium for MSSA aerosols; the microbe species with higher RRs also indicate which species are more resistant to the stress of air sampling. Therefore, the descending order of resistance to the stress from impaction (sampling by Andersen impactor) was as follows: *Bacillus subtilis* endospores or *Penicillium* spores ($RR = 2 \times 10^{-1}$) > Gram-negative *Escherichia coli* ($RR = 1.5 \times 10^{-2}$) > nonenveloped viruses ($RR = 1 \times 10^{-2}$ to 2×10^{-2}; Li and Lin 1999a, 1999b; Li et al. 2003; Tseng and Li 2005). Compared with previous studies, the RR findings of Gram-positive MSSA were similar to those using hardy endospores and fungal spores, but they were much higher than the RRs of *E. coli* and nonenveloped viruses. The RR results from previous studies were all obtained using nonselective agar; it has been suggested that the recovery of a microorganism onto an agar medium surface is highly species dependent.

Nuclepore Filter. The average RR values of the Nuclepore filter sampler (Figure 4) for MSSA on all four agar media did not differ significantly at the three RH levels. However, the average RR for MSSA on all four agar media at a RH of 85% (from 1.4×10^{-1} to 2×10^{-1}) was significantly higher than the RRs at RHs of 30% and 55% (from 1×10^{-2} to 4×10^{-2}; $p < .05$). Previous studies have shown that a filter may not be suitable

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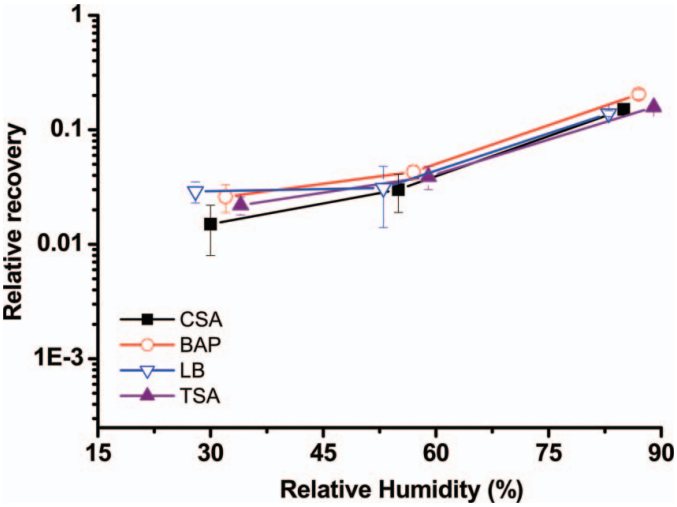

FIG. 4. The effects of relative humidity on the RR of CSA, BAP, LB, and TSA when collecting airborne MSSA using a Nuclepore filter. (RR = C_{test}/C_{susp}; C_{test}: CFU/m^3 on the evaluated agar medium, the number of culturable bacteria particles per m^3 of air on the evaluated agar medium. C_{susp}: CFU/mL in the suspension, the number of culturable bacteria particles in the suspension on the evaluated agar medium). (Color figure available online.)

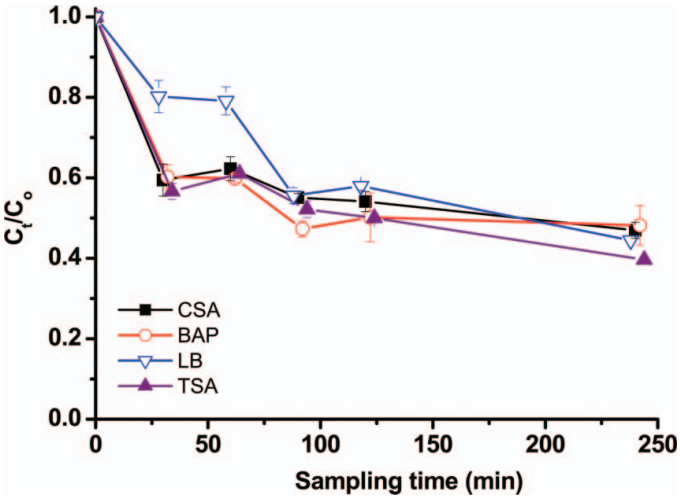

FIG. 5. Sampling stress of MSSA recovered on CSA, BAP, LB, and TSA by closed-face filter sampling. (Color figure available online.)

for collecting fragile airborne bacteria because the filter dries out during extended sampling, placing additional dehydration stress on the collected microorganisms (Crook 1995; Li and Lin 1999b). Our results demonstrated that the dehydration stress on MSSA by filter sampling may be significant at dry and moderate RHs. A higher RR observed at a high RH level might be due to the formation of a moisture film that protects the culturability of MSSA. Therefore, the observed loss of MSSA culturability (reflected by the lower RR at RHs of 30% and 55%) in samples collected using a Nuclepore filter is probably related primarily to biological stress from filtration and dehydration during the sampling and extraction process (Chen and Li 2005; Tseng and Li 2005).

