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Performance of CHROMagar VRE Medium for the Detection of Airborne Vancomycin-Resistant/Sensitive *Enterococcus* Species

Pao-Kuei Hsiao,[1] Chieh-Chen Cheng,[2] Kai-Chih Chang,[3] Lih-Ming Yiin,[2] Chia-Jung Hsieh,[2] and Chun-Chieh Tseng[2]

[1]*Institute of Environmental Health, National Taiwan University, Taipei, Taiwan*
[2]*Department and Graduate Institute of Public Health, Tzu Chi University, Hualien, Taiwan*
[3]*Department of Laboratory Medicine and Biotechnology, Tzu Chi University, Hualien, Taiwan*

Vancomycin-sensitive and vancomycin-resistant *Enterococcus* (VSE and VRE) species have become a significant health problem. CHROMagar medium, which permits direct, color-based identification of target pathogens, could potentially be used to rapidly monitor airborne VSE and VRE. In this study, the efficiency of CHROMagar VRE medium without vancomycin supplementation (CVSE) for collecting airborne vancomycin-sensitive *Enterococcus faecalis* was evaluated in a chamber study. Subsequently, the performance of bioaerosol samplers combined with CVSE and CHROMagar VRE (CVRE) was evaluated in a hospital environment, a wastewater treatment plant, and a pig-rearing facility. Our results demonstrated that an Andersen impactor was much more effective than a Nuclepore filter for collecting airborne *E. faecalis* at relative humidity levels of 30% and 55%. In addition, approximately 10% of the isolated environmental *Enterococcus* strains were vancomycin-resistant. The average sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the colony identification were 58.5%, 81.3%, 5.5%, and 99.1%, respectively, for CVSE and 100%, 88.3%, 8.4%, and 100%, respectively, for CVRE. These findings indicate that the use of CHROMagar might provide a rapid method for detecting airborne VSE or VRE, shortening the detection time to 24–48 h. However, any mauve-colored colonies recovered on CVSE or CVRE by air sampling should be subjected to further identification tests.

1. INTRODUCTION

Enterococci are gram-positive cocci that are commonly found in the intestines of humans. Within the genus *Enterococcus*, *E. faecalis*, and *E. faecium* are opportunistic pathogens

that are critically important to human health and are frequently identified as the cause of urinary tract, blood stream, and wound infections (Jett et al. 1994). Since the widespread introduction of vancomycin into clinical practice, vancomycin-resistant *Enterococcus* (VRE) strains have emerged worldwide as important nosocomial pathogens (Russell 2002). Although VRE is transmitted by close contact with infected persons or with contaminated surfaces (Otter et al. 2011), evidence suggests that airborne dispersal and transmission may also be important (Savini et al. 2012). Therefore, comprehensive monitoring and accurate and timely identification of airborne VRE are critical for early awareness and preventative measures.

To date, very limited research has been conducted regarding the presence of VRE or vancomycin-sensitive *Enterococcus* (VSE) in the air. Studies have demonstrated that *Enterococcus* may be inhaled in hospital wards (Muzslay et al. 2012), poultry houses (Brodka et al. 2012), swine feeding facilities (Predicala et al. 2002), and wastewater treatment plants (Karra and Katsivela 2007). The use of chromogenic media has become a key method for the rapid identification of microorganisms in clinical samples. Chromogenic media exploit enzyme substrates that release colored dyes upon hydrolysis by a specific bacterial enzyme, resulting in pigmentation of the target pathogen colonies (Perry and Freydiere 2007). Among the various chromogenic media, CHROMagar can be used to detect VRE in clinical stool and rectal swab samples with high sensitivity (98–99%) and specificity (94–99%) (Peltroche-Llacsahuanga et al. 2009; Kallstrom et al. 2010; Stamper et al. 2010). However, the performance of CHROMagar may differ depending on the sample type (i.e., clinical vs. environmental). We previously demonstrated that the sensitivity (71%) and specificity (65%) of CHROMagar for the identification of airborne methicillin-resistant *Staphylococcus aureus* (MRSA) isolates were significantly lower than for the identification of clinical isolates (Hsiao et al. 2012). The differing efficiencies of CHROMagar for clinical and environmental samples may be related to the presence of environmental

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compounds in the environmental samples, which might interfere with the hydrolytic process and suppress mauve color formation (Hsiao et al. 2012). Moreover, the competing effects of other environmental bacterial species may also decrease the performance of this system (Perez et al. 2003; Diederen et al. 2005).

Although mauve-colored colonies should be verified by further identification processes (Hsiao et al. 2012), CHROMagar still shortens the detection time from >3 days to 24–48 h because of its ability to isolate target bacteria from the air and detect antibiotic resistance in a single step. Because of these advantages, the performance of CHROMagar in airborne VRE and VSE sampling is worthy of further analysis. In addition to questions about the relative sensitivity and specificity of CHRO-Magar, bacterial colony survival rates on different agar media are also of concern. Damaged bacteria may be able to multiply and form colonies on nonselective agar medium but not on selective media containing inhibiting agents or extremely high concentrations of inorganic salts (Stewart et al. 1995). Therefore, the resulting impaction stress, which influences the recovery and injury of the collected bacteria, may be different for CHROMagar than for nonselective agar.

The purposes of this study were to evaluate the bacterial colony survival rate on CHROMagar during the collection of airborne vancomycin-sensitive *E. faecalis* in a chamber study and to evaluate the performance of CHROMagar for identifying airborne VSE and VRE in a hospital environment, a wastewater treatment plant, and a swine confinement-style building. To evaluate the bacterial colony survival rate on CHROMagar compared with other nonselective agar, three agar media, lysogeny broth agar (LB), Mueller Hinton agar (MH), and tryptic soy agar (TSA), were also used in the chamber study. In addition, two different bioaerosol samplers, an Andersen one-stage impactor and a Nuclepore polycarbonate filter, were investigated with the four types of agar medium. An environmental monitoring program for detecting airborne VSE and VRE using the two different samplers was conducted to test the colony identification performance of CHROMagar. After sampling, all colonies recovered using CHROMagar that demonstrated positive (mauve) or negative results were verified using conventional laboratory tests and polymerase chain reaction (PCR).

