Journal of Hospital Infection 99 (2018) 256–262

Available online at www.sciencedirect.com

Journal of Hospital Infection

journal homepage: www.elsevier.com/locate/jhin

Pre–post evaluation of effects of a titanium dioxide coating on environmental contamination of an intensive care unit: the TITANIC study

B. de Jong [a], A.M. Meeder [a], K.W.A.C. Koekkoek [b], M.A. Schouten [c], P. Westers [d], A.R.H. van Zanten [a,*]

[a] Department of Intensive Care, Gelderse Vallei Hospital, Ede, The Netherlands
[b] Department of Internal Medicine, Gelderse Vallei Hospital, Ede, The Netherlands
[c] Department of Microbiology, Gelderse Vallei Hospital, Ede, The Netherlands
[d] Julius Centre, University Medical Center Utrecht, Utrecht, The Netherlands

ARTICLE INFO

Article history:
Received 9 December 2016
Accepted 9 April 2017
Available online 15 April 2017

Keywords:
Titanium dioxide
Healthcare-associated infection
Self-disinfecting
Environment
Surface
Intensive care unit
Antimicrobial
Cleaning
Coating
Hospital-acquired infection
Nosocomial infection

SUMMARY

Background: Among patients admitted to European hospitals or intensive care units (ICUs), 5.7% and 19.5% will encounter healthcare-associated infections (HAIs), respectively, and antimicrobial resistance is emerging. As hospital surfaces are contaminated with potentially pathogenic bacteria, environmental cleanliness is an essential aspect to reduce HAIs.
Aim: To address the efficacy of a titanium dioxide coating in reducing the microbial colonization of environmental surfaces in an ICU.
Methods: A prospective, controlled, single-centre pilot study was conducted to examine the effect of a titanium dioxide coating on the microbial colonization of surfaces in an ICU. During the pre- and post-intervention periods, surfaces were cultured with agar contact plates (BBL RODAC plates). Factors that were potentially influencing the bacterial colonization of surfaces were recorded. A repeated measurements analysis within a hierarchic multi-level framework was used to analyse the effect of the intervention, controlling for the explanatory variables.
Findings: The mean ratio for the total number of colony-forming units (cfus) in a room between the pre- and post-intervention periods was 0.86 (standard deviation 0.57). The optimal model included the following explanatory variables: intervention (P=0.065), week (P=0.002), culture surfaces (P<0.001), ICU room (P=0.039), and interaction between intervention and week (P=0.002) and between week and culture surfaces (P=0.031). The effect of the intervention on the number of cfus from all culture plates in Week 4 between the pre- and post-intervention periods was -0.47 (95% confidence interval -0.24 to - 0.70).
Conclusion: This study found that a titanium dioxide coating had no effect on the microbial colonization of surfaces in an ICU.

* Corresponding author. Address: Department of Intensive Care Medicine, Gelderse Vallei Hospital, Willy Brandtlaan 10, 6716 RP Ede, The Netherlands. Tel.: +31 318 434115; fax: +31 318 434115.
E-mail address: zantena@zgv.nl (A.R.H. van Zanten).

http://dx.doi.org/10.1016/j.jhin.2017.04.008

Introduction

It has been estimated that approximately 5.2% of patients admitted to general wards and 19.5% of patients admitted to intensive care units (ICUs) in European hospitals encounter at least one healthcare-associated infection (HAI) [1]. With 81,000 patients admitted each day, this results in 37,000 deaths, 16 million additional days of hospital stay and direct costs of at least 7 billion Euros per year [1,2].

Furthermore, antimicrobial resistance (AMR) is emerging in Europe. Invasive bacterial isolates from hospitalized patients show an ongoing trend of increasing resistance to key antimicrobial groups [3]. As antibiotics are the only effective treatment for serious infections, increasing AMR confers great concern towards patient safety worldwide.

Despite emerging resistance, hospitals struggle to implement and comply with infection control programmes [4]. Key healthcare-associated pathogens have the potential to persist for weeks, months and even years in the hospital environment, and their presence is related to increased risk of HAIs [5–16]. As hospital surfaces are contaminated with pathogens, environmental cleanliness (i.e. the result of the process of cleaning, disinfecting and monitoring) seems to be an important factor to reduce HAIs. However, in general, less than 50% of hospital surfaces are cleaned and disinfected adequately [7]. For that reason, novel materials and cleaning technologies have been developed, such as ultraviolet germicidal irradiation or hydrogen peroxide vapour systems [17,18]. However, in daily clinical practice, these techniques are laborious, expensive and – most importantly – can only be applied for terminal disinfection. Self-disinfecting surfaces may circumvent these problems. Once applied, antimicrobial surfaces will reduce the burden of nosocomial pathogens continuously, and potentially prevent transmission, colonization and HAIs.

