Journal of Microbiology, Immunology and Infection (2016) xx, 1–10

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jmii.com

ORIGINAL ARTICLE

Bio-Kil, a nano-based disinfectant, reduces environmental bacterial burden and multidrug-resistant organisms in intensive care units

Wen-Sen Lee [a], Tai-Chin Hsieh [a], Justine C. Shiau [b], Tsong-Yih Ou [a], Fu-Lun Chen [a], Yu-Hsin Liu [a], Muh-Yong Yen [c,*], Po-Ren Hsueh [d,]**

[a] *Division of Infectious Disease, Department of Internal Medicine, Wan Fang Medical Center and School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan*
[b] *Department of Biology, Eberly College of Science, Pennsylvania State University, State College, PA, USA*
[c] *Division of Infectious Disease, Taipei City Hospital and National Yang-Ming University, School of Medicine, Taipei, Taiwan*
[d] *Departments of Laboratory Medicine and Internal Medicine, National Taiwan University Hospital, National Taiwan University College of Medicine, Taipei, Taiwan*

Received 24 December 2015; received in revised form 14 April 2016; accepted 25 April 2016
Available online ■ ■ ■

KEYWORDS
Bio-Kil;
environmental decontamination;
healthcare-associated infections;
multidrug-resistant organisms

Abstract *Background/Purpose:* This prospective before-after study was intended to investigate the effect of Bio-Kil on reducing environmental bacterial burden and healthcare-associated infections (HAIs) in intensive care units (ICUs) at the Municipal Wan-Fang Hospital, Taipei, Taiwan in 2014.
Methods: Four rooms in the medical and surgical ICUs were investigated and designated as study rooms ($n = 2$) or control rooms ($n = 2$). Routine disinfection was performed during the pre-intervention period in both room types. Bio-Kil was applied to the fomites and surroundings of the study rooms during the intervention period. Total bacterial burden and proportion of colonization of fomites and surroundings by multidrug-resistance organisms (MDROs) were determined before and after the intervention. The demographic characteristics, underlying conditions, and clinical outcomes of patients were analyzed.

* Corresponding author. Division of Infectious Disease, Taipei City Hospital and National Yang-Ming University, School of Medicine, Number 10, Section 4, Zen-Ai Road, Taipei 106, Taiwan.
** Corresponding author. Department of Laboratory Medicine, National Taiwan University Hospital, National Taiwan University College of Medicine, Number 7 Chung-Shan South Road, Taipei 100, Taiwan.
E-mail addresses: myyen1121@gmail.com (M.-Y. Yen), hsporen@ntu.edu.tw (P.-R. Hsueh).

http://dx.doi.org/10.1016/j.jmii.2016.04.008

Please cite this article in press as: Lee W-S, et al., Bio-Kil, a nano-based disinfectant, reduces environmental bacterial burden and multidrug-resistant organisms in intensive care units, Journal of Microbiology, Immunology and Infection (2016), http://dx.doi.org/10.1016/j.jmii.2016.04.008

Results: After application of Bio-Kil, the bacterial burden declined in both groups, although the reduction was greater in the study rooms as compared with the control rooms ($p = 0.001$). During the pre-intervention period, 16 patients were admitted to control rooms and 18 patients to study rooms. After the intervention, 22 patients were admitted to control rooms and 21 patients to study rooms. The number of cases of new-onset sepsis declined in the intervention group (from 33% to 23.8%), but increased in the control group (from 25% to 40.9%); however, there was no significant difference in incidence of new-onset sepsis between the study and control rooms after intervention.
Conclusion: Application of Bio-Kil reduced the environmental bacterial burden and MDROs in ICUs. Further studies are needed to evaluate the efficacy of this nanotechnology-based disinfectant in reducing HAIs.

Introduction

There is compelling evidence that contaminated inanimate surfaces are major sources of hospital-acquired infections (HAIs).[1–4] Many important nosocomial pathogens, such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), carbapenem-resistant *Enterobacteriaceae* (CRE), carbapenem-resistant *Pseudomonas aeruginosa* (CRPA), and carbapenem-resistant *Acinetobacter calcoaceticus-baumannii* complex (CRAB), can survive for days to weeks on dry inanimate surfaces.[5] Patients admitted to rooms previously colonized with these pathogens are at increased risk of colonization or infection.[6–8] Additionally, previous research found that the frequency of MRSA contamination was similar for healthcare personnel who either had direct contact with a colonized/infected patient or with contaminated surfaces.[9] Therefore, decontamination and disinfection of hospital environmental surfaces is an important infection-control measure.

Environmental decontamination involves the use of water, detergents, and, increasingly, a disinfectant or microbiocide, although the effectiveness of any agent depends on how it is applied and the meticulousness of the decontamination. A previous study of environmental disinfection of intensive care units (ICUs) indicated that $< 50\%$ of surfaces were cleaned adequately; however, after implementation of an infection-control intervention program, including the use of ultraviolet monitors as surrogate markers for bacterial contamination, $\sim 82\%$ of fomites were adequately disinfected.[10] Other studies revealed that many room surfaces remained inadequately disinfected.[11,12] Several manufacturers have developed room-disinfection units that can decontaminate environmental surfaces and objects by non-touch methods that employ either UV radiation[13] or hydrogen-peroxide vapor.[14] These methods, however, can only be used for terminal disinfection of a discharged room and cannot be used for daily room disinfection.[11,15] Recently, self-disinfecting surfaces were developed to reduce the biological burden on environmental surfaces during hospitalization.[16]

Bio-Kil [3-(Trimethoxysilyl) propyloctadecyldimethyl ammonium chloride; Cargico Group, Taipei, Taiwan) is an antimicrobial nanomaterial consisting of inorganic metal components and organic quaternary ammonium components.[17] Bio-Kil molecules have a high-affinity structure and a strong electric field that attracts pathogens. The strong electrical charge damages the membrane proteins of microorganisms, thereby killing the pathogens. Bio-Kil forms a permanent covalent bond with the surface of many products, including plastic, paint, and textiles.[17] Previous research indicated that use of Bio-Kil in an ICU effectively reduced the bacterial burden in that environment[18,19]; however, the effect of this reduction in bacterial burden on the incidence of HAIs has not yet been documented. Therefore, we performed this prospective before–after study to determine the efficacy of Bio-Kil on HAIs and bacterial colonization in ICUs.