Previous studies also demonstrated a descending order of resistance to stress from filtration (sampling by Nuclepore filter) at moderate RHs in the following sequence: *B. subtilis* endospores or *Penicillium* spores (RR = 1.5×10^{-1} to 3.8×10^{-1}) > Gram-negative *E. coli* (RR = 7×10^{-2}) > nonenveloped viruses (RR = 1.5×10^{-2}) > enveloped viruses (RR = 1×10^{-4}; Li et al. 2003; Chen and Li 2005; Tseng and Li 2005). Therefore, the RR findings for Gram-positive MSSA were similar to those of *E. coli* and nonenveloped viruses and were much higher than those of enveloped viruses. However, the RR values were much lower than the hardy *B. subtilis* endospores and *Penicillium* spores. Obviously, collection with the Andersen impactor would demonstrate a higher recovery rate of airborne MSSA than the filtration process.

Sampling Stress on Closed-Face Filter Cassettes

As shown in Figure 5, all curves representing the C_t/C_0 values were characterized by an initially rapidly decreasing phase that eventually transitioned into gradual lag rates after pure air was passed through the filter. Finally, the C_t/C_0 values declined with increased sampling time. When the sampling time was less than 100 min, the average C_t/C_0 values for MSSA on LB were significantly higher than those on the other three agar media ($p < .05$); however, the C_t/C_0 values on all of the evaluated agar media did not differ significantly from each other after 100 min of sampling. The sampling time for the Nuclepore filter was 60 min in both our chamber and field studies. After 60 min of sampling, the average C_t/C_0 values were 0.62 for CSA, 0.60 for BAP, 0.79 for LB, and 0.61 for TSA. Therefore, the recovery rate of MSSA collected by a closed-face filter cassette may not exceed 0.5 for sampling times of less than 60 min.

For safety reasons, MSSA was used as a surrogate for MRSA during our chamber experiments because it has the same strain and particle size. However, for antibiotic-resistant bacteria, the involvement of a plasmid might change a defense-associated protein in a cytoplasmic membrane or in the cell wall. MRSA are able to resist antibiotics because they possess unique types of penicillin-binding proteins (PBPs) in their cytoplasmic membranes (Stapleton and Taylor 2002). If PBPs are altered, the antibiotic cannot inhibit cell-wall formation and the bacteria are thus rendered resistant. This plasmid-mediated process not only affects bacterial resistance to antibiotics but may also produce resistance to other oxidative stress from biocides (Suller and Russell 1999), disinfectants (Rutala et al. 1997), and photocatalysis (Tsai et al. 2010). Although the possibility of this mechanism being manifested depends on the species, the altered PBPs that MRSA possess in their cytoplasmic membranes make possible changes to their susceptibility to the sampling stress from impaction and filtration.

In summary, the recovery of MSSA did not depend on the RH when using an Andersen impactor, whereas the recovery of MSSA strongly depended on the RH when using a Nuclepore

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filter. The Andersen impactor likely performed better than the Nuclepore filter in sampling airborne MSSA. When using the Nuclepore filter, the sampling was conducted for 60 min in order to increase yields. However, a major disadvantage of this method was the filtration and dehydration that occurred during the sampling and extraction processes may have caused enough biological stress to cause the bacteria to lose some culturability. The recovery of MSSA on CSA was lower than it was on LB when using an Andersen impactor. The other two nonselective agars, BAP and the commonly used TSA, did not perform better than CSA. A sampling time of over 190 min is not recommended when using a Nuclepore filter because more than half of the *S. aureus* on the filter could lose culturability during the filtration process.