Titanium dioxide (TiO_2) is a well-known nanotechnological material and has many applications in daily life. Due to its high refractive index, the principal use of TiO_2 is as a whitening and opacifying agent (e.g. paints, ink, cosmetics, toothpaste, paper and food or food packaging). Furthermore, it is used in sunscreens as a UV filter. Finally, being a photocatalyst, TiO_2 has applications for self-cleaning surfaces, water and air purification, and as an antimicrobial or self-disinfecting coating. The latter effect is due to the formation of hydroxyl radicals (•OH), superoxide anions ($•O_2^-$) and hydrogen peroxide, leading to inactivation, membrane damage and killing of micro-organisms, and ultimately decomposition of all cell components [19,20].

In order to address the efficacy of a mixed TiO_2 coating in reducing the microbial colonization of environmental surfaces in an ICU, a prospective, open-label, controlled, single-centre pilot study was performed.

Methods

Design overview

This study comprised two consecutive six-week periods: the control period and the test period. Each period started after thorough cleaning of the rooms with a wash-in phase of two weeks with regular use of the specific ICU rooms. During the next four-week phase, microbiological culturing was performed. The intervention (i.e. coating of the rooms) took place in the test period directly after initial cleaning of the rooms.

After the control period, the conventional fluorescent tubes (TL tubes) were changed to full spectrum TL tubes with an average intensity of 278 $\mu W/cm^2$ measured straight below and at 180 cm from the light source (Fullspectrum Sunlight, FreshlightSolutions, Utrecht, The Netherlands). Furthermore, the spectrogram (Avaspec-ULS2048L-RS-EVO, Serial No. 1601203U1, Avantes, Apeldoorn, the Netherlands) showed peak intensity of 1.47 $\mu W/cm^2/nm$ at a wavelength of 450 nm. Between the wavelengths of 490 and 680 nm, an area of higher intensity was seen, with a maximum of 0.84 $\mu W/cm^2/nm$.

The local medical ethics committee of Gelderse Vallei Hospital approved the study, and informed consent was obtained from the patients or their legal representatives. This trial is registered at ClinicalTrials.gov (No. NCT02348346).

Setting

Gelderse Vallei Hospital is a university-affiliated teaching hospital with a 17-bed level III ICU. Four isolation rooms with a non-recirculating ventilation system and a constant positive pressure inside the rooms were selected for this study.

The patient population comprises both medical and surgical patients. All invasive ventilated patients were treated with selective oropharyngeal decontamination [21].

Standard cleaning regimen

Standard daily cleaning is performed by mopping the floor with water using ultra microfibre cloths (Ultra Microfiber Reflex ProTex cloths, BioFriends Nederland B.V., Nunspeet, The Netherlands) and wiping contact surfaces of non-medical equipment with an alcohol-based interior cleaner (Taski Sprint 200 NC, Diversey, Utrecht, The Netherlands). Contact surfaces of medical equipment, beds and computers are wiped with alcohol 70% at 08:00 am, 4:00 pm and 11:00 pm. Terminal cleaning involves mopping the floor with water and wiping both the medical and non-medical equipment with alcohol 70%, and is more extensive compared with standard cleaning. The walls, ceilings and doors are not cleaned on a regular basis.

In the case of isolation measures (e.g. patient with multi-drug-resistant micro-organism or respiratory viruses), terminal cleaning is performed using Incidin (Ecolab B.V., Nieuwegein, The Netherlands) and no alcohol.

No active surveillance of the environment is performed for any micro-organisms.

Mixed TiO_2 coating

This study used a TiO2 coating containing silver supported zeolite, peroxotitanic acid and water (Miracle Titanium MVX, Maeda Kougyou, Kitakyushu, Japan) as provided by the FreshlightSolutions company (http://www.freshlightsolutions.com, Utrecht, The Netherlands). Laboratory studies show conflicting results for the antibacterial activity of MVX, but with log reductions up to 4 for both *Staphylococcus aureus* and *Escherichia coli* (unpublished data, test methods JIS R 1702:2006 or ISO 27447:2009).

Environmental cleaning and coating

Both the control and test periods started after thorough cleaning of the four rooms involved in the study. First, cleaning was performed by wiping all surfaces and equipment in the room with water, using ultra microfibre cloths (Ultra Microfiber Reflex ProTex cloths, BioFriends Nederland B.V.). Next, all plastic and metal surfaces were cleaned and degreased by spraying with an interior cleaner (Speedball Original, Diversey) and wiping with a cloth. Surfaces of medical and non-medical equipment were cleaned with 70% alcohol.