Methods

Study design and setting

This prospective, open-label, before-after study with control and intervention groups was conducted in the Medical and Surgical ICUs (MICUs and SICUs) of the Municipal Wan-Fang Hospital, a 750-bed teaching hospital in Taipei, Taiwan, from May 2014 to October 2014. Two rooms each from the MICU (MICU-15 and MICU-16) and SICU (SICU-21 and SICU-22) were selected for study. MICU-16 and SICU-22 were designated as the study rooms, and MICU-15 and SICU-21 served as the control rooms. These rooms were selected for the study, because they were situated at the far end of the ICU and were separated from the other rooms. The primary objective of this study was to assess the efficacy of Bio-Kil in reducing bacterial burden in ICUs. The secondary objective was to evaluate whether reducing bacterial burden in patient surroundings prevented nosocomial infections. The study protocol was approved by the Taipei Medical University-Joint Institutional Review Board (study protocol TMU-JIRB 201311029).

The three periods of this study were the pre-intervention period (from May 1, 2014 to July 22, 2014), the Bio-Kil-setup period (from July 29, 2014 to August 4, 2014), and the intervention period (from August 4, 2014 to October 20, 2014). During the pre-intervention period, environmental samples were collected from 17 sites from each study area and from patients twice weekly. During the

Please cite this article in press as: Lee W-S, et al., Bio-Kil, a nano-based disinfectant, reduces environmental bacterial burden and multidrug-resistant organisms in intensive care units, Journal of Microbiology, Immunology and Infection (2016), http://dx.doi.org/10.1016/j.jmii.2016.04.008

Bio-Kil-setup period, the study rooms, including fomites and their surroundings, were disinfected with Bio-Kil. The room walls, ceilings, and air-conditioning filters were also treated with Bio-Kil, and the surfaces of instruments (mechanical ventilators, telephones, and computer key pads) were covered with Bio-Kil-embedded silicon pads. During this period, no specimen collection was performed.

During the intervention period, textiles, including pillow cases, bed sheets, and mattresses, were replaced with textiles that had been washed in a solution of Bio-Kil as previously described.[19] The nurses assigned to care for patients in the study rooms also wore clothing embedded with Bio-Kil. Specimen collection followed the same methods employed during the pre-intervention period.

Routine infection-control measures, environmental decontamination, textile washing, and replacement

During the pre-intervention and intervention periods, textile washing and replacement, infection-control practices of nurses, physicians, and visitors, and disinfection of environments and instruments in all ICUs were performed according to hospital regulations. Daily disinfection of rooms in the ICUs comprised the application of a 500-ppm solution of sodium hypochlorite for routine cleaning. Based on the status of environmental contamination found during the 1st week of the pre-intervention period and for ethical considerations, the frequency of changing pillow cases increased from every other day to daily from the 2nd week to the end of this study period (including pre-intervention and intervention periods). Additionally, the cleaners who performed the routine disinfection measures in the control and study rooms were trained in more stringent cleaning practices and to focus on areas that had been shown in the pre-intervention period as having a high degree of bacterial burden. Otherwise, the frequency of cleaning and changing of textiles was the same in all study rooms in the pre-intervention and intervention periods. All staff members were monitored for adherence to hand-hygiene practices in both ICUs throughout the study period.

Specimen collection, bacterial identification, and susceptibility testing

Specimens for microbiological isolation were collected twice weekly (Monday and Thursday) from the anterior nares and rectum of patients, high-contact areas in patient environments (Figure 1), and from nurse uniforms. We collected the specimens from swab cultures of fomites, surroundings, and healthcare-related sites after the daily routine cleaning work throughout both study periods. Samples were collected from 17 sites from each study area (control and study), and all specimens were recorded separately. For analytical purposes, these collection sites were classified as: (1) fomites if they were in direct contact with the patient (pillow cases, bed sheets, mattresses, and bed rails); (2) patient surroundings if they had no direct contact with patients (walls surrounding patient beds, curtains, electrocardiogram monitors, dining tables, cupboards, and chart covers); or (3) healthcare worker-related sites (surfaces of working stations, telephone handsets, computer keyboards, and sleeves and fronts of nurse uniforms).

Moistened, sterile, cotton swabs were used to evenly wipe a designated square (10 cm $\times$ 10 cm) on each collection site. Samples were then inoculated into trypticase soy agar and inoculated at 35°C under 5% CO_2 conditions. After 48 hours, colony forming units (CFUs/100 cm^2) were determined. Bacterial species identification and antimicrobial susceptibility testing were performed using the Becton Dickinson Phoenix System (Becton Dickinson Diagnostics, Sparks, MD, USA). Antimicrobial susceptibility results were interpreted according to Clinical and Laboratory Standards Institute guidelines.[20] We compared mean bacterial counts from specimens before and after application of Bio-Kil in the intervention group and during the pre-intervention and intervention periods in the control group. In this study, multidrug-resistant organisms (MDROs), including MRSA, VRE, CRE, CRPA, and CRAB, and the incidence of MDROs from each collection were determined.

Patient characteristics

For all patients admitted to the assigned rooms, demographic characteristics, underlying conditions, Acute Physiology and Chronic Health Evaluation II (APACHE II) score within 24 hours of admission, microorganisms isolated from all clinical specimens, and clinical outcomes were determined. A patient was defined as colonized with a MDRO if any MDRO was isolated from a clinical specimen within 3 months prior to admission to the assigned room.

Inclusion criteria and outcome definitions

Only patients who resided in an assigned room for $>$ 24 hours were included in the analysis. New-onset sepsis was defined as the development of sepsis after 48 hours of admission to assigned rooms or within 48 hours of leaving those rooms.[21] The 30-day mortality rate was defined as overall mortality in the 30 days after admission to the assigned room. Patients were assessed for development of any allergic reaction (skin rash or erythema on the next day) related to Bio-Kil, such as contact dermatitis or related respiratory reactions.

Statistical analysis

We used the Chi-square test or Fisher's exact test for categorical comparisons of data. Differences in means of continuous variables were tested by the independent *t* test. Changes in bacterial colony counts and incidence of new-onset sepsis in the pre- and post-intervention periods were determined by a two-proportion *z* test. Binary logistic regression analysis was used to identify factors associated with the development of new-onset sepsis and 30-day mortality. All significant variables from the univariate analyses were included in a multivariate logistic regression model for calculation of odds ratios (ORs). A $p < 0.05$ was considered statistically significant, and all tests were two-tailed. All statistical analyses were performed with the SPSS version 21 (IBM Corp., Armonk, NY, USA).