Field Study

Total Concentration of Airborne Bacteria in the Hospital

During the sampling period, ambient temperatures ranged from 20°C to 24°C, CO_2 concentrations ranged from 659 to 940 ppm, and RHs ranged from 54% to 64% in the hospital. Using different agar media in an Andersen impactor, the average bacterial concentrations varied widely from 70 to 1294 CFU/m^3 (Figure 6a). The H had the highest total bacterial concentration of the three sampling locations ($p < .05$), and the bacterial concentrations of P and R were not significantly different from each other. Human activity might play a role in these concentration differences. In addition, the selective agar media CSA and C-MRSA were able to detect and measure the total concentrations of airborne bacteria in the hospital. The total bacterial concentration measured by CSA was not significantly different from that detected by the commonly used LB and TSA agar, but the concentration measured by C-MRSA was much lower than concentrations detected by the other three agar media ($p < .01$). Although CSA and C-MRSA are selective differential media for *S. aureus* and MRSA, other bacterial species can still grow on them. However, only the target colonies are mauve colored. In contrast to CSA, some antibiotic-related supplements are added to the C-MRSA. Therefore, bacteria without antibiotic resistance are usually unable to grow on C-MRSA. This may explain the low total bacterial concentration measured by C-MRSA. In comparison with the Andersen impactor, the Nuclepore filter significantly underestimated the concentration of airborne bacteria (from 1.7 to 5.1 times lower) no matter which agar medium was used (Figure 6b). The highest total bacterial concentration was found in H, and there were no significant differences among the total concentrations measured by CSA, TSA, and LB agar media. As occurred with the Andersen impactor, the Nuclepore filter with C-MRSA measured the lowest bacterial concentration.

Concentration of Airborne S. aureus in the Hospital Determined by CSA and C-MRSA

The concentrations presented in Table 2 were determined using conventional laboratory tests and real-time PCR on all

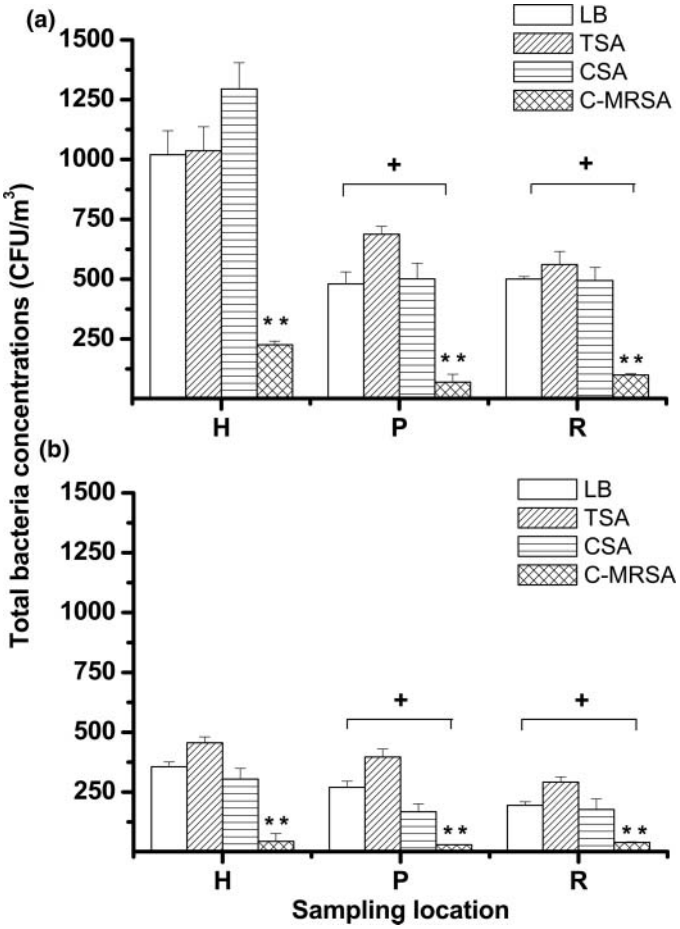

FIG. 6. Total airborne bacteria concentrations recovered on CSA and C-MRSA, LB and TSA by (a) Andersen impactor and (b) Nuclepore filter sampling in the waiting areas of the outpatient hall (H), pediatrics department (P), and respiratory care department (R). $^{+}p < .05$ compared to the respective H groups; $^{*}p < .01$ compared to respective LB, TSA, and CSA groups.

of the recovered colonies on CSA or C-MRSA. Both CSA and C-MRSA combined with an Andersen impactor demonstrated that the order of total *S. aureus* concentrations (regardless of the presence or absence of antibiotic resistance) was H > P > R. Similarly, the concentrations of airborne MRSA and ORSA in H and P were higher than the concentrations in R. Within the sampling period, there were twice as many people in H and P than in R; if more patients were sicker or stayed longer in H or P, then more people were present during the sampling times, which may account for the greater concentration of MRSA and ORSA measured at those times. The detected concentrations on CSA and C-MRSA with the Nuclepore filter were much lower than those detected by the Andersen impactor (except ORSA recovered on C-MRSA in P). There was no MRSA or ORSA detected by the Nuclepore filter in some samples from P and R. This result, along with our chamber study, suggests that the loss of *S. aureus* culturability in air samples collected using a Nuclepore filter may be related to the stress of filtration

TABLE 2
Average recovered concentrations of airborne *S. aureus* from different sampling locations in the hospital