2. MATERIAL AND METHODS

2.1. Chamber Study

2.1.1. Test Microorganism

Because most clinical *Enterococcus* isolates are *E. faecalis* (80–90%) rather than *E. faecium* (5–10%) (Treitman et al. 2005), a single strain of vancomycin-sensitive *E. faecalis* (ATCC 29212) was used as a surrogate for both VSE and VRE species in the chamber study. An active *E. faecalis* culture was inoculated in lysogeny broth and incubated for 24 h at 37°C. Following cultivation, the microbe pellets were aseptically washed and resuspended in sterile distilled water to prepare nebulized suspensions.

2.1.2. Aerosol Preparation and Test System

A Collision three-jet nebulizer (BGI Collision Nebulizer, BGI Inc., Waltham, MA, USA) was used to nebulize the *E. faecalis* stock in deionized water at 20 psi with dry, filtered, compressed laboratory air. The aerosolized suspension was then diluted with filtered, compressed air at 50 l/min. Because the concentration of airborne *Enterococcus* rarely exceeds 1,000 colony forming units per cubic meter of air (CFU/m^3) in the environment (Predicala et al. 2002), *E. faecalis* at 1,000 CFU/m^3 was used to simulate field conditions. Preliminary analyses (data not shown) showed that nearly 1,000 CFU/m^3 of airborne *E. faecalis* could be generated by an *E. faecalis* suspension in a nebulizer at 1,000 CFU/ml. Therefore, the *E. faecalis* concentration in the nebulizer was approximately 1,000 CFU/ml (coefficient of variation% (CV%) = 5.0%) in each experiment. To determine the concentration of the *E. faecalis* suspension in the nebulizer, *E. faecalis* was cultured in a flask and incubated at 37°C until reaching an OD_{600} of 1.0 (1×10^8 CFU/ml). The *E. faecalis* culture was then serially diluted to obtain a final concentration of approximately 1×10^3 CFU/ml. Because relative humidity (RH) strongly affects bacterial culturability, collection was performed at three RH levels (30%, 55%, and 85%).

2.1.3. Test Samplers and Sample Processing

To evaluate the bacterial colony survival rate on assorted agar media, an Andersen 1-STG impactor (Andersen Samplers, Inc., Atlanta, GA, USA) and a Nuclepore filter sampler (Nuclepore®, Costar Corp., Cambridge, MA, USA) were used to collect *E. faecalis* aerosols. The Andersen impactor was operated at a sampling flow rate of 28.3 l/min and a sampling time of 7 min. The Nuclepore filter sampler consisted of a polycarbonate membrane with a 0.4-μm pore size and a 37-mm diameter supported by cellulose pads. The sampling flow rate of the Nuclepore filter sampler was 20 l/min, and the sampling time was 60 min.

Commercially available media for the selective and differential growth of vancomycin-resistant *E. faecalis* and vancomycin-resistant *E. faecium* (VRE.faes) that rely on colony color-based identification include CHROMagar VRE (CVRE; CHROMagar Microbiology, Paris, France). According to the manufacturer, CVRE without vancomycin supplementation (CVSE) can distinguish vancomycin-sensitive *E. faecalis* and vancomycin-sensitive *E. faecium* (VSE.faes) from other bacterial strains based on colony color. Because *E. faecalis* was used as a surrogate for VRE in the chamber study, only CVSE (not CVRE) was evaluated for airborne collection of *E. faecalis* in a chamber. In addition to CVSE, bacterial colony survival rates on several nonselective media such as LB (REF 244520), MH (REF 225250), and TSA agar (REF 236950) (Difco Laboratory, Detroit, MI, USA) were compared. After sampling, the plates of collection medium (CVSE, LB, MH, and TSA) from the Andersen impactor were incubated at 37°C for 24 h. For filter sampling, filters were eluted after sampling by rinsing with 1 ml sterile deionized water. Then, 0.1 ml of eluate was inoculated separately on the various agars and incubated at 37°C for 24 h.

Finally, CFU/m^3 was calculated based on the dilution ratio, plated volume, sampling time, and flow rate.

2.1.4. Calculation of the Bacterial Colony Survival Rate

Microbial collection and survival in bioaerosol samplers strongly depend on the type of aerosolization, the type of sampler, the sampling time, the sampling flow rate, and the collection medium. Because we generated *E. faecalis* aerosols using the same method and the same bioaerosol sampler (Andersen impactor or Nuclepore filter sampler) at a constant operating flow rate, sampling time, and RH, the impact stress on the bacterial colony was directly related to the components of the loaded agar (Juozaitis et al. 1994; Stewart et al. 1995). However, the bacterial colony survival rate on a collection medium can be strongly affected by the culture preparation process (Lin and Li 1998, 1999; Chen and Li 2005; Tseng and Li 2005). Therefore, to better understand the colony survival rate on each agar medium evaluated for collecting *E. faecalis* aerosols, the culturability of microorganisms in the liquid suspension used as the source of the bioaerosols needed to be evaluated (Li et al., 2003). To accomplish this, we used a parameter called colony survival (CS) as an indicator to assess the biological performance of the tested sampler for each agar medium:

$$CS = \frac{C_{test}}{C_{susp}},$$

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

2.1.5. Dehydration Stress of Filter Sampling

The dehydration stress on *E. faecalis* induced by the Nuclepore filter sampler was evaluated in a chamber at an RH of 55% according to a previously published method (Wang et al., 2001). *E. faecalis* was cultured overnight to a concentration of 1×10^8 CFU/ml. To determine the effect of dehydration stress on *E. faecalis*, bacterial cultures were serially diluted to 1×10^5 CFU/ml. The bacterial culture was then spiked onto 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 for 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 with 1 ml sterile deionized water, which was used for bacterial culture on CVSE, LB, MH, and TSA agar. The dehydration stress on *E. faecalis* 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* hours and 0 h, respectively.