Please cite this article in press as: Lee W-S, et al., Bio-Kil, a nano-based disinfectant, reduces environmental bacterial burden and multidrug-resistant organisms in intensive care units, Journal of Microbiology, Immunology and Infection (2016), http://dx.doi.org/10.1016/j.jmii.2016.04.008

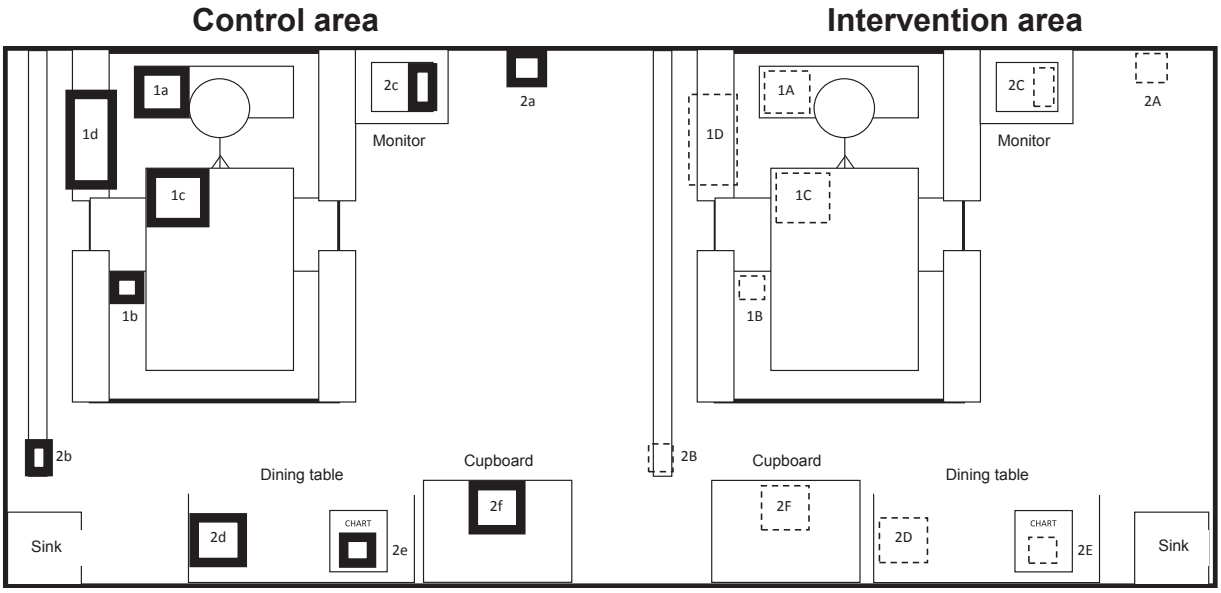

Figure 1. Schematic of patient areas and sites of specimen collection. Fomites (inanimate objects in direct contact with patients): 1a/1A, pillowcase; 1b/1B, bed sheet; 1c/1C, mattress; and 1d/1D, bed rails. Patient surroundings (inanimate objects with no direct patient contact): 2a/2A, wall surrounding patient bed; 2b/2B, curtains; 2c/2C, electrocardiogram monitor; 2d/2D, dining table; 2e/2E, patient chart cover; and 2f/2F, bedside cupboard.

Results

Counts of bacteria and MDROs before and after intervention

We collected specimens 41 times during the pre-intervention period (20 from MICUs and 21 from SICUs) and 47 times during the intervention period (24 from MICUs and 23 from SICUs). A total of 697 samples collected during the pre-intervention period and 799 samples collected during the intervention period were analyzed according to the sites from which they had been obtained: fomites, surroundings (Table 1), and healthcare worker-related sites (Figure 2). Mean CFU/100 cm^2 and proportion of isolation of MDROs from each sample collection are summarized in Table 1.

In the control and study rooms, the mean bacterial colony counts from samples isolated from fomites were significantly greater than those isolated from the surroundings during each period. After the intervention, the average colony counts from samples taken from fomites declined similarly in control rooms (78.3%) and study rooms (75.3%); however, during the intervention period, bacterial counts in the surroundings of the study rooms were significantly lower than those observed in the surroundings of the control rooms (80.5% vs. 3.3%, $p = 0.001$; Figure 3).

Relative to the control rooms, the study rooms also had greater declines in overall MDROs isolated from fomites (45.8% vs. 34.6%, $p = 0.002$) and from the surroundings (83.1% vs. 65.1%, $p = 0.004$) after application of Bio-Kil (Figure 4). However, analysis of MRSA, VRE, and CRAB colonies did not reveal a significant decline, most likely because of the limited case numbers in the study. There was only one isolate of CRPA during the pre-intervention period and one isolate of CRE during the post-intervention period (data not shown) (see Figure 5).

Patient characteristics

Table 2 shows the demographic characteristics, underlying conditions, and clinical outcomes of all patients. During the pre-intervention period, 16 patients were admitted to control rooms, and 18 patients were admitted to study rooms. During this period, control patients were more likely to be on ventilator support as compared with intervention patients (68.8% vs. 33.3%, $p = 0.039$), but less likely to suffer from heart disease (18.8% vs. 72.2%, $p = 0.002$). There were no significant differences in disease severity on admission (APACHE II score) or in the prevalence of previous bacterial colonization between the two groups. After the intervention, 22 patients were admitted to control rooms and 21 patients were admitted to study rooms. Patients admitted to the study rooms had higher mean APACHE II scores on admission (20 vs. 14, $p = 0.004$) and were more likely to have cancer (33.3% vs. 0%, $p = 0.033$).

Clinical outcomes

During the pre-intervention period, the incidence of new-onset sepsis was higher in the study rooms as compared with the control rooms (33.3% vs. 25%); however, during the intervention period, the incidence of new-onset sepsis decreased in the study rooms and increased in the control rooms (from 33% to 23.8% vs. from 25% to 40.9%, $p = 0.001$). Interestingly, when the incidence of new-onset sepsis was compared between the control and study rooms, we found that there was no significant difference in proportion between the two groups (40.9% vs. 23.8%, $p = 0.232$). Possible infection foci and microorganisms isolated from patients with new-onset sepsis are summarized in Table 2. The most common infection focus in patients with new-onset sepsis was the respiratory tract, and

Please cite this article in press as: Lee W-S, et al., Bio-Kil, a nano-based disinfectant, reduces environmental bacterial burden and multidrug-resistant organisms in intensive care units, Journal of Microbiology, Immunology and Infection (2016), http://dx.doi.org/10.1016/j.jmii.2016.04.008

Table 1 Bacterial colony counts and MDROs isolated during the pre-intervention and intervention periods in control and study beds.