For the test period, all rooms were coated directly after the cleaning was performed and after at least 1 h of drying. Coating was performed using a high-pressure spraying system. First, a primer was sprayed on all surfaces except for the windows, computer screens and medical equipment (i.e. ventilator screen and equipment, electronic perfusors, docking stations). After drying, the mixed TiO_2 coating was applied on exactly the same surfaces as the primer. After at least 2 h of drying, the rooms could be used for regular care.

Microbiology

During the pre- and post-intervention periods, culturing was started after the wash-in phase and took place every Monday around 8:00 pm; this continued for four weeks. Surface microbial testing was performed using sets of agar contact plates (BBL RODAC plates, surface 24 cm^2, Becton Dickinson B.V., Breda, The Netherlands) for *S. aureus* (type S), Enterobacteriaceae (type E) and a non-selective plate (type T). Culturing per surface was performed with a set of plates (type S, E and T) and with a contact time of 10 s per plate.

In each room, cultures were taken from the wall, floor, bed rail, door and door handle, ceiling, computer keyboard, bedside table, monitor arm, medical equipment cabinet, footboard plateau and the sink.

The plates were incubated aerobically at 37°C, and the number of colony-forming units (cfus) was counted manually after 48 h of incubation as the sum of cfus per plate.

Potential confounders between the intervention and bacterial colonization of the ICU environment were recorded. Patient-associated confounders were length of stay in the ICU, duration of mechanical ventilation, number of X-ray procedures, severity of illness (SOFA score, APACHE-II score), patient's nosocomial infections according to criteria of the Centers for Disease Control and Prevention, antibiotic defined daily doses and type of patient (medical or surgical) [22,23]. Potential environmental-associated confounders were number of admissions per room, strict isolation measures applied (yes/no), number of door passages (people counter HPC001, Highlight) and the nurse:patient ratio per shift (1:1 or 1:2).

In-vitro study

After the clinical study, an in-vitro study was performed to evaluate the effect of MVX in a controlled environment (i.e. an empty office room with no entrance of patients, HCWs or other visitors, using the same culture methods and agar contact plates, indoor lighting and TiO_2 coating as used in the clinical study). *E. coli* (ATCC strain 25922) and *S. aureus* (ATCC strain 25923) were used for inoculation. After subsequent testing and sampling with the RODAC plates, the concentration for the inocula to result in as many cfus as possible without confluent growth was determined for a suspension with 150 cfus/mL for both *E. coli* and *S. aureus*.

Each morning, an overnight culture of *E. coli* and *S. aureus* was used to make the suspension which was transported to the test room.

Four plastic samples of 30 x 65 cm, with 18 marked squares of 10 x 10 cm on each sample, were used. Two samples were coated with MVX and the others were the control samples.

After sterilization of the samples (70% ethanol) and 10 min of drying, each square was inoculated by a suspension of *E. coli* or *S. aureus* using a sterile cotton swab. The swab was soaked in the solution; excess fluid was pressed out until the swab was moist. Each square on a test sample was subsequently swabbed horizontally and vertically.

Samples were not covered, and three cultures were taken directly after placement in the test room, followed by culture rounds after 1, 2, 4, 6 and 8 h. S plates were used for *S. aureus* and E plates were used for *E. coli*.

After sampling, the RODAC plates were transported directly to the laboratory where they were incubated overnight at 37°C. A laboratory analyst counted the number of cfus the following day.

Study endpoints

The ratio of the sum of the cfus for all types of contact plates (type S, type E and type T) in the post-intervention period compared with the sum of the cfus for all types of contact plates in the pre-intervention period was the primary endpoint. The secondary endpoints were the ratios of the sum of the cfus for each different type of contact plate (type S, type E or type T) in the post-intervention period compared with the sum of the cfus for each different type of contact plate in the pre-intervention period.

Statistical analysis

Results are described as medians with interquartile range (IQR), means with standard deviations (SD), or as numbers and percentages (%), as appropriate.

A repeated measurements analysis within a hierarchic multilevel framework was used to analyse the effect of the intervention, controlling for study week, ICU room and culture surfaces on the $\log_{10}$-transformed sum of the cfus of all contact plates (type S, type E and type T) for each ICU room [24]. The variable 'ICU room' reflects all the potential confounders. Only the intercept was considered as a random effect in the model. The analysis was started with a full model including all marginal and two-way interaction effects. Using the likelihood ratio test, the full model was reduced step-by-step to the optimal model (type I error 5%). Statistical tests were performed using SPSS Statistics for Windows Version 21 (IBM Corp., Armonk, NY, USA).