2.1.6. Statistical Analysis

In the chamber studies, all experiments were performed in triplicate. The CS or C_t/C_0 values of *E. faecalis* on the evaluated medium were compared using the nonparametric Kruskal–Wallis test followed by Scheffe's test to evaluate statistically significant differences ($p < 0.05$). These statistical tests were also used to compare the CS values among the three different RH levels using CVSE or CVRE.

2.2. Field Study

2.2.1. Sampling Location

We collected aerosol samples from the outpatient hall (H) and the wastewater treatment plant (W) of a medical center in Taiwan. At site H, the central air conditioning was supplied by air-handling units. Samples from site W were collected from the influent and effluent tanks. Sampling was also conducted in a swine confinement-style building (S) because *Enterococcus* species are frequently found in the air of swine facilities (Predicala et al. 2002).

2.2.2. Aerosol Sampling

At each sampling site, parallel aerosol samples were collected using an Andersen impactor and a Nuclepore filter sampler. We collected a total of 282 aerosol samples, including 207 impactor samples and 75 filter samples. At site W, we collected aerosol samples at the influent and effluent tanks for wastewater treatment, including the influent and effluent pumping room, through which workers pass. At site S, we selected enclosed environments, such as farrowing and nursery units in the swine building, for air sampling. The air pump and sampling apparatus were placed approximately 1 m from patient seating at site H and in the central walkway at sites W and S. The sampling heights were 1 m above the floor at site H and 1.5 m above the floor at sites W and S, within the breathing zones of the seated patients and occupational workers, respectively. CVSE and CVRE agar media were used in conjunction with each sampler to identify airborne VSE.faes and VRE.faes, respectively. To compare the total bacterial concentrations in the aerosol, the nonselective media LB, MH, and TSA were also investigated. The sample processing was identical to that used in the chamber study. All evaluated agar media (CVSE, CVRE, LB, MH, and TSA) containing the field samples were incubated at 37°C for 24 h.

2.2.3. Bacterial Identification and Antibiotic Susceptibility Test

CVSE and CVRE are selective and differential media for VSE.faes and VRE.faes, respectively. In both cases, the target colonies are mauve in color. After 20–24 h of cultivation, colonies that appeared mauve-positive or mauve-negative were identified using conventional laboratory tests in sequence, including Gram staining, catalase testing, PYR testing, bile-esculin testing, and 6.5% NaCl tolerance testing, to verify the results. A total of 9,532 colonies on both media were subjected

to conventional laboratory tests. *Enterococcus* species are Gram positive, catalase negative, PYR positive, and bile-esculin positive and grow in broth containing 6.5% NaCl.

2.2.4. *Molecular Identification of* E. Faecalis *and* E. Faecium *from Aerosol Samples*

Any sample identified as an *Enterococcus* species that passed all of the conventional laboratory tests was validated by PCR performed on a DNA thermal cycler GeneAmp PCR system 2700 (Applied Biosystems, Foster City, CA, USA). For DNA extraction, suspensions from airborne samples were heated at 95°C for 15 min and centrifuged for 2 min at 14,000 rpm to sediment the debris. The clear supernatant was used as a DNA template for PCR analysis. To identify *E. faecalis*, a primer pair (EfF: 5′-CCGAGTGCTTGCACTCAATTGG-3′; EfR: 5′-CTCTTATGCCATGCGGCATAAAC-3′) was used to specifically amplify the *E. faecalis* 16S rRNA gene, as described previously (Sedgley et al. 2005). The primer pair EM1A/EM1B (EM1A: 5′-TTGAGGCAGACCAGATTGACG-3′; EM1B: 5′-TATGACAGCGACTCCGATTCC-3′) was also used to amplify an *E. faecium*-specific genomic fragment according to a previously described method (Cheng et al. 1997). The expected product sizes of the respective PCR amplicons were 138 and 658 bp. The resulting PCR products were analyzed by agarose gel electrophoresis (Figure 1).

2.2.5. *Vancomycin Susceptibility Test*

Following verification of *E. faecalis* or *E. faecium*, room-temperature MH agar plates were used to evaluate each of the isolates for antibiotic resistance. The vancomycin susceptibilities were assessed using the disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI 2005) guidelines. To evaluate the performance of CVRE, *E. faecalis* or *E. faecium* displaying vancomycin resistance was defined as vancomycin-resistant *Enterococcus* (VRE).
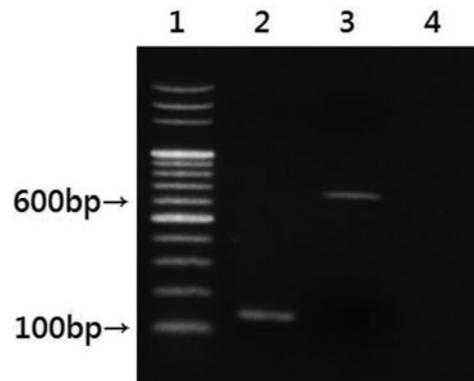

FIG. 1. Identification of *E. faecalis* and *E. faecium* in airborne field samples by gel analysis of PCR products. Lane 1: 1 kb DNA ladder; lane 2: *E. faecalis*; lane 3: *E. faecium*; lane 4: sterile water.

2.2.6. *Data Analysis*

The performance of CVSE or CVRE was assessed in a 2×2 contingency table format (Table 1). The performance statistics reflect sensitivity $[a/(a+b)]$ or $[e/(e+f)]$ and specificity $[d/(c+d)]$ or $[h/(g+h)]$. Sensitivity refers to the proportion of VSE.faes and VRE.faes with a positive test result (mauve color) using CVSE or CVRE. Specificity refers to the proportion of non-VSE.faes or non-VRE.faes with a negative test result (nonmauve color) using CVSE or CVRE. In addition to sensitivity and specificity, the positive predictive values [PPV; $(a/(a+c)$ or $e/(e+g)]$ and negative predictive values [NPV; $d/(b+d)$ or $h/(f+h)]$ 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.