	Pre-intervention period				Intervention period				Percentage change		p[a]
	Control rooms		Study rooms		Control rooms		Study rooms		Control rooms	Study rooms	
	Mean bacterial colony count (CFU/100 cm^2) in each specimen collection[b]										
	Specimen collection (n)	Mean CFUs/100 cm^2	Specimen collection (n)	Mean CFUs/100 cm^2	Specimen collection (n)	Mean CFUs/100 cm^2	Specimen collection (n)	Mean CFUs/100 cm^2			
Fomites	41	11098	41	12962	47	2409	47	3208	−78.3	−75.3	0.001
Surroundings	41	1835	41	3252	47	1775	47	635	−3.3	−80.5	0.001
	Incidence of MDROs (percentage of total specimens)[c]										
	Specimen collection (n)	MDRO incidence (%)	Specimen collection (n)	MDRO incidence (%)	Specimen collection (n)	MDRO incidence (%)	Specimen collection (n)	MDRO incidence (%)			
Fomites											
All MDROs[d]	41	19.5	41	19.5	47	12.8	47	10.6	−34.6	−45.8	0.002
MRSA	41	9.8	41	2.4	47	2.1	47	0	−78.2	−100.0	0.261
VRE	41	4.9	41	9.8	47	10.6	47	4.2	118.1	−56.6	0.261
CRAB	41	9.8	41	9.8	47	0	47	6.4	−100.0	−34.9	0.135
Patient surroundings											
All MDROs	41	12.2	41	12.1	47	4.3	47	2.1	−65.1	−83.1	0.004
MRSA	41	4.9	41	4.9	47	0	47	0	−100.0	−100.0	0.333
VRE	41	7.3	41	2.4	47	0	47	0	−100.0	−100.0	0.135
CRAB	41	2 .4	41	2.4	47	4.3	47	2.1	74.5	−57.9	0.135

[a] Values based on the two-proportion z test; $p < 0.05$ was considered significant.
[b] Mean bacterial colonies in each specimen collection in CFU/100 cm^2.
[c] MDROs include MRSA, VRE, CRE, CRPA, and CRAB.
[d] If one single type of MDRO was isolated at the same time from different specimens of the same patient, the incidence was calculated as one.

CFU = colony forming unit; CRAB = carbapenem-resistant *Acinetobacter calcoaceticus baumannii* complex; CRE = carbapenem-resistant *Enterobacteriaceae*; CRPA = carbapenem-resistant *Pseudomonas aeruginosa*; MDRO = multidrug-resistance organisms; MRSA = methicillin-resistant *Staphylococcus aureus*; VRE = vancomycin-resistant enterococci.

Please cite this article in press as: Lee W-S, et al., Bio-Kil, a nano-based disinfectant, reduces environmental bacterial burden and multidrug-resistant organisms in intensive care units, Journal of Microbiology, Immunology and Infection (2016), http://dx.doi.org/10.1016/j.jmii.2016.04.008

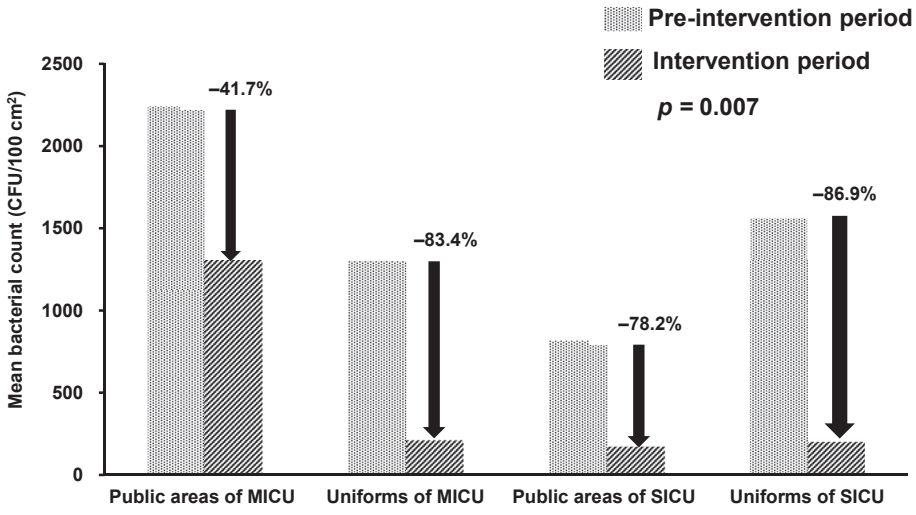

Figure 2. Mean bacterial colony counts (CFUs/100 cm^2) in different locations of the MICU and the SICU before and after the intervention. Public areas: surfaces of workstations, telephone handsets, and computer keyboards. Uniforms: sleeves and fronts of nurse uniforms. CFU = colony forming unit; MICU = medical intensive care unit; SICU = surgical intensive care unit.

the majority of cases of sepsis in both groups were due to *Enterobacteriaceae* (60% in the intervention group and 44% in the control group) (see Figure 6).

Safety and side effects

There were no cases of contact dermatitis or other adverse effects during the study period. Additionally, none of the nurses who wore clothes treated with Bio-Kil reported any discomfort.

Discussion

The application of Bio-Kil resulted in a self-disinfecting surface with broad-spectrum antimicrobial activity that produced little or no toxicity to humans.[16] The efficacy of these self-disinfecting surfaces in the prevention of HAIs has not yet been proven. To the best of our knowledge, the present study is the first to evaluate the clinical outcomes of patients who reside in ICU rooms that have been treated with Bio-Kil.