Results

In total, 24 and 29 ICU patients were admitted to the study ICU rooms during the pre- and post-intervention periods, respectively. Eighty-three percent of patients were medical. For the potential confounders (both patient and environment associated), there were no clinically relevant differences

between the two periods (Table I). There were no infectious disease clusters or outbreaks during the study period.

In both the pre- and post-intervention periods, 192 sets of RODAC plates were used with a total of 384 sets or 1152 culture plates available for microbiological analysis. The sum of cfus for all rooms in the post- vs pre-intervention period was 11,934 and 17,683 cfus with, respectively, 187 and 276 cfus per room (mean) and a mean ratio of 0.86 ± 0.57 per room. The mean ratios for the type S, type T and type E plates were 0.71 ± 0.38, 0.94 ± 0.64 and 0.25 ±0.50 per room, respectively (Table II).

The optimal model for the effects on the $\log_{10}$-transformed sum of the cfus included the following explanatory variables: week (P=0.002), culture surfaces (P<0.001), ICU room (P=0.039), and interaction between intervention and week (P=0.002) and between week and culture surfaces (P=0.031). The intervention itself had no effect on the number of cfus (P=0.065). However, the intervention had a significant interaction effect and so had to be included in the model.

The effect of the intervention on the $\log_{10}$ sum of all cfus of all culture plates in Week 4 between the pre- and post-intervention periods was -0.47 (95% confidence interval -0.24 to - 0.70). The effects of the intervention on the $\log_{10}$ sum of all cfus of all culture plates in Weeks 1, 2 and 3 were 0.087, 0.086 and -0.134, respectively.

Discoloration of furniture and equipment was noted as a side effect of the coating, as well as wall surfaces requiring painting after the experiments. The texture of coated surfaces was not found to change.

In-vitro study

At baseline, there were 400 cfus (mean) of *S. aureus* for both the control and coated samples, and this reduced to 363 and 181 cfus after 8 h, respectively.

Table I
Patient- and environment-associated potential confounders for both the pre- and post-intervention periods[a]

	Pre-intervention period	Post-intervention period
Patient-associated confounders		
Number of patients	*N*=24	*N*=29
Medical patients	20 (83)	24 (83)
Nosocomial infections	1 (1–2)	1 (0–1)
LOS-ICU (h)	62 (35–158)	47 (24–135)
Duration of MV (h)	42 (7–97)	24 (0–120)
X-ray procedures	3 (1–5)	2 (1–4)
APACHE-II score	22 (18–26)	20 (14–29)
SOFA score	5 (3–7)	5 (3–8)
Antibiotic DDDs	3 (0–17)	6 (2–14)
Environment-associated confounders		
Isolation measures, yes	10 (42)	9 (31)
Door passages/day	169 (55–236)	236 (134–330)
Nursing shifts	8 (5–19)	6 (2–17)
Shifts with nurse: patient ratio 1:2	133 (44)	229 (56)

LOS-ICU, length of stay in the intensive care unit; MV, mechanical ventilation; APACHE, Acute Physiology and Chronic Health Evaluation; SOFA, Sequential Organ Failure Assessment; DDDs, defined daily doses.
[a] Data are presented as median (interquartile range) or *N* (%).

Table II
Number of colony-forming units per room (mean) for three types of RODAC plates and mean ratios (with standard deviation) per room for the post-intervention period vs the pre-intervention period

RODAC plates	Pre-intervention period	Post-intervention period	Mean ratio/room
Staphylococcus aureus	116	65	0.71 (0.38)
Enterobacteriaceae	0	0	0.25 (0.50)
Non-selective	161	121	0.94 (0.64)
Total	276	187	0.86 (0.57)

For *E. coli*, there were 400 and 31 cfus (mean) for the control and coated samples at baseline, and this reduced to 10 and 4 cfus within 1 h, respectively (Figure 1).

As hardly any Enterobacteriaceae spp. were found in either the control or intervention periods, this study focused on experiments with *S. aureus*. Plastic samples were coated with MVX with different concentrations of silver (5–20%) or a thin or thick layer (by estimation) of TiO_2. These results showed 400 cfus at baseline for all different samples, and no reduction or a minimal reduction to 350 CFUs after 8 h.

Discussion

These data show that a mixed TiO_2 coating had no effect on the number of cfus in an ICU environment.