2.2.7. *Statistical Analysis*

In field studies, the total concentrations of airborne bacteria at H, W, and S were compared using the nonparametric Kruskal–Wallis test followed by Scheffe's test to evaluate

TABLE 1
Contingency table for air sampling performance of CVSE and CVRE

	CHROMagar VSE (CVSE)			CHROMagar VRE (CVRE)		
	VSE.faes	Non-VSE.faes	Total	VRE.faes	Non-VRE.faes	Total
Mauve	a	c	$a+c$	e	g	$e+g$
Nonmauve	b	d	$b+d$	f	h	$f+h$
Total	$a+b$	$c+d$	$a+b+c+d$	$e+f$	$g+h$	$e+f+g+h$

VSE.faes or VRE.faes = the collected colonies that were verified as vancomycin-sensitive *E. faecalis* and vancomycin-sensitive *E. faecium* (VSE.faes) or vancomycin-resistant *E. faecalis* and vancomycin-resistant *E. faecium* (VRE.faes), respectively, using conventional laboratory tests (Gram staining, catalase testing, PYR testing, bile-esculin testing, and NaCl tolerance testing) and PCR.

Non-VSE.faes or non-VRE.faes = the collected colonies that were verified as not being VSE.faes or VRE.faes, respectively, using conventional laboratory tests and PCR.

Mauve = the collected colonies that were mauve on CVSE or CVRE.

Nonmauve = the collected colonies that were not mauve on CVSE or CVRE.

statistically significant differences ($p < 0.05$). The statistical tests were also utilized to compare the total bacterial concentrations detected using the five different agar media. The nonparametric Wilcox rank-sum (Mann–Whitney) test was utilized to examine the differences in bacterial concentrations between two sampler types or two agar media. The difference in bacterial identification performance between two sampler types or two agar media (CVSE and CVRE) was also analyzed by the same statistical tests.

3. Results

3.1. Chamber Study

3.1.1. Bacterial Colony Survival Rates on the Tested Agar Media

The CS values of the Andersen one-stage impactor for *E. faecalis* on CVSE, LB, MH, and TSA (Figure 2a) ranged from 0.6 to 1.2 at all three RH levels (30%, 55%, and 85%). The RH did not affect the CS of *E. faecalis* on any of the evaluated agars ($p = 0.07$). In addition, the CS of *E. faecalis* on CVSE
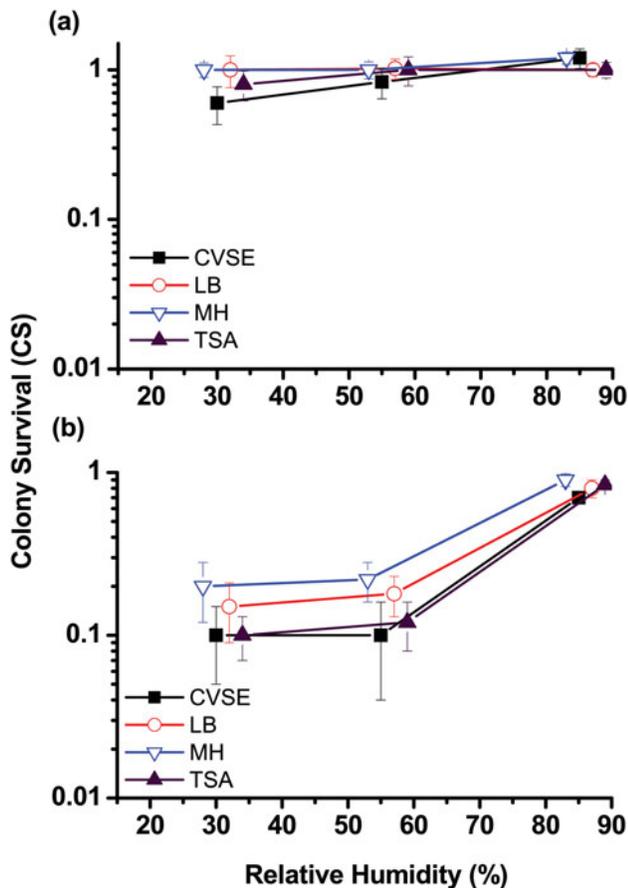
FIG. 2. Effects of RH on colony survival (CS) on four culture media (CVSE, LB, MH, and TSA) when collecting airborne *E. faecalis* using (a) an Andersen impactor and (b) a Nuclepore filter sampler. The *y*-axis CS represents the bacterial colony survival rate on different culture media on a logarithmic scale. Experiments were performed in triplicate, and the data shown are the mean ± standard error of the mean.
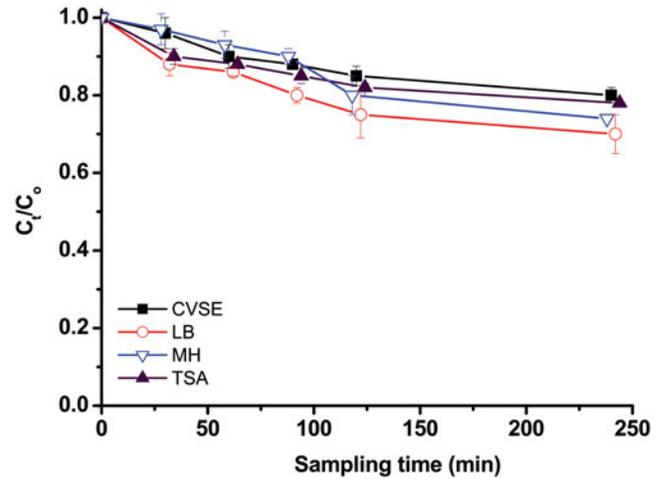
FIG. 3. Dehydration stress of *E. faecalis* recovered on four culture media (CVSE, LB, MH, and TSA) by closed-face filter sampling. The *y*-axis C_t/C_0 represents dehydration stress in *E. faecalis* induced by the Nuclepore filter sampler in arithmetic scale. Experiments were performed in triplicate, and the data shown are the mean ± standard error of the mean.

was only slightly lower than that on LB, MH, or TSA at RHs of 30% or 55%, and the results were not significantly different ($p = 0.10$). The average CS values of the Nuclepore filter sampler (Figure 2b) for *E. faecalis* on all four agar media did not differ significantly at any of the RH levels ($p = 0.21$). However, the average CS for *E. faecalis* on all four agar media at an RH of 85% (from 0.7 to 0.9) was significantly higher than the CS values at RHs of 30% and 55% (from 0.1 to 0.2; $p < 0.05$).