We found that total bacterial counts in samples from fomites were ~80- to 100-fold higher than those observed in samples taken from the surroundings. Numerous studies evaluated environmental contamination by MDROs,[1,3,4,7,22,23] but most focused on contamination of frequently contacted surfaces in hospital environments. Fomites play an important role in transmission of microorganism-related diseases.[24] Textiles and clothing that are in direct contact with patients can harbor large numbers of bacteria and can be a source of hospital

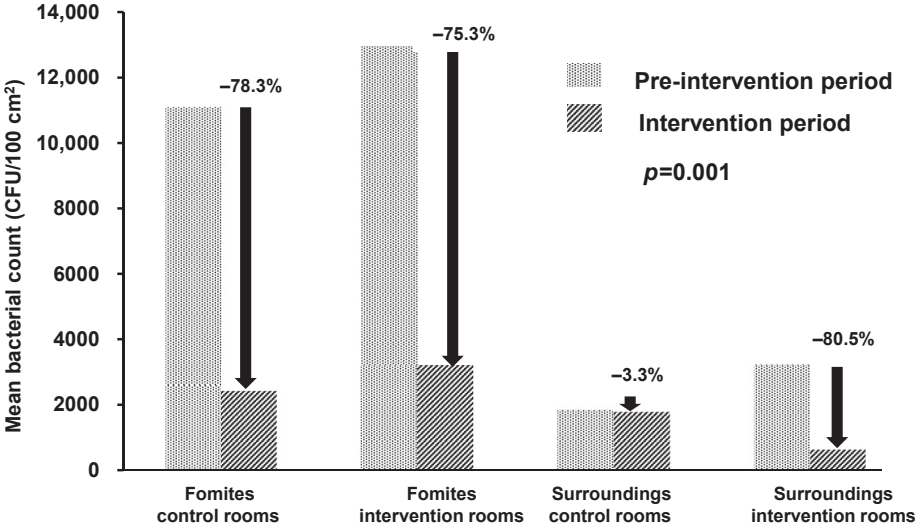

Figure 3. Mean bacterial colony counts (CFUs/100 cm^2) in samples taken from fomites and patient surroundings before and after the intervention. CFU = colony forming unit.

Please cite this article in press as: Lee W-S, et al., Bio-Kil, a nano-based disinfectant, reduces environmental bacterial burden and multidrug-resistant organisms in intensive care units, Journal of Microbiology, Immunology and Infection (2016), http://dx.doi.org/10.1016/j.jmii.2016.04.008

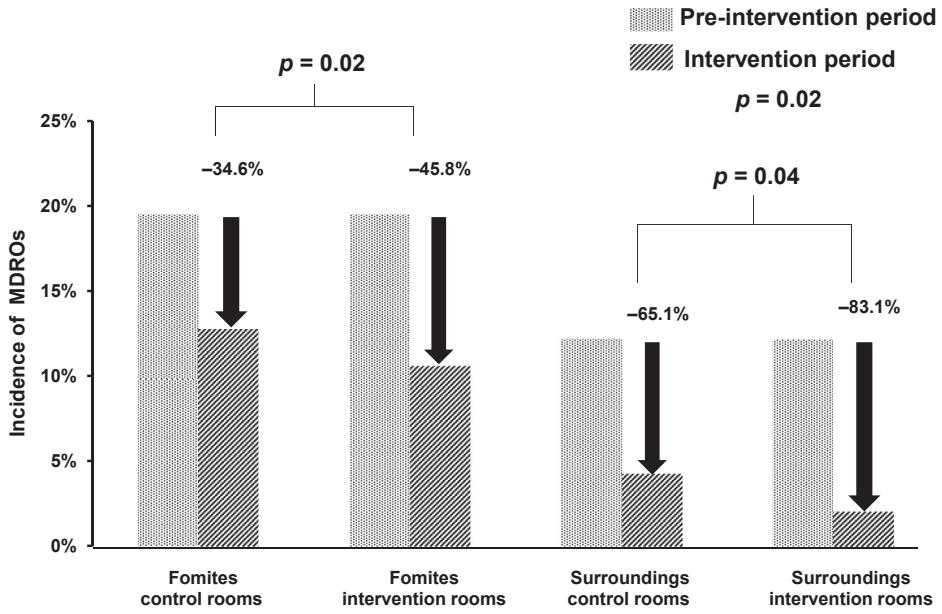

Figure 4. Incidence of MDROs in samples taken from fomites and patient surroundings before and after the intervention. MDROs include MRSA, VRE, CRAB, CRE, and CRPA. MDRO incidence refers to the percentage of specimens that were MDRO. CRAB = carbapenem-resistant *Acinetobacter calcoaceticus baumannii* complex; CRE = carbapenem-resistant Enterobacteriaceae; CRPA = carbapenem-resistant *Pseudomonas aeruginosa*; MRDO = multidrug-resistant organisms; MRSA = methicillin resistant *Staphylococcus aureus*; VRE = vancomycin resistant enterococci.

contamination. We found that fomites and textiles coated with Bio-kil were less likely to be a source of bacterial growth as compared with untreated surfaces, thereby reducing the risk of fomite transmission of infection in the ICU setting (Figure 2).

Our findings confirmed those in previous reports that Bio-Kil reduces bacterial colonization of patient environments.[18,19] An interesting finding was that bacterial colony counts declined in study rooms and in control rooms. The fact that the frequency of changing pillowcases was increased from every other day to daily after the pre-intervention period, and that the cleaners who performed the routine disinfection measures in the control and study rooms were retrained to focus on areas shown during the pre-intervention period to have a high degree of bacterial

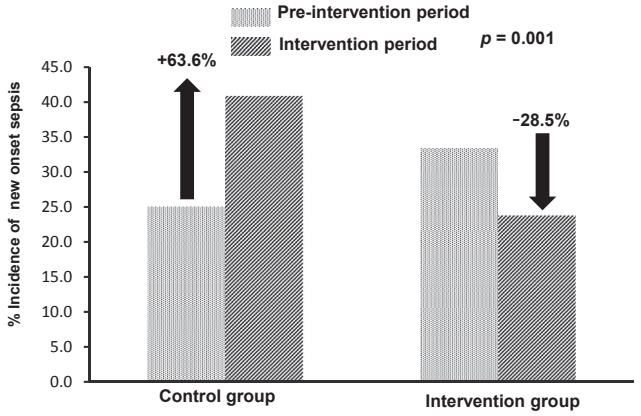

Figure 5. Incidence of new-onset sepsis before and after intervention.

burden during the intervention period most likely contributed to this specific finding. Nonetheless, the number of colony counts in samples taken from patient surroundings after the application of Bio-Kil was significantly lower as compared with samples taken from control rooms (Figure 3). We proposed that the fomites were more affected by vigilant disinfection as compared with the surroundings, because they were not as close to the patient bedside as fomites. These results indicated that disinfection with Bio-Kil resulted in markedly lower bacterial burden as compared with stringent manual surface cleaning in ICUs.