A previous clinical study examined the efficacy of TiO_2 in addition to terminal cleaning in preventing environmental contamination [25]. According to the company that provided the coating, the nanocoating could be activated by sunlight or fluorescent light. In that study, a total of 698 swabs were taken at three–six-month intervals over a two-year period, and randomly in the case of a patient with meticillin-resistant *S. aureus* being admitted to the hospital for more than 48 h. Sampled surfaces were the doors and door handles, bedside lockers, taps, light switches, monitors, rulers, curtains, blood pressure cuffs or light handles. Sampling was not performed systematically with respect to the wards (49% ICU, 42% general ward, 9% medium care), coated vs non-coated surfaces (19% and 81% respectively), planned vs ad-hoc sampling (33% and 67% respectively) and the different surfaces. Of the samples, 12.1% and 4.4% were positive for the non-coated and coated surfaces, respectively. Adjusting for some confounding variables, TiO_2 had no effect on positive culture results. It should be noted that this study had some relevant limitations. First, as stated by the authors, it was not a cross-sectional observational study. Second, subsequent measurements were taken with uneven intervals. Furthermore, mutually correlated independent variables increased the likelihood of positive cultures varying between different surfaces and measurements. For these reasons, multiple logistic regression testing should not be used. Third, the study used a very limited set of assumed confounders. Despite the fact that the present study was designed in such a manner to limit these methodological limitations, no significant benefit of the coating was observed in this study, except for the cultures from doors.

PubMed was searched for studies published before 30th April 2015 using the terms 'antimicrobial agents' and 'titanium(dioxide)' with all word variations and synonyms.

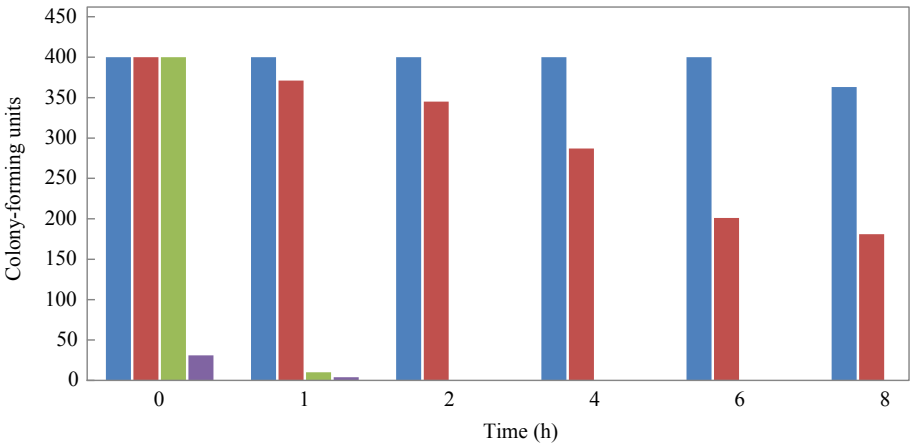

Figure 1. Number of colony-forming units (mean) for *Staphylococcus aureus* and *Escherichia coli* on plastic samples without (controls) or with MVX coating. Blue bars, control sample, *S. aureus*; red bars, MVX-coated sample, *S. aureus*; green bars, control sample, *E. coli*; purple bars, MVX-coated sample, *E. coli*.

Many laboratory studies have assessed the efficacy of TiO_2 to kill bacteria, viruses, fungi or yeasts. However, most of these tests were performed in an aqueous solution with variable concentrations of TiO_2, or applied TiO_2-coated surfaces were tested in water. Despite their high efficacy, these test results cannot be extrapolated to the application of TiO_2 in a hospital environment without these specific conditions. In-vitro studies using methods more comparable to the hospital setting show promising results, using activation through UV light ($\lambda<400$ nm) but with a much higher intensity than conventional indoor light [26–33]. Studies using conventional indoor lighting show extremely variable survival rates for *E. coli* and/or *S. aureus* from 97% to 0%, with reaction times from 60 min to 24 h [30,34–38].

These heterogeneous results may be due to factors that influence the efficacy of TiO_2 photocatalysis such as air humidity, anatase phase quantity (one of the three mineral forms of TiO_2), shape and dose of TiO_2 nanoparticles, UV light intensity and wavelength, thickness of the coating, target organism, and initial concentration and composition of the coated surface [26,31–34,38–44].