	Sampler							
	Andersen impactor				Nuclepore filter			
Media[a]	H	P	R	Mean	H	P	R	Mean
CSA								
MSSA[b]	3.7	3.3	2.3	3.1	2.9	2.2	1.8	2.3
MRSA[c]	3.7	3.2	1.7	2.9	0.6	N.D.	0.6	0.4
ORSA[d]	3.0	3.6	2.7	3.1	0.6	N.D.	N.D.	0.2
S. aureus[e]	8.0	6.9	5.4	6.8	3.5	2.2	2.4	2.7
C-MRSA								
MSSA	2.7	1.9	0.6	1.7	0.9	N.D.	N.D.	0.3
MRSA	7.4	3.2	1.9	4.1	2.8	2.6	N.D.	1.8
ORSA	6.2	2.5	1.9	3.5	3.7	2.6	N.D.	2.1
S. aureus	10.1	5.0	2.5	5.9	4.6	2.6	N.D.	2.4
								Unit: CFU/m^3

Note: N.D. means "not detected."
[a]Which agar media was used with the bioaerosol sampler.
[b]The concentration of airborne methicillin-sensitive S. aureus.
[c]The concentration of airborne methicillin-resistant *S. aureus*.
[d]The concentration of airborne oxacillin-resistant *S. aureus.*
[e]The concentration of total airborne *S. aureus*.

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and dehydration, especially when sampling at a moderate RH. Nevertheless, the highest total concentrations of *S. aureus* were still found in H on both CSA and C-MRSA by Nuclepore filter sampling.

Combining the data from the Andersen impactor and Nuclepore filter, the MSSA concentration detected by CSA was significantly higher than that detected by C-MRSA ($p < .05$). However, there was no significant difference in the MRSA and ORSA concentrations detected by CSA and C-MRSA. In the absence of antibiotic resistance, MSSA is difficult to grow on C-MRSA because the antibiotic-related supplements in C-MRSA suppress MSSA growth. In contrast, both MRSA and ORSA can grow on CSA because CSA cannot distinguish between *S. aureus* with or without antibiotic resistance. As shown in Table 2, we observed that the concentrations of airborne MRSA and ORSA were different. However, there was no statistically significant difference in MRSA and ORSA concentrations ($p > .05$). In addition, the recovered total concentration of *S. aureus* ranged from 2.2 to 10.1 CFU/m^3. These concentrations were lower than those found in a previous study conducted in indoor and outdoor residential environments (from 12.6 to 15.4 CFU/m^3; Gandara et al. 2006). The concentrations of airborne *S. aureus* were not particularly high during our sampling periods; however, the health risks are still difficult to calculate because very few studies have examined *S. aureus* in indoor air. MRSA or ORSA detected in P should be of particular concern because they are significant pathogens in children and have effects ranging from minor soft tissue lesions to life-threatening infections (Marcinak and Frank 2003).

Performance of CSA for Air Sampling

The performance of CSA as a selective differential medium for *S. aureus* in air samples was evaluated. As shown in the contingency table (Table 3), conventional laboratory tests and real-time PCR were used to evaluate CSA, and its performance was determined on the basis of sensitivity, specificity, PPV, and NPV.

TABLE 3
Air sampling performance of CSA in a 2 × 2 contingency table format using conventional laboratory tests and real-time PCR as comparison tests

	Sampler			
	Andersen		Filter	
	S. aureus	Non-*S. aureus*	*S. aureus*	Non-*S. aureus*
Mauve	62	350	14	113
Nonmauve	195	3614	42	942
Sensitivity (%)	24.1		25.0	
Specificity (%)	91.2		89.3	
PPV (%)	15.0		11.0	
NPV (%)	94.9		95.7	

The type of sampler that was used did not affect the performance of CSA. Combining the data from the Andersen impactor and the Nuclepore filter, CSA exhibited a low sensitivity (24%) but a high specificity (90%) in the detection of airborne *S. aureus*. Similarly, the PPV was low (13%) but the NPV was relatively high (95%) for CSA. Compared to clinical specimens and environmental seawater samples in previous studies, the sensitivity and PPV of CSA were in the range of 91%–100% (Gaillot et al. 2000; Carricajo et al. 2001; Chihara et al. 2009; Goodwin and Pobuda 2009). These differences may be related to the different comparison tests that have been used in *S. aureus* identification, such as PCR or other conventional laboratory tests. However, the relatively low sensitivity indicated that CSA did not recognize airborne *S. aureus.* In our chamber study, CSA recognized all recovered MSSA colonies, which were detected by the mauve color. However, this phenomenon was not observed in the field. The low sensitivity of CSA in field applications may be related to environmental interference, bacterial enzyme expression, and competing effects between different bacterial strains.