3.1.2. Dehydration Stress on Closed-Face Filter Cassettes

As shown in Figure 3, all curves representing the C_t/C_0 values declined with increasing sampling time and decreased gradually after pure air was passed through the filter. The C_t/C_0 values for all of the evaluated agar media did not differ significantly from each other ($p = 0.59$). The sampling time for the Nuclepore filter sampler was 60 min in both our chamber and field studies. Following 60 min of sampling, the average C_t/C_0 values were 0.90 for CVSE, 0.86 for LB, 0.93 for MH, and 0.88 for TSA. Therefore, the recovery rate on *E. faecalis* collected by a closed-face filter cassette was not less than 0.8 for sampling times of less than 60 min.

3.2. Field Study

3.2.1. Total Concentration of Airborne Bacteria at the Three Sampling Sites

During the sampling period, the mean ambient RHs were 64% in site H, 59% in site W, and 73% in site S. The average bacterial concentrations for the different agar media in an Andersen impactor varied from 138 to 49,638 CFU/m^3 (Figure 4a). Site S had the highest total bacterial concentration of the three sampling locations ($p < 0.05$), while the bacterial concentrations at sites H and W were not significantly different from each other ($p = 0.83$). The total bacterial concentration measured using CVSE was not significantly different from that detected
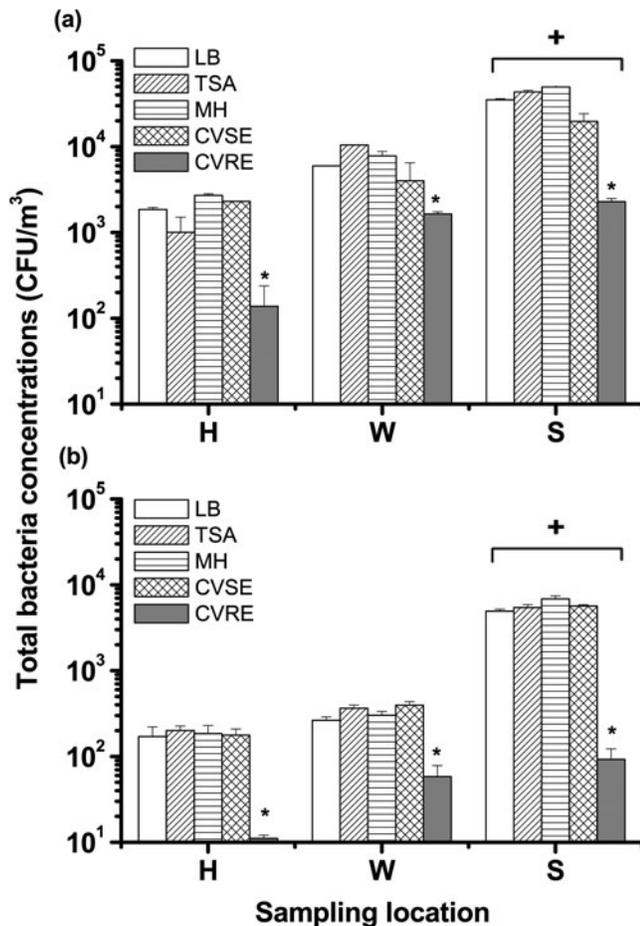
FIG. 4.   Total airborne bacterial concentrations recovered on five culture media (CVSE, CVRE, LB, TSA, and MH) after sampling using (a) an Andersen impactor or (b) a Nuclepore filter sampler in an outpatient hall (H), wastewater treatment plant (W), and swine confinement-style building (S). $^{+}p < 0.05$ compared to the respective H and W groups. $^{*}p < 0.05$ compared to the respective LB, TSA, MH, and CVSE groups.
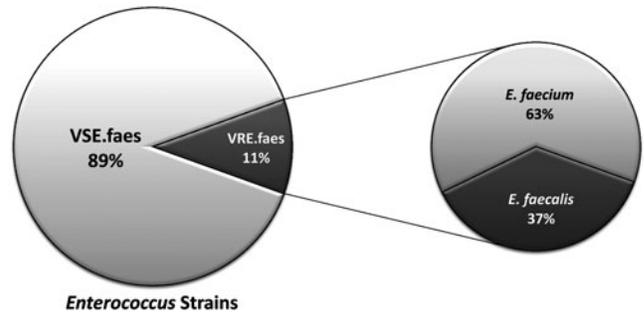

FIG. 5.   Distribution of vancomycin-resistant *Enterococcus* strains in the air of a hospital, wastewater treatment plant, and pig-rearing facility. Approximately 10% of the isolated *Enterococcus* strains were vancomycin-resistant, and more than half of the vancomycin-resistant strains were *E. faecium*.

using the nonselective agars LB, MH, and TSA ($p = 0.23$); however, the concentration measured using CVRE was significantly lower than that detected using the other three agar types ($p < 0.05$). In comparison with the Andersen impactor, the Nuclepore filter sampler greatly underestimated the concentration of airborne bacteria (3.5–28.7-fold lower; $p < 0.05$), regardless of the agar medium used (Figure 4b). Filter sampling also detected the highest total bacterial concentration at site S, and there were no significant differences among the total concentrations measured using CVSE, LB, MH, and TSA ($p = 0.13$). As with the Andersen impactor, the CVRE medium yielded the lowest bacterial concentration for the Nuclepore filter sampler ($p < 0.05$).