The negative results of this study and the heterogeneity of the laboratory studies is difficult to explain. No specific information on the characterization of the product used in the present study limits the interpretation of product characteristics regarding the cause of the findings. However, the non-UV indoor lighting used during the study with a much lower intensity than lighting used in most in-vitro studies might be an important factor. Furthermore, the number of door passages suggests a highly dynamic environment with a high risk of continuous environmental contamination by bioaerosols, or contact with healthcare workers (HCWs) or visitors. The efficacy of a self-disinfecting coating should at least exceed the rate of environmental contamination. During the in-vitro study, the log reduction was less than 2 in an 8-h period, and therefore the results have to be considered non-significant for the clinical environment. The results for *E. coli* suggest early bacterial killing of Gram-negative rods. However, as hardly any Enterobacteriaceae were cultured, this may have no clinical value. Finally, studies show a higher efficacy of silver-containing TiO_2 with a dose-dependent effect [29,33,37,38,43,45–52], but this could not be demonstrated during the in-vitro study as different silver concentrations had no effect on the cfus for *S. aureus*.

The efficacy of the TiO_2 coating used in the present clinical and laboratory experiments was insufficient, possibly due to low-intensity non-UV indoor lighting and/or a low rate of bacterial killing.

A limitation of this study is the absence of information about the characteristics of the TiO_2 coating (e.g. morphology, microstructure, elemental state or quality of indoor lighting to optimally activate the coating without posing a risk to patients, HCWs or visitors). Furthermore, it was not possible to ascertain the presence and/or quality of the coating during the study. Wear and tear might have damaged or removed the coating from some of the tested surfaces, possibly reducing its efficacy.

Further testing of the TiO_2 coating in laboratory settings is recommended, changing characteristics such as morphology, microstructure and elemental state of the nanoparticles. Furthermore, indoor lighting of various qualities could be tested. Further clinical testing will only be warranted when marked killing can be shown in laboratory tests for various clinically relevant pathogens associated with HAIs.

In conclusion, this study showed no effect of a mixed TiO_2 coating on the microbial colonization of surfaces in an ICU environment. Additional laboratory tests have shown TiO_2 to reduce *E. coli* and to have a limited effect on *S. aureus*. This may, at least in part, explain the limited clinical findings, as Gram-negative colonization was rare and Gram-positive colonization was common. Limited exposure to UV light may have played a role. Further research should focus on the characterization and further improvement of TiO_2 coatings activated by non-UV indoor lighting.

Conflict of interest statement
None declared.

Funding sources
None.

References

[1] European Centre for Disease Prevention and Control. Point prevalence survey of healthcare associated infections and