Microbes form colored colonies on the CHROMagar media because the media release colored dyes upon hydrolysis by a specific bacterial enzyme. Environmental interference has been demonstrated to inhibit PCR amplification of air samples (Chen et al. 2009). Therefore, some compounds may interfere with the hydrolytic process and suppress mauve color formation. Moreover, previous studies have demonstrated that the enzymatic expression by microbes may vary on the basis of the geographical origin of the species (Perez et al. 2003). Some bacterial species that were recovered from different geographical locations cannot express the specific enzyme that was detected by the CHROMagar, which may result in low sensitivity. Finally, the competing effects of different microbial species grown on the same CHROMagar plate may also affect its sensitivity (Perez et al. 2003; Diederen et al. 2005). The specific enzyme that was expressed by *S. aureus* may interfere with other bacterial species and result in the inability of CSA to detect *S. aureus*.

PPV and NPV are the other two important indicators that investigators have used to determine whether the mauve and nonmauve colonies were *S. aureus*. The PPV was directly proportional to the percentage of *S. aureus* (i.e., the concentration of *S. aureus* to total bacteria) in the air. In our study, the percentage of *S. aureus* ranged from 0.7% to 4.3%, which was similar to the percentage (from 0% to 10%) that was observed in a previous study (Gandara et al. 2006). Therefore, the low percentage of *S. aureus* may be present in low PPV of CSA (13%). With an increasing percentage of *S. aureus* in the air, it is more likely that a mauve colony will be *S. aureus* rather than a false positive (i.e., the mauve colony is not *S. aureus*). In addition, some coagulase-negative *Staphylococcus* spp., including *S. cohnii*, *S. intermedius*, and *S. schleiferi*, yielded a faint mauve color with CSA (Chihara et al. 2009), which may also decrease the PPV of CSA in air sampling. Although the sensitivity and PPV of CSA were low, CSA exhibited high specificity and NPV in air sampling. This result suggests that a nonmauve colony that is recovered using CSA can be reported as a specimen that is not *S. aureus*. However, a mauve colony should be further confirmed using conventional procedures or PCR.

TABLE 4
Air sampling performance of C-MRSA in a 2 × 2 contingency table format using conventional laboratory tests and real-time PCR as comparison tests.

	Sampler			
	Andersen		Filter	
	MRSA	Non-MRSA	MRSA	Non-MRSA
Mauve	27	203	5	20
Nonmauve	11	378	2	37
Sensitivity (%)	71.1		71.4	
Specificity (%)	65.1		64.9	
PPV (%)	11.7		20.0	
NPV (%)	97.2		94.9	

Performance of C-MRSA in Air Sampling

The performance of C-MRSA as a selective differential medium for MRSA in air samples is summarized in Table 4. Conventional laboratory tests and real-time PCR combined with antibiotic susceptibility testing were used as comparison tests for C-MRSA. The performance of C-MRSA with an Andersen impactor was not significantly different from that with a Nuclepore filter. Compared to CSA, the performance of C-MRSA exhibited trade-offs concerning sensitivity and specificity. The sensitivity of C-MRSA (71%) was higher than that of CSA (24%). However, the specificity (65%) was lower than that of CSA (90%). However, the PPV (16%) and NPV (96%) of C-MRSA were similar to those of CSA. These findings confirm those of a previous study by Goodwin and Pobuda, showing the sensitivity (78%) and low PPV (25%) for identification of MRSA in seawater samples (Goodwin and Pobuda 2009).

The sensitivity and specificity of C-MRSA were different from those of CSA. The higher sensitivity of C-MRSA may indicate that the performance of C-MRSA in recognizing airborne MRSA was better than that of CSA in recognizing *S. aureus.* To date, the components of the chromogenic and selective mix in CHROMagar are proprietary. Therefore, it is difficult to explain the differences between the performances of CSA and C-MRSA. Some antibiotic supplements, such as ciprofloxacin, methicillin, oxacillin, or cefoxitin, may be incorporated into C-MRSA to suppress MSSA strains (Perry and Freydiere 2007). Therefore, the selective mixes and antibiotics in C-MRSA may help to suppress other bacteria species (especially for antibiotic-sensitive strains) and improve the performance of C-MRSA in recognizing airborne MRSA. The percentage of MRSA to total bacteria (from 0% to 2.7%) was lower than the percentage of total *S. aureus* in the air. Therefore, the identification of airborne

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MRSA by C-MRSA also revealed a low PPV. Similar to CSA, C-MRSA exhibited a high NPV in air sampling. This result suggests that a nonmauve colony that is recovered using C-MRSA in air sampling can be reported as negative. However, a mauve colony should be subjected to further identification processes.