3.2.2.   Concentration of Airborne VSE and VRE as Determined Using CVSE and CVRE

Tables 2 and 3 show the median concentrations of VSE and VRE, which were determined using conventional laboratory

tests and PCR on all of the recovered colonies on CVSE or CVRE media. CVSE combined with either the Andersen impactor or the Nuclepore filter sampler demonstrated that the order of VSE concentrations by sampling site was $S > W > H$. Similarly, the concentration of airborne VRE at site S was also higher than the concentrations at sites H and W. The detected concentrations on CVSE and CVRE media using the Nuclepore filter sampler were 3.1- to 6.0-fold lower than those detected by the Andersen impactor. In addition, several filter samples had no detectable VSE.faes or VRE.faes at site H. Combining the data from the Andersen impactor and the Nuclepore filter sampler, no VSE.faes colonies were detected on the CVRE agar. In addition, there were no significant differences in the VRE.faes concentrations detected by CVSE and CVRE ($p = 0.61$). As shown in Figure 5, we observed that approximately 90% of the airborne *E. faecalis* and *E. faecium* were VSE, while the remaining 10% of the isolates were VRE. Figure 5 also shows that *E. faecium* accounted for 63% of the airborne isolates of VRE.faes; *E. faecalis* accounted for the remaining 37% of isolates.

3.2.3.   Performance of CVSE for Air Sampling

The performance of CVSE as a selective differential medium for VSE.faes in air samples was evaluated. As shown in the contingency table (Table 4), conventional laboratory tests and PCR were used to evaluate CVSE, and its performance was determined based on sensitivity, specificity, PPV, and NPV. The type of sampler that was used did not affect the performance of CVSE ($p = 0.77$). Combining the data from the Andersen impactor and the Nuclepore filter sampler to calculate the mean sensitivity, specificity, PPV, and NPV, CVSE exhibited low sensitivity (58.5%) but relatively high specificity (81.3%) for the detection of airborne VSE.faes. Similarly, the PPV was low (5.5%) but the NPV was relatively high (99.1%) for CSA.

3.2.4.   Performance of CVRE in Air Sampling

The performance of CVRE as a selective differential medium for VRE.faes in air samples is summarized in Table 5. Conventional laboratory tests combined with PCR and vancomycin susceptibility testing were used as comparison tests for CVRE.

TABLE 2

Airborne *Enterococcus* concentrations recovered from different locations by Andersen impactor sampling

Media[a]	Outpatient hall (*H*)	Wastewater treatment plant (*W*)	Swine confinement-style building (*S*)	Mean
CVSE				
VSE.faes[b]	15.1 (6.7–30.3)	32.0 (21.9–38.7)	641.1 (605.8–689.9)	229.4
E. faecalis	11.8 (3.4–28.6)	28.6 (16.8–33.7)	526.7 (521.6–538.4)	189.0
E. faecium	3.3 (1.7–6.7)	1.7 (1.7–16.8)	111.1 (79.1–158.2)	38.7
VRE.faes[c]	3.4 (1.7–6.7)	10.1 (6.7–20.2)	53.8 (45.4–58.9)	22.4
E. faecalis	0 (N.D.-1.7)	3.4 (3.4–6.7)	20.2 (15.1–25.2)	7.9
E. faecium	1.7 (1.7–5.0)	6.7 (3.4–13.5)	32.0 (25.2–40.4)	13.5
CVRE				
VRE.faes	1.7 (N.D.-3.4)	16.8 (10.1–18.5)	58.9 (52.2–62.3)	25.8
E. faecalis	N.D.	6.7 (3.4–11.8)	21.9 (16.8–26.9)	9.5
E. faecium	1.7 (N.D.-3.4)	8.4 (6.7–11.8)	37.0 (32.0–42.1)	15.7

Data are median concentrations, with ranges in parentheses. Unit: CFU/m^3.

N.D. = not detected.

Media[a] = the agar medium used with the bioaerosol sampler.

VSE.faes[b] = the concentration of airborne vancomycin-sensitive *E. faecalis* and *E. faecium*.

VRE.faes[c] = the concentration of vancomycin-resistant *E. faecalis* and *E. faecium*.

The performance of CVRE with the Andersen impactor was not significantly different from that with the Nuclepore filter sampler ($p = 1.0$). Combining the Andersen impactor and the Nuclepore filter sampler data, the mean sensitivity of CVRE (100%) was higher than that of CVSE (58.5%), although the difference was not significant ($p = 0.10$). Moreover, the mean specificity (88.3%), PPV (8.4%), and NPV (100%) of CVRE were similar to that of CVSE, respectively.

4. DISCUSSION

4.1. Chamber Study

We did not find any significant differences between TSA and CVSE when sampling airborne *E. faecalis*. This result is surprising given that TSA is considered an excellent general agar for culturing a variety of bacteria. A previous study suggested that impaction onto an agar surface may injure bacteria, particularly

TABLE 3

Airborne *Enterococcus* concentrations recovered from different locations by Nuclepore filter sampling

Media[a]	Outpatient hall (*H*)	Wastewater treatment plant (*W*)	Swine confinement-style building (*S*)	Mean
CVSE				
VSE.faes[b]	2.8 (2.8–8.3)	5.6 (2.8–11.1)	106.6 (100.0–113.9)	38.3
E. faecalis	2.8 (2.8–8.3)	2.8 (2.8–8.3)	91.5 (86.1–94.4)	32.3
E. faecium	N.D.	1.4 (N.D.-2.8)	13.9 (13.9–22.2)	5.1
VRE.faes[c]	N.D.	8.0 (5.6–8.3)	9.7 (8.3–13.9)	5.9
E. faecalis	N.D.	2.8 (2.8–5.6)	4.4 (2.8–5.6)	2.4
E. faecium	N.D.	4.2 (2.8–5.6)	5.5 (2.8–8.3)	3.2
CVRE				
VRE.faes	N.D.	11.1 (8.3–16.7)	13.9 (8.3–19.4)	8.3
E. faecalis	N.D.	5.1 (2.8–8.3)	2.8 (N.D.-2.8)	2.6
E. faecium	N.D.	6.1 (2.8–11.1)	11.1 (5.6–19.4)	5.7

Data are median concentrations, with ranges in parentheses. Unit: CFU/m^3.