antimicrobial use in European acute care hospitals. Stockholm: ECDC; 2013.
[2] European Centre for Disease Prevention and Control. Annual epidemiological report on communicable diseases in Europe 2008. Stockholm: ECDC; 2008.
[3] European Centre for Disease Prevention and Control. Antimicrobial resistance surveillance in Europe 2014. Annual report of the European Antimicrobial Resistance Surveillance Network (EARS-Net). Stockholm: ECDC; 2015.
[4] Zingg W, Holmes A, Dettenkofer M, Goetting T, Secci F, Clack L, et al. Hospital organisation, management, and structure of prevention of health-care associated infection: a systematic review and expert consensus. Lancet Infect Dis 2015;15:212–24.
[5] Esteves DC, Pereira VC, Souza JM, Keller R, Simões RD, Winkelstroter Eller LK, et al. Influence of biological fluids in bacterial viability on different hospital surfaces and fomites. Am J Infect Control 2016;44:311–4.
[6] Hota B. Contamination, disinfection, and cross-colonization: are hospital surfaces reservoirs for nosocomial infection? Clin Infect Dis 2004;39:1182–9.
[7] Weber DJ, Anderson D, Rutala WA. The role of the surface environment in healthcare-associated infections. Curr Opin Infect Dis 2013;26:338–44.
[8] Steinberg JP, Denham ME, Zimring C, Kasali A, Hall KK, Jacob JT. The role of the hospital environment in the prevention of healthcare-associated infections by contact transmission. Health Environ Res Design J 2013;7(Suppl.):46–73.
[9] Moore G, Muzslay M, Wilson AP. The type, level, and distribution of microorganisms within the ward environment: a zonal analysis of an intensive care unit and a gastrointestinal surgical ward. Infect Control Hosp Epidemiol 2013;34:500–6.
[10] Dancer SJ. The role of environmental cleaning in the control of hospital-acquired infection. J Hosp Infect 2009;73:378–85.
[11] Dancer SJ. Importance of the environment in methicillin-resistant *Staphylococcus aureus* acquisition: the case for hospital cleaning. Lancet Infect Dis 2008;8:101–13.
[12] Oberauner L, Zachow C, Lackner S, Högenauer C, Smolle KH, Berg G. The ignored diversity: complex bacterial communities in intensive care units revealed by 16S pyrosequencing. Sci Rep 2013;3:1413.
[13] Crnich CJ, Safdar N, Maki DG. The role of the intensive care unit environment in the pathogenesis and prevention of ventilator-associated pneumonia. Respir Care 2005;50:813–36.
[14] Joseph NM, Sistla S, Dutta TK, Badhe AS, Rasitha D, Parija SC. Role of intensive care unit environment and health-care workers in transmission of ventilator-associated pneumonia. J Infect Dev Ctries 2010;4:282–91.
[15] Wolf I, Bergervoet PW, Sebens FW, van den Oever HL, Savelkoul PH, van der Zwet WC. The sink as a correctable source of extended-spectrum beta-lactamase contamination for patients in the intensive care unit. J Hosp Infect 2014;87:126–30.
[16] Mahida N, Beal A, Trigg D, Vaughan N, Boswell T. Outbreak of invasive group A streptococcus infection: contaminated patient curtains and cross-infection on an ear, nose and throat ward. J Hosp Infect 2014;87:141–4.
[17] Weber DJ, Rutala WA, Anderson DJ, Chen LF, Sickbert-Bennett EE, Boyce JM. Effectiveness of ultraviolet devices and hydrogen peroxide systems for terminal room decontamination: focus on clinical trials. Am J Infect Control 2016;44(Suppl.):e77–84.
[18] Otter JA, Yezli S, Schouten MA, van Zanten AR, Houmes-Zielman G, Nohlmans-Paulssen MK. Hydrogen peroxide vapor decontamination of an intensive care unit to remove environmental reservoirs of multidrug-resistant Gram-negative rods during an outbreak. Am J Infect Control 2010;38:754–6.
[19] Fujishima A, Zhang X, Tryk DA. TiO_2 photocatalysis and related surface phenomena. Surf Sci Rep 2008;63:515–82.
[20] Foster HA, Ditta IB, Varghese S, Steele A. Photocatalytic disinfection using titanium dioxide: spectrum and mechanism of antimicrobial activity. Appl Microbiol Biotechnol 2011;90:1847–69.
[21] De Smet AM, Kluytmans JA, Cooper BS, Mascini EM, Benus RF, van der Werf TS, et al. Decontamination of the digestive tract and oropharynx in ICU patients. N Engl J Med 2009;360:20–31.
[22] Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. Am J Infect Control 2008;36:309–32.
[23] WHO Collaborating Centre for Drug Statistics Methodology. ATC/DDD index 2015. Geneva: WHO; 2015. Available at: http://www.whocc.no/atc_ddd_index [last accessed September 2015].
[24] Snijders TAB, Bosker RJ. Multilevel analysis: an introduction to basic and advanced multilevel modelling. 2nd ed. London: Sage Publications; 2012.
[25] Leng CW, Soe TA, Wui LW, Yen JLF, Kurup A, Lin LM, et al. Efficacy of titanium dioxide compounds in preventing environmental contamination by meticillin resistant *Staphylococcus aureus* (MRSA). Int J Infect Control 2013;9(3). http://dx.doi.org/10.3396/IJIC.v9i3.022.13.
[26] Chen F, Yang X, Xu F, Wu Q, Zhang Y. Correlation of photocatalytic bactericidal effect and organic matter degradation of TiO2 part 1: observation of phenomena. Environ Sci Tech 2009;43:1180–4.
[27] Joost U, Juganson K, Visnapuu M, Mortimer M, Kahru A, Nõmmiste E, et al. Photocatalytic antibacterial activity of nano-TiO2 (anatase)-based thin films: effects on *Escherichia coli* cells and fatty acids. J Photochem Photobiol B Biol 2015;142:178–85.
[28] Kong H, Song J, Jang J. Photocatalytic antibacterial capabilities of TiO2-biocidal polymer nanocomposites synthesized by a surface-initiated photopolymerization. Environ Sci Technol 2010;44:5672–6.
[29] Yu B, Leung KM, Guo Q, Lau WM, Yang J. Synthesis of Ag-TiO2 composite nano thin film for antimicrobial application. Nanotechnology 2011;22:115603.
[30] Chawengkijwanich C, Hayata Y. Development of TiO2 powder-coated food packaging film and its ability to inactivate *Escherichia coli* in vitro and in actual tests. Int J Food Microbiol 2008;123:288–92.
[31] Ditta IB, Steele A, Liptrot C, Tobin J, Tyler H, Yates HM, et al. Photocatalytic antimicrobial activity of thin surface films of TiO2, CuO and TiO2/CuO dual layers on *Escherichia coli* and bacteriophage T4. Appl Microbiol Biotechnol 2008;79:127–33.
[32] Nakano R, Ishiguro H, Yao Y, Kajioka J, Fujishima A, Sunada K, et al. Photocatalytic inactivation of influenza virus by titanium dioxide thin film. Photochem Photobiol Sci 2012;11:1293–8.
[33] Nhung le TT, Nagata H, Takahashi A, Aihara M, Okamoto T, Shimohata T, et al. Sterilization effect of UV light on bacillus spores using TiO2 films depends on wavelength. J Med Invest 2012;59:53–8.
[34] Chung CJ, Lin HI, Tsou HK, Shi ZY. He JL.An antimicrobial TiO2 coating for reducing hospital-acquired infection. J Biomed Mater Res B Appl Biomater 2008;85:220–4.
[35] Fu G, Vary PS, Lin CT. Anatase TiO2 nanocomposites for antimicrobial coatings. J Phys Chem 2005;109:8889–98.
[36] He Z, Xu Q, Tan TT. Understanding antibacterial performance on ambient light activated TiO2-InVo4 nanostructured films. Nanoscale 2011;3:4977–83.
[37] Hebeish AA, Abdelhady MM, Youssef AM. TiO2 nanowire and TiO2 nanowire doped Ag-PVP nanocomposite for antimicrobial and self-cleaning cotton textile. Carbohydr Polym 2013;91:549–59.
[38] Pham TD, Lee BK. Effects of Ag doping on the photocatalytic disinfection of *E. coli* in bioaerosol by Ag-TiO2/GF under visible light. J Colloid Interface Sci 2014;428:24–31.
[39] Shintani H, Kurosu S, Miki A, Hayashi F, Kato S. Sterilization efficiency of the photocatalyst against environmental microorganisms in a health care facility. Biocontrol Sci 2006;11:17–26.