Because of the widespread use of antibiotics, the number of resistant or virulent bacterial strains such as MRSA may increase. There is an immediate need to develop an environmental monitoring program for rapid identification of airborne MRSA. On the basis of the similar PPV and NPV, C-MRSA may become more valuable than CSA because C-MRSA had higher sensitivity. From a health perspective, MRSA poses higher threats to health than MSSA. A high level of sensitivity in a test is desirable for detecting airborne microbes that cause serious disease. Even through C-MRSA, with a low PPV, may lead to false positive results, additional simple tests (such as Gram staining and coagulase tests) would improve the performance of C-MRSA. Several alternative methods, such as real-time PCR, are available to rapidly identify *S. aureus* or MRSA. However, these methods are expensive, require specialized equipment, and do not necessarily indicate that any of the detected microbes are infectious. In this study, we demonstrated that C-MRSA was used in air sampling to achieve MRSA isolation without additional susceptibility testing. Therefore, these advantages may enable C-MRSA to rapidly identify the concentration profiles of airborne MRSA in the hospital. Although the low PPVs of CSA and C-MRSA suggest that a positive result should also be verified using conventional procedures, these media may reduce the detection time of *S. aureus* or MRSA by 24–48 h, depending upon the methods that are used.

CONCLUSIONS

The recovery efficiencies of four agar media (CSA, BAP, LB, and TSA) in collecting airborne MSSA were evaluated in a chamber experiment. The Andersen impactor preserved MSSA culturability better than the Nuclepore filter. The unsuitability of the Nuclepore filter was possibly due to sampling stress during filtration and due to dehydration during the sampling and extraction processes. In addition, the recovery efficiency of CSA for collecting MSSA was only lower than LB when using an Andersen impactor. The other two nonselective agars, BAP and TSA, did not perform better than CSA. The field results revealed that CSA can also be used to detect the total bacterial aerosol; the detected concentrations did not differ significantly from those detected by the commonly used TSA and LB. Moreover, for detection of low concentrations of airborne *S. aureus* or MRSA, CSA or C-MRSA combined with an Andersen impactor would result in a lower detection limit than they would when combined with a Nuclepore filter. For colony identification in the field, CSA had a high specificity and C-MRSA had a better sensitivity. However, the PPV and NPV are more important indicators than the sensitivity and specificity of CSA or C-MRSA during detection of airborne MSSA or MRSA. Both CSA and C-MRSA had a low PPV and a high NPV, suggesting that a Gram-stain and coagulase test should be performed on the mauve colonies in order to exclude the species that are coagulase negative or that have gram stains that are not consistent with *S. aureus* or MRSA.

REFERENCES

Brown, D. F., Edwards, D. I., Hawkey, P. M., Morrison, D., Ridgway, G. L., Towner, K. J., et al. (2005). Guidelines for the Laboratory Diagnosis and Susceptibility Testing of Methicillin-Resistant *Staphylococcus aureus* (MRSA). *J. Antimicrob. Chemother.*, 56:1000–1018.

Carricajo, A., Treny, A., Fonsale, N., Bes, M., Reverdy, M. E., Gille, Y., et al. (2001). Performance of the Chromogenic Medium CHROMagar Staph Aureus and the Staphychrom Coagulase Test in the Detection and Identification of *Staphylococcus aureus* in Clinical Specimens. *J. Clin. Microbiol.*, 39:2581–2583.

Chen, P. S., and Li, C. S. (2005). Sampling Performance for Bioaerosols by Flow Cytometry with Fluorochrome. *Aerosol Sci. Technol.*, 39:231–237.

Chen, P. S., Lin, C. K., Tsai, F. T., Yang, C. Y., Lee, C. H., Lia, Y. S., et al. (2009). Quantification of Airborne Influenza and Avian Influenza Virus in a Wet Poultry Market Using a Filter/Real-Time qPCR Method. *Aerosol Sci. Technol.*, 43:290–297.

Chihara, S., Hayden, M. K., Minogue-Corbett, E., and Singh, K. (2009). Shortened Time to Identify *Staphylococcus* Species from Blood Cultures and Methicillin Resistance Testing Using CHROMAgar. *Int. J. Microbiol.* 2009:636502.

Clinical and Laboratory Standards Institute. (2005). *Performance Standard for Antimicrobe Susceptibility Testing; 15th Informantional Supplement*. Clinical and Laboratory Standards Institute, Wayne, PA.

Cooper, B. S., Stone, S. P., Kibbler, C. C., Cookson, B. D., Roberts, J. A., Medley, G. F., et al. (2004). Isolation Measures in the Hospital Management of Methicillin Resistant *Staphylococcus aureus* (MRSA): Systematic Review of the Literature. *BMJ*, 329:533.