N.D. = not detected.

Media[a] = the agar medium used with the bioaerosol sampler.

VSE.faes[b] = the concentration of airborne vancomycin-sensitive *E. faecalis* and *E. faecium*.

VRE.faes[c] = the concentration of vancomycin-resistant *E. faecalis* and *E. faecium*.

TABLE 4
Contingency table for air sampling performance of CVSE
using conventional laboratory tests and PCR

| | Sampler | | | |
| | Andersen | | Filter | |
	VSE. faes	Non-VSE. faes	VSE. faes	Non-VSE. faes
Mauve	82	1067	8	200
Nonmauve	55	5198	6	781
Sensitivity (%)	59.9		57.1	
Specificity (%)	83.0		79.6	
Positive predictive values (%)	7.1		3.8	
Negative predictive values (%)	99.0		99.2	

when the bacteria are impacted onto a selective agar medium such as CVSE (Stewart et al. 1995). It is usually difficult to culture injured bacteria on selective media due to nutritional limitations and the presence of inhibiting agents. Nonselective agars, such as LB or TSA, offer a more effective means for preserving bacterial culturability. However, because CVSE is specifically used for the isolation and direct differentiation of VSE.faes, the components and selective mix that characterize CVSE may help *E. faecalis* to recover and may enable a CS level similar to that achieved with TSA.

When using the Nuclepore filter, sampling was conducted for 60 min to increase yields; under these conditions, less than 20% of the *E. faecalis* lost culturability on the filter. If filter sampling was continued for more than 240 min, more than

TABLE 5
Contingency table for air sampling performance of CVRE
using conventional laboratory tests and PCR

| | Sampler | | | |
| | Andersen | | Filter | |
	VRE. faes	Non-VRE. faes	VRE. faes	Non-VRE. faes
Mauve	15	182	3	30
Nonmauve	0	1719	0	186
Sensitivity (%)	100		100	
Specificity (%)	90.4		86.1	
Positive predictive values (%)	7.6		9.1	
Negative predictive values (%)	100		100	

half of the *E. faecalis* on the filter demonstrated a decrease in culturability during the dehydration process. Previous studies have shown that this is likely due to dehydration, which occurred during the sampling and the extraction processes, and might have caused enough biological stress to reduce the culturability of the bacteria (Chen and Li 2005; Tseng and Li 2005).

The parameter CS represents the colony survival rate of *E. faecalis* aerosols on each agar medium; isolates with higher CS values correspond to species that are more resistant to the stress of air sampling. The following levels of resistance to stress from impaction and filtration have previously been reported (listed in descending order): *Bacillus subtilis* endospores or *Penicillium* spores (CS = 0.15–0.38) > gram-positive *S. aureus* (CS = 0.01–0.1) > gram-negative *Legionella pneumophila* (CS = 0.02) > nonenveloped viruses (CS = 0.01–0.02) > gram-negative *Escherichia coli* (CS = 0.006) (Li and Lin 1999a,b; Li et al. 2003; Tseng and Li 2005; Chang et al., 2010; Hsiao et al. 2012). The CS of gram-positive *E. faecalis* in the present study (CS = 0.6–1.2) was much higher than those reported for *S. aureus* and *E. coli*. *Enterococcus* strains have been reported to survive for at least 1 week and for as long as 4 months on dry surfaces (Wendt et al., 2001). Resistance to dry conditions may not only promote the transmissibility of *Enterococcus* strains but also increase their resistance to sampling stress. Thus, the colony survival rate of a microorganism on an agar medium surface is highly species-dependent. However, collection with the Andersen impactor yielded a higher colony survival rate for airborne *E. faecalis* than that obtained via the filtration process at RHs of 30% and 55%.

4.2. Field Study

The overall average total bacterial concentration in the swine buildings (site *S*) measured in the current study using the culture method was 4.9×10^4 CFU/m^3, in agreement with the findings of previous studies (Chang et al. 2001; Chi and Li 2005). Although CVSE and CVRE are selective differential media for VSE.faes and VRE.faes, other bacterial species can still grow on them. However, only the target colonies are mauve-colored. Unlike CVSE, some antibiotic-related supplements are added to CVRE. Therefore, the low total bacterial concentration measured by CVRE is likely due to the inability of antibiotic-sensitive bacteria to grow on CVRE.

Tables 2 and 3 show that the detected concentrations in the CVSE and CVRE media using the Nuclepore filter sampler were lower than those detected with the Andersen impactor. This result, along with our chamber study (Figure 2), suggests that the loss of *E. faecalis* culturability in air samples collected using a Nuclepore filter may be related to the stress of impaction and dehydration at a moderate RH. In contrast, Figure 3 demonstrates that the dehydration effect of filter sampling was low when sampling was conducted for 60 min at a RH of 55%. However, the dehydration stress test only accounted for the stress caused by air flow, not for stress resulting from the impaction of bacterial particles onto the filter (Wang et al. 2001). The overall

stress from filter sampling should be referred to as the sampling indicator CS. This indicator includes the aerosolization of *E. faecalis* and thus could contain all the stresses (impaction, dehydration, and extraction) that airborne *E. faecalis* may suffer during aerosolization and filter sampling. The microbial species with higher CSs are also likely more resistant to the overall stress caused by aerosolization and air sampling.