The National Health Insurance (NHI) system in Taiwan covers a wide range of out- and inpatient services and allows people in Taiwan to have access to comprehensive medical care. However, the introduction of the NHI system has increased the numbers of bedridden patients and the long-term use of invasive medical devices,[25] which has, in turn, led to increases in rates of HAIs caused by MDROs, especially CRAB, CRE, and VRE.[26] Moreover, the increase in average life expectancy and the growth of the elderly population in Taiwan have led to an increased number of people living in long-term care facilities,[25] which can be reservoirs of MDROs. A previous study in Chicago, USA reported a higher prevalence of *Klebsiella pneumoniae* carbapenemase-producing *Enterobacteriaceae* in long-term care hospitals relative to short-stay ICUs.[27] In Taiwan, the incidence of community acquired infections due to pathogens once classified as nosocomial has also increased recently.[28,29] In our study, ~15–30% of patients were colonized with an MDRO at admission. Ake et al[30] observed that an initially clean and new trauma-surgical unit quickly became contaminated by MDROs. The researchers used

Please cite this article in press as: Lee W-S, et al., Bio-Kil, a nano-based disinfectant, reduces environmental bacterial burden and multidrug-resistant organisms in intensive care units, Journal of Microbiology, Immunology and Infection (2016), http://dx.doi.org/10.1016/j.jmii.2016.04.008

Table 2 Demographic factors, underlying conditions, and clinical outcomes of enrolled patients.

	Pre-intervention			Intervention		
	Control rooms (n = 16)	Study rooms (n = 18)	p	Control rooms (n = 22)	Study rooms (n = 21)	p
Male	7 (43.8)	10 (55.6)	0.492	11 (50.0)	11 (52.4)	0.876
Age in years	70 (±15)	77 (±17)	0.220	65 (±17.2)	73 (±14.2)	0.121
APACHE II score	20 (±8)	21 (±8)	0.752	14 (±6.6)	20 (±6.6)	0.004
Length of ICU stay	10.8 (±10.3)	9.2 (±7.7)	0.624	22 (±4.9)	21 (±6.1)	0.578
Underlying condition						
Mechanical ventilation	11 (68.8)	6 (33.3)	0.039	10 (45.5)	13 (61.9)	0.280
Bed-ridden status	7 (43.8)	5 (27.8)	0.331	7 (31.8)	9 (42.9)	0.454
DM	5 (31.3)	5 (27.8)	0.824	3 (13.6)	6 (28.6)	0.229
ESRD	3 (18.8)	3 (16.7)	0.874	5 (22.7)	5 (23.8)	0.933
Cirrhosis	1 (6.3)	1 (5.6)	0.932	0 (0.0)	3 (14.3)	0.066
Cancer	5 (31.3)	2 (11.1)	0.147	0 (0.0)	7 (33.3)	0.003
Heart disease	3 (18.8)	13 (72.2)	0.002	11 (50.0)	8 (38.1)	0.432
COPD	5 (31.3)	4 (22.2)	0.551	1 (4.5)	5 (23.8)	0.068
Previous microbiological results						
Previous colonization with MDROs	5 (31.3)	2 (11.1)	0.147	6 (27.3)	4 (19.1)	0.523
Types of MDROs colonized[a]			0.136			0.244
CRAB	2 (40.0)	2 (100.0)		4 (67.7)	2 (50.0)	
MRSA	1 (20.0)	0		0	1 (25.0)	
CRPA	1 (20.0)	0		1 (16.7)	0	
VRE	1 (20.0)	0		1 (16.7)	1 (25.0)	
CRE	0	0		0	1 (25.0)	
Sepsis at admission	10 (62.5)	6 (33.3)	.089	11 (50.0)	10 (47.6)	0.876
Healthcare-associated infection	5 (31.3)	4 (22.2)	0.551	7 (31.8)	7 (33.3)	0.916
Antibiotic use at the time of study	13 (81.3)	13 (72.2)	0.387	16(72.7)	14 (70.0)	0.845
Clinical outcomes						
New-onset sepsis[b]	4 (25.0)	6 (33.3)	0.595	9 (40.9)	5 (23.8)	0.232
Sites of infection			0.335			0.713
Respiratory tract	3 (75.0)	5 (83.3)		6 (66.7)	4 (80.0)	
Urinary tract	1 (0.0)	0		1 (11.1)	1 (20.0)	
Wound infection	0	0		2 (22.2)	0	
Bloodstream infection	0	1 (16.7)		0	0	
Colonized MDROs related sepsis[c]	2 (50.0)	1 (16.7)	0.260	1 (22.1)	1 (20.0)	0.923
Pathogens isolated from patients with new-onset sepsis			0.350			0.286
Acinetobacter species	1 (25.0)	1 (16.7)		3 (33.3)	0	
Stenotrophomonas species	0	1 (16.7)		1 (11.1)	0	
Pseudomonas aeruginosa	1 (25.0)	0		1 (11.1)	0	
MRSA	1 (25.0)	0		0	1 (20.0)	
Enterococcus species	1 (25.0)	0		0	0	
Enterobacteriaceae	0	2 (33.4)		4 (44.4)	3 (60.0)	
No organisms isolated	0	2 (33.4)		0	1 (20.0)	
30-d mortality[d]	4 (25.0)	6 (33.3)	0.595	5 (22.7)	6 (28.6)	0.661

[a] Includes types of MDROs isolated from colonized patients within 3 months before admission to the study bed. One patient (Patient A) was colonized with both MRSA and CRAB.
[b] Indicates that sepsis developed 48 hours after admission to or within 48 hours of leaving the study bed.
[c] Indicates percentage of new-onset sepsis caused by the same MDROs colonizing the patient.
[d] Indicates crude mortality after admission to assigned beds.
Data are presented as n (%) or mean (±SD).
APACHE II = Acute Physiology and Chronic Health Evaluation II; COPD = chronic obstructive pulmonary disease; CRAB = carbapenem-resistant *Acinetobacter calcoaceticus baumannii* complex; CRE = carbapenem-resistant Enterobacteriaceae; CRPA = carbapenem-resistant *Pseudomonas aeruginosa*; DM = diabetes mellitus; ESRD = end-stage renal disease; ICU = intensive care unit; MDRO = multidrug-resistant organisms; MRSA = methicillin-resistant *Staphylococcus aureus*; VRE = vancomycin-resistant enterococci.