Crook, B. (1995). Non-Inertial Samplers: Biological Perspectives, in *Bioaerosols Handbook*, C.S. Cox and C.M. Wathes, eds. Lewis Publishers, Boca Raton, FL, pp. 269–283.

Diederen, B., van Duijn, I., van Belkum, A., Willemse, P., van Keulen, P., and Kluytmans, J. (2005). Performance of CHROMagar MRSA Medium for Detection of Methicillin-resistant *Staphylococcus aureus*. *J. Clin. Microbiol.*, 43:1925–1927.

Fang, H., and Hedin, G. (2003). Rapid Screening and Identification of Methicillin-Resistant *Staphylococcus aureus* from Clinical Samples by Selective-Broth and Real-Time PCR Assay. *J. Clin. Microbiol.*, 41:2894–2899.

Francis, J. S., Doherty, M. C., Lopatin, U., Johnston, C. P., Sinha, G., Ross, T., et al. (2005). Severe Community-Onset Pneumonia in Healthy Adults Caused by Methicillin-Resistant *Staphylococcus aureus* Carrying the Panton-Valentine Leukocidin Genes. *Clin. Infect. Dis.*, 40:100–107.

Gaillot, O., Wetsch, M., Fortineau, N., and Berche, P. (2000). Evaluation of CHROMagar Staph. aureus, a New Chromogenic Medium, for Isolation and Presumptive Identification of *Staphylococcus aureus* from Human Clinical Specimens. *J. Clin. Microbiol.*, 38:1587–1591.

Gandara, A., Mota, L. C., Flores, C., Perez, H. R., Green, C. F., and Gibbs, S. G. (2006). Isolation of *Staphylococcus aureus* and Antibiotic-Resistant *Staphylococcus aureus* from Residential Indoor Bioaerosols. *Environ. Health Perspect.*, 114:1859–1864.

Gibbs, S. G., Green, C. F., Tarwater, P. M., Mota, L. C., Mena, K. D., and Scarpino, P. V. (2006). Isolation of Antibiotic-Resistant Bacteria from the Air Plume Downwind of a Swine Confined or Concentrated Animal Feeding Operation. *Environ. Health Perspect.*, 114:1032–1037.

Goodwin, K. D., and Pobuda, M. (2009). Performance of CHROMagar Staph aureus and CHROMagar MRSA for Detection of *Staphylococcus aureus*

Aerosol Science and Technology 2012.46:297-308.

in Seawater and Beach Sand—Comparison of Culture, Agglutination, and Molecular Analyses. *Water Res.*, 43:4802–4811.

Ho, M., McDonald, L. C., Lauderdale, T. L., Yeh, L. L., Chen, P. C., and Shiau, Y. R. (1999). Surveillance of Antibiotic Resistance in Taiwan, 1998. *J. Microbiol. Immunol. Infect.,* 32:239–249.

Hulten, K. G., Kaplan, S. L., Lamberth, L. B., Slimp, K., Hammerman, W. A., Carrillo-Marquez, M., et al. (2010). Hospital-acquired S*taphylococcus aureus* Infections at Texas Children's Hospital, 2001–2007. *Infect. Control Hosp. Epidemiol.*, 31:183–190.

Juozaitis, A., Willeke, K., Grinshpun, S. A., and Donnelly, J. (1994). Impaction onto a Glass Slide or Agar Versus Impingement into a Liquid for the Collection and Recovery of Airborne Microorganisms. *Appl. Environ. Microbiol.*, 60:861–870.

Li, C. S., and Lin, Y. C. (1999a). Sampling Performance of Impactors for Bacterial Bioaerosols. *Aerosol Sci. Technol.*, 30:280–287.

Li, C. S., and Lin, Y. C. (1999b). Sampling Performance of Impactors for Fungal Spores and Yeast Cells. *Aerosol Sci. Technol.*, 31:226–230.

Li, C. S., Tseng, C. C., Lai, H. H., and Chang, C. W. (2003). Ultraviolet Germicidal Irradiation and Titanium Dioxide Photocatalyst for Controlling *Legionella pneumophila*. *Aerosol Sci. Technol.*, 37:961–966.

Lin, W. H., and Li, C. S. (1998). The Effect of Sampling Time and Flow Rates on the Bioefficiency of Three Fungal Spore Sampling Methods. *Aerosol Sci. Technol.*, 28:511–522.

Lin, W. H., and Li, C. S. (1999). Collection Efficiency and Culturability of Impingement into a Liquid for Bioaerosols of Fungal Spores and Yeast Cells. *Aerosol Sci. Technol.*, 30:109–118.