During the sampling period, VRE were widespread in the swine building and were present in the air. Although the swine building had the highest total bacterial concentration, the widespread use of antibiotics there (Gordoncillo et al. 2012) may account for the greater concentration of VRE measured at this site. In the absence of antibiotic resistance, VSE.faes are difficult to culture on CVRE medium because the antibiotic-related supplements in CVRE suppress VSE.faes growth. By contrast, both VSE.faes and VRE.faes can grow on CVSE because CVSE cannot distinguish between resistant and sensitive strains of *E. faecalis* and *E. faecium*. In the present study, *E. faecalis* accounted for the majority of airborne VSE.faes isolates, in agreement with previous *Enterococcus* species identifications from clinical isolates (Treitman et al. 2005). However, the concentration of vancomycin-resistant *E. faecium* was higher than that of *E. faecalis*. Evidence suggests that the prevalence of vancomycin-resistant *E. faecium* is increasing and that resistance to vancomycin is far more commonly associated with *E. faecium* than with *E. faecalis* (Suppola et al. 1998).

There are no previous reports of the utilization of CVSE and CVRE to detect *Enterococcus* species in environmental samples. Previous studies using clinical specimens demonstrated that the sensitivity and PPV of CVSE were in the range of 84.2–95.9% (Peltroche-Llacsahuanga et al. 2009; Kallstrom et al. 2010; Stamper et al. 2010), compared with the range of 3.8–59.9% observed in the current study. These differences may be related to variations in the tests used for *Enterococcus* identification. However, the relatively low sensitivity observed in the current study indicates that CVSE is not particularly effective for the recognition of airborne VSE.faes. The low sensitivity of CVSE in field applications is similar to our previous observations for the use of CHROMagar to detect airborne *S. aureus* (71%). The low sensitivity may be related to environmental interference, bacterial enzyme expression, and competition between different bacterial strains (Perez et al. 2003; Diederen et al. 2005).

Bacteria form colored colonies on CHROMagar because the media release colored dyes upon hydrolysis by a specific bacterial enzyme. Environmental interference can inhibit PCR amplification of target bacterial sequences from air samples (Chen et al. 2009). Therefore, some compounds may interfere with the hydrolytic process and suppress mauve color formation on CHROMagar plates. Moreover, previous studies have demonstrated that bacterial enzymatic expression may vary based on the geographical origin of the species (Perez et al. 2003). Some bacterial species recovered from different geographical locations do not express the specific enzyme detected by CHROMagar, decreasing the sensitivity of this medium. Finally, compe-

tition between different microbial species on the same CHROMagar plate may also affect its sensitivity (Perez et al. 2003; Diederen et al. 2005). The specific enzymes expressed by *E. faecalis* and *E. faecium* may interfere with other bacterial species and render CVSE unable to detect VSE.faes.

PPV and NPV are two important indicators that have been used to determine whether the mauve and nonmauve colonies on CHROMagar are VSE.faes or VRE.faes. PPV is directly proportional to the percentage of VSE.faes (i.e., the ratio of VSE.faes to total bacteria) in the air. In our study, the percentage of VSE.faes ranged from 0.4 to 2.2%. Therefore, the low percentage of VSE.faes may be reflected in the low PPV of CVSE (5%). As the percentage of VSE.faes in the air increases, it is more likely that a mauve colony will be VSE.faes rather than a false positive (i.e., a mauve colony that is not VSE.faes). False-positive colonies on CHROMagar are most likely to be *Candida* species, gram-negative rods, *Enterococcus* species (other than *E. faecalis* and *E. faecium*), gram-positive rods, or *Streptococcus* species (Malhotra-Kumar et al. 2008). Although the sensitivity and PPV of CVSE were low, CVSE yielded high specificity and NPV in air sampling (99%). This result suggests that a nonmauve colony recovered using CVSE can be reported as a specimen that is not VSE.faes, while a mauve colony should be further verified.

In addition, our results demonstrated that the sensitivity and specificity of CVRE differ from those of CVSE. Antibiotic supplements, such as vancomycin, are incorporated into CVRE to suppress VSE.faes strains. The selective mixes and antibiotics in CVRE may help to suppress the growth of other bacterial species (particularly vancomycin-sensitive strains) and improve the performance of CVRE in recognizing airborne VRE.faes. The percentage of VRE.faes isolates among the total number of bacteria (0–3.4%) was low in the air. Therefore, the identification of airborne VRE by CVRE also revealed a low PPV (8%). Similar to CVSE, CVRE exhibited a high NPV (100%), suggesting that a nonmauve colony recovered using CVRE in air sampling can be reported as negative, while a mauve colony should be subjected to further identification analyses.

Our study has some limitations. First, vancomycin-sensitive *E. faecalis* was used as a surrogate for VRE during our chamber experiments for safety reasons. However, for antibiotic-resistant bacteria, the altered binding proteins in the cytoplasmic membranes of VRE strains suggest the possibility of changes in their susceptibility to the sampling stress associated with impaction and filtration. Second, our study was only conducted in limited environments. When CVSE or CVRE are applied in other occupational fields, the enzymatic expression and competing effects of different microbial species may vary between different geographical locations. These factors may affect the performance of CHROMagar in detecting airborne VSE.faes or VRE.faes.

5. CONCLUSIONS

The widespread use of antibiotics has resulted in an increase in the number of antibiotic-resistant bacterial strains. There is

an immediate need to develop an environmental monitoring program for the rapid identification of airborne VRE. Based on the similar PPV and NPV results, CVRE may become more valuable than CVSE because CVRE has high sensitivity and specificity. From a health perspective, VRE poses a greater threat to human health than VSE. A high level of sensitivity and specificity in a test is desirable for detecting airborne microbes that cause serious disease. Although the low PPV of CVRE may lead to false-positive results, additional simple tests (such as Gram staining, catalase testing, PYR testing, and bile-esculin testing) can improve the performance of CVRE. In this study, we demonstrated that CVRE could be used in air sampling to isolate VRE.faes without additional susceptibility testing. Therefore, these advantages may enable CVRE to rapidly identify the concentration profiles of airborne VRE.faes in hospital or occupational environments. Although the low PPVs of CVSE and CVRE suggest that a positive result should also be verified using conventional procedures, these media may reduce the detection time of VSE.faes or VRE.faes by 24–48 h, depending on the methods used.

FUNDING

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