Please cite this article in press as: Lee W-S, et al., Bio-Kil, a nano-based disinfectant, reduces environmental bacterial burden and multidrug-resistant organisms in intensive care units, Journal of Microbiology, Immunology and Infection (2016), http://dx.doi.org/10.1016/j.jmii.2016.04.008

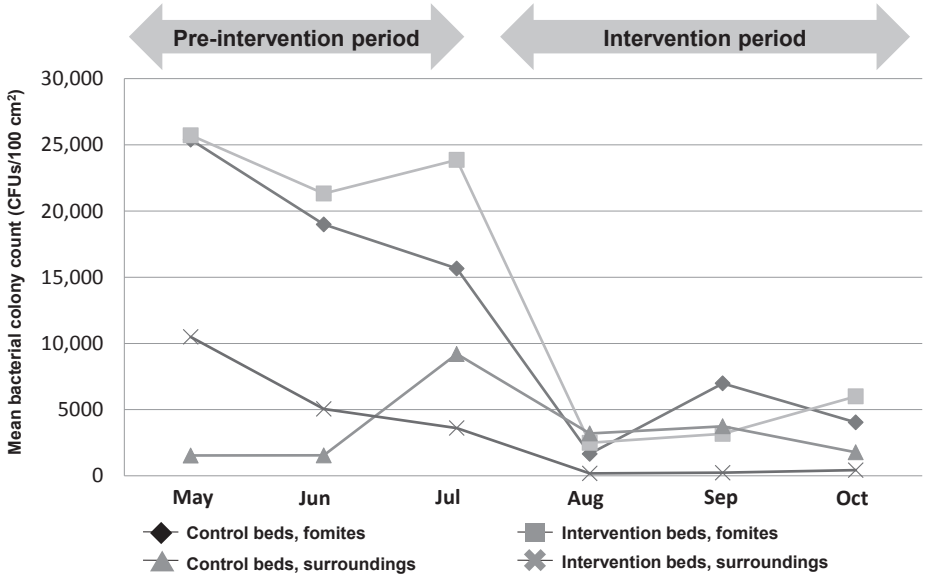

Figure 6. Changes in mean bacterial colony counts (CFUs/100 cm^2) over time during the pre-intervention period (April 1 to July 15) and the intervention period (July 22 to October 31). CFU = colony forming unit.

molecular techniques to link these MDROs to patients colonized by MDROs from the community.[30] Thus, MDROs can spread from a hospital to the community and from the community to a hospital. Previous studies showed that patients colonized or infected with an MDRO can serve as sources of environmental contamination in hospitals.[8,31–33] Surfaces and textiles that have been coated with Bio-Kil can reduce the likelihood of contamination of the hospital environment, especially when they are used or worn by heavily colonized patients. Reduction of the bacterial burden in one area of a hospital environment can reduce the total bacterial burden by interrupting the chain of transmission. Future studies should be conducted to assess the benefit of these self-disinfection surfaces when present at the time of admission of patients from communities with a high prevalence of MDROs.

Finally, we found that the incidence of new-onset sepsis was markedly, although not statistically, lower in the study rooms, despite the fact that patients admitted to study rooms were significantly sicker (higher APACHE II scores at admission) and significantly older. Previous research indicated that the sources of pathogens causing HAIs in the ICU were patient endogenous flora (40–60%), cross-infection via the hands of personnel (20–40%), antibiotic-driven changes in flora (20–25%), and other sources, including contamination of the environment (20%).[34] Nevertheless, bacterial colonization is still an important step in the development of infection in susceptible hosts. Even though Bio-Kil effectively reduced the bacterial burden and proportion of MDROs in ICU surroundings, it did not significantly reduce the incidence of HAIs in our study.

There were several limitations in this study. First, this was a single-center study involving a small sample size of patients. Second, patients were also followed-up with for a relatively short duration. Third, although the frequency of changing pillowcases was increased from every other day to daily beginning the 2nd week of the pre-intervention period, the duration of the pre-intervention period (83 days; changes every other day) became 76 days (changes every day), and the intervention period was also 77 days (no change). The study background and duration of both periods (76 vs. 77) was nearly as the same. The clinical statistical analysis of both groups was not affected significantly. Decontamination and disinfection of hospital environmental surfaces is an important infection-control measure. During the intervention period, the incidence of new-onset sepsis decreased in the study rooms and increased in the control rooms (from 33% to 23.8% vs. from 25% to 40.9%; $p = 0.001$). Nevertheless, to the best of our knowledge, this is the first study evaluating the potential use of a nano-based environmental disinfectant product in the prevention of HAIs.

In conclusion, application of Bio-Kil reduced the environmental bacterial burden in ICUs, but did not reduce the incidence of HAIs. Proven hospital control measures, such as tighter protocols for sterilization of fomites and strict adherence to hand-washing protocols, should be used to reduce environmental colonization by MDROs.

Conflicts of interest

None declared.

Acknowledgments

This study was financially supported by a grant (10301-62-027) from the Department of Health, Taipei City Government. This work was also supported in part by Cargico Group, Taiwan. Cargico Group had no influence on the design or analysis of the study. We thank Miss Wei-Wen Chen for her assistance in statistical analysis.

Please cite this article in press as: Lee W-S, et al., Bio-Kil, a nano-based disinfectant, reduces environmental bacterial burden and multidrug-resistant organisms in intensive care units, Journal of Microbiology, Immunology and Infection (2016), http://dx.doi.org/10.1016/j.jmii.2016.04.008