Macher, J. M., and Willeke, K. (1992). Performance Criteria for Bioaerosol Samplers. *J. Aerosol Sci.*, 23:647–650.

Marcinak, J. F., and Frank, A. L. (2003). Treatment of Community-Acquired Methicillin-Resistant *Staphyococcus aureus* in Children. *Curr. Opin. Infect. Dis.,* 16:265–269.

Merlino, J., Leroi, M., Bradbury, R., Veal, D., and Harbour, C. (2000). New Chromogenic Identification and Detection of *Staphylococcus aureus* and Methicillin-Resistant *S. aureus*. *J. Clin. Microbiol.*, 38:2378–2380.

Negrini Tde, C., Duque, C., de Oliveira, A. C., Hebling, J., Spolidorio, L. C., and Spolidorio, D. M. (2009). *Staphylococcus aureus* Contamination in a Pediatric Dental Clinic. *J. Clin. Pediatr. Dent.*, 34:13–18.

Pape, J., Wadlin, J., and Nachamkin, I. (2006). Use of BBL CHROMagar MRSA Medium for Identification of Methicillin-Resistant *Staphylococcus aureus* Directly from Blood Cultures. *J. Clin. Microbiol.*, 44:2575–2576.

Perez, J. M., Cavalli, P., Roure, C., Renac, R., Gille, Y., and Freydiere, A. M. (2003). Comparison of Four Chromogenic Media and Hektoen Agar for Detection and Presumptive Identification of *Salmonella* Strains in Human Stools. *J. Clin. Microbiol.*, 41:1130–1134.

Perry, J. D., and Freydiere, A. M. (2007). The Application of Chromogenic Media in Clinical Microbiology. *J. Appl. Microbiol.*, 103:2046–2055.

Rutala, W. A., Stiegel, M. M., Sarubbi, F. A., and Weber, D. J. (1997). Susceptibility of Antibiotic-Susceptible and Antibiotic-Resistant Hospital Bacteria to Disinfectants. *Infect. Control Hosp. Epidemiol.*, 18:417–421.

Sacar, S., Sayin Kutlu, S., Turgut, H., Cevahir, N., Hircin Cenger, D., and Tekin, K. (2010). Epidemiology and Associated Factors for Nosocomial Methicillin-Resistant *Staphylococcus aureus* Infection in a Tertiary-Care Hospital. *Epidemiol. Infect.*, 138:697–701.

Shiomori, T., Miyamoto, H., Makishima, K., Yoshida, M., Fujiyoshi, T., Udaka, T., et al. (2002). Evaluation of Bedmaking-Related Airborne and Surface Methicillin-Resistant *Staphylococcus aureus* Contamination. *J. Hosp. Infect.,* 50:30–35.

Stapleton, P. D., and Taylor, P. W. (2002). Methicillin Resistance in *Staphylococcus aureus*: Mechanisms and Modulation. *Sci. Prog.*, 85:57–72.

Stewart, S. L., Grinshpun, S. A., Willeke, K., Terzieva, S., Ulevicius, V., and Donnelly, J. (1995). Effect of Impact Stress on Microbial Recovery on an Agar Surface. *Appl. Environ. Microbiol.*, 61:1232–1239.

Suller, M. T., and Russell, A. D. (1999). Antibiotic and Biocide Resistance in Methicillin-Resistant *Staphylococcus aureus* and Vancomycin-Resistant Enterococcus. *J. Hosp. Infect.,* 43:281–291.

Tsai, T. M., Chang, H. H., Chang, K. C., Liu, Y. L., and Tseng, C. C. (2010). A Comparative Study of the Bactericidal Effect of Photocatalytic Oxidation by TiO_2 on Antibiotic-Resistant and Antibiotic-Sensitive Bacteria. *J. Chem. Technol. Biotech. ,* 85:1642–1653.

Tseng, C. C., and Li, C. S. (2005). Collection Efficiencies of Aerosol Samplers for Virus-Containing Aerosols. *J. Aerosol Sci.*, 36:593–607.

Wendt, C., Havill, N. L., Chapin, K. C., Boyce, J. M., Dickenson, R., Eigner, U., et al. (2010). Evaluation of a New Selective Medium (BD BBL™ CHROMagar™ MRSA II) for the Detection of Methicillin-Resistant *Staphylococcus aureus* (MRSA) from Different Specimens. *J. Clin. Microbiol*, 48:2223–2227.

Wilson, R. D., Huang, S. J., and McLean, A. S. (2004). The Correlation Between Airborne Methicillin-Resistant *Staphylococcus aureus* with the Presence of MRSA Colonized Patients in a General Intensive Care Unit. *Anaesth. Intensive Care*, 32:202–209.

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