References

1. Boyce JM. Environmental contamination makes an important contribution to hospital infection. *J Hosp Infect* 2007;**65**:50—4.
2. Dettenkofer M, Spencer RC. Importance of environmental decontamination-a critical view. *J Hosp Infect* 2007;**65**:55—7.
3. Weber DJ, Anderson D, Rutala WA. The role of the surface environment in healthcare-associated infections. *Curr Opin Infect Dis* 2013;**26**:338—44.
4. Otter JA, Yezli S, Salkeld JA, French GL. Evidence that contaminated surfaces contribute to the transmission of hospital pathogens and an overview of strategies to address contaminated surfaces in hospital settings. *Am J Infect Control* 2013;**41**:S6—11.
5. Kramer A, Schwebke I, Kampf G. How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. *BMC Infect Dis* 2006;**6**:130.
6. Drees M, Snydman DR, Schmid CH, Barefoot L, Hansjosten K, Vue PM, et al. Prior environmental contamination increases the risk of acquisition of vancomycin-resistant enterococci. *Clin Infect Dis* 2008;**46**:678—85.
7. Nseir S, Blazejewski C, Lubret R, Wallet F, Courcol R, Durocher A. Risk of acquiring multidrug-resistant Gram-negative bacilli from prior room occupants in the intensive care unit. *Clin Microbiol Infect* 2011;**17**:1201—8.
8. Huang SS, Datta R, Platt R. Risk of acquiring antibiotic-resistant bacteria from prior room occupants. *Arch Intern Med* 2006;**166**:1945—51.
9. Stiefel U, Cadnum JL, Eckstein BC, Guerrero DM, Tima MA, Donskey CJ. Contamination of hands with methicillin-resistant *Staphylococcus aureus* after contact with environmental surfaces and after contact with the skin of colonized patients. *Infect Control Hosp Epidemiol* 2011;**32**:185—7.
10. Carling PC, Parry MF, Bruno-Murtha LA, Dick B. Improving environmental hygiene in 27 intensive care units to decrease multidrug-resistant bacterial transmission. *Crit Care Med* 2010;**38**:1054—9.
11. Carling PC, Parry MF, Von Beheren SM. Identifying opportunities to enhance environmental cleaning in 23 acute care hospitals. *Infect Control Hosp Epidemiol* 2008;**29**:1—7.
12. Goodman ER, Platt R, Bass R, Onderdonk AB, Yokoe DS, Huang SS. Impact of an environmental cleaning intervention on the presence of methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci on surfaces in intensive care unit rooms. *Infect Control Hosp Epidemiol* 2008;**29**:593—9.
13. Boyce JM, Havill NL, Moore BA. Terminal decontamination of patient rooms using an automated mobile UV light unit. *Infect Control Hosp Epidemiol* 2011;**32**:737—42.
14. Passaretti CL, Otter JA, Reich NG, Myers J, Shepard J, Ross T, et al. An evaluation of environmental decontamination with hydrogen peroxide vapor for reducing the risk of patient acquisition of multidrug-resistant organisms. *Clin Infect Dis* 2013;**56**:27—35.
15. Rutala WA, Weber DJ. Disinfectants used for environmental disinfection and new room decontamination technology. *Am J Infect Control* 2013;**41**:S36—41.
16. Weber DJ, Rutala WA. Self-disinfecting surfaces: review of current methodologies and future prospects. *Am J Infect Control* 2013;**41**:S31—5.
17. Cargico. Bio-Kil technology catalogs. Available from: http://www.cargico.com. [Accessed 11 January 2015].
18. Chen YL, Yeh MY, Huang SY, Liu CM, Sun CC, Lu HF, et al. Feasibility study for epidemic prevention and control in a regional hospital. *Mol Med Rep* 2012;**5**:859—65.
19. Hsueh PR, Huang HC, Young TG, Su CY, Liu CS, Yen MY. Bacteria killing nanotechnology Bio-Kil® technology effectively reduces bacterial burden in intensive care units. *Eur J Clin Microbiol Infect Dis* 2014;**33**:591—7.
20. Clinical and Laboratory Standard Institute (CLSI). *Performance standard for antimicrobial suspectibility testing; twenty fourth informational supplement*. Wayne (PA): CLSI; 2014. M100-S24.
21. Dellinger RP, Levy MM, Rhodes A, Annane D, Gerlach H, Opal SM, et al. Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock: 2012. *Intensive Care Med* 2013;**39**:165—228.
22. Havill NL. Best practices in disinfection of noncritical surfaces in the health care setting: creating a bundle for success. *Am J Infect Control* 2013;**41**:S26—30.
23. Sydnor ER, Perl TM. Hospital epidemiology and infection control in acute-care settings. *Clin Microbiol Rev* 2011;**24**:141—73.
24. Boone SA, Gerba CP. Significance of fomites in the spread of respiratory and enteric viral disease. *Appl Environ Microbiol* 2007;**73**:1687—96.
25. Tseng SH, Chien LJ, Chang FY. National action plan to eliminate central line-associated bloodstream infections in Taiwan. *J Microbiol Immunol Infect* 2014;**47**:265—7.
26. Tseng SH, Ke YF, Chang FY. National action plan to combat antimicrobial resistance in Taiwan. *J Microbiol Immunol Infect* 2014;**47**:167—70.
27. Lin MY, Lyles-Banks RD, Lolans K, Hines DW, Spear JB, Petrak R, et al. The importance of long-term acute care hospitals in the regional epidemiology of *Klebsiella pneumoniae* carbapenemase-producing Enterobacteriaceae. *Clin Infect Dis* 2013;**57**:1246—52.
28. Fan NC, Chen HH, Chen CL, Ou LS, Lin TY, Tsai MH, et al. Rise of community-onset urinary tract infection caused by extended-spectrum beta-lactamase-producing *Escherichia coli* in children. *J Microbiol Immunol Infect* 2014;**47**:399—405.
29. Chang YT, Lin CY, Lu PL, Lai CC, Chen TC, Chen CY, et al. *Stenotrophomonas maltophilia* bloodstream infection: comparison between community-onset and hospital-acquired infections. *J Microbiol Immunol Infect* 2014;**47**:28—35.
30. Ake J, Scott P, Wortmann G, Huang XZ, Barber M, Wang Z, et al. Gram-negative multidrug-resistant organism colonization in a US military healthcare facility in Iraq. *Infect Control Hosp Epidemiol* 2011;**32**:545—52.
31. Hayden MK, Blom DW, Lyle EA, Moore CG, Weinstein RA. Risk of hand or glove contamination after contact with patients colonized with vancomycin-resistant enterococcus or the colonized patients' environment. *Infect Control Hosp Epidemiol* 2008;**29**:149—54.
32. Manian FA, Griesenauer S, Senkel D, Setzer JM, Doll SA, Perry AM, et al. Isolation of *Acinetobacter baumannii* complex and methicillin-resistant *Staphylococcus aureus* from hospital rooms following terminal cleaning and disinfection: can we do better? *Infect Control Hosp Epidemiol* 2011;**32**:667—72.
33. Vernon MO, Hayden MK, Trick WE, Hayes RA, Blom DW, Weinstein RA, et al. Chlorhexidine gluconate to cleanse patients in a medical intensive care unit: the effectiveness of source control to reduce the bioburden of vancomycin-resistant enterococci. *Arch Intern Med* 2006;**166**:306—12.
34. Weinstein RA. Epidemiology and control of nosocomial infections in adult intensive care units. *Am J Med* 1991;**91**:179.

Please cite this article in press as: Lee W-S, et al., Bio-Kil, a nano-based disinfectant, reduces environmental bacterial burden and multidrug-resistant organisms in intensive care units, Journal of Microbiology, Immunology and Infection (2016), http://dx.doi.org/10.1016/j.jmii.2016.04.008