[40] Chang JT, Su CW, He JL. Photocatalytic TiO2 film prepared using arc ion plating. Surf Coat Technol 2006;200:3027—34.
[41] Aminedi R, Wadhwa G, Das N, Pal B. Shape-dependent bactericidal activity of TiO2 for the killing of Gram-negative bacteria *Agrobacterium tumefaciens* under UV torch irradiation. Environ Sci Pollut Res Int 2013;20:6521—30.
[42] Arun TA, Madhavan AA, Chacko DK, Anjusree GS, Deepak TG, Thomas S, et al. A facile approach for high surface area electrospun TiO2 nanostructures for photovoltaic and photocatalytic applications. Dalton Trans 2014;43:4830—7.
[43] Paret ML, Vallad GE, Averett DR, Jones JB, Olson SM. Photocatalysis: effect of light-activated nanoscale formulations of TiO2 on *Xanthomonas perforans* and control of bacterial spot of tomato. Phytopathology 2013;103:228—36.
[44] Chen F, Yang X, Wu Q. Antifungal capability of TiO2 coated film on moist wood. Build Environ 2009;44:1088—93.
[45] Choi JY, Kim KH, Choy KC, Oh KT, Kim KN. Photocatalytic antibacterial effect of TiO2 film formed on Ti and TiAg exposed to *Lactobacillus acidophilus*. J Biomed Mater Res B Appl Biomater 2007;80:353—9.
[46] Yu KP, Huang YT, Yang SC. The antifungal efficacy of nano-metals supported TiO2 and ozone on the resistant *Aspergillus niger* spore. J Hazard Mater 2013;261:155—62.
[47] Selvam S, Rajiv Gandhi R, Suresh J, Gowri S, Ravikumar S, Sundrarajan M. Antibacterial effect of novel synthesized sulfated β-cyclodextrin crosslinked cotton fabric and its improved antibacterial activities with ZnO, TiO2 and Ag nanoparticles coating. Int J Pharmaceut 2012;434:366—74.
[48] Zhao Y, Xing Q, Janjanam J, He K, Long F, Low KB, et al. Facile electrochemical synthesis of antimicrobial TiO2 nanotube arrays. Int J Nanomed 2014;9:5177—87.
[49] Pant HR, Pandeya DR, Nam KT, Baek WI, Hong ST, Kim HY. Photocatalytic and antibacterial properties of a TiO2/nylon-6 electrospun nanocomposite mat containing silver nanoparticles. J Hazard Mater 2011;189:465—71.
[50] Tallósy SP, Janovák L, Ménesi J, Nagy E, Juhász Á, Balázs L, et al. Investigation of the antibacterial effect of silver-modified TiO2 and ZnO plasmonic photocatalysts embedded in polymer thin films. Environ Sci Pollut Res 2014;21:11155—67.
[51] Yao Y, Ohko Y, Sekiguchi Y, Fujishima A, Kubota Y. Self-sterilization using silicone catheters coated with Ag and TiO2 nanocomposite thin film. J Biomed Mater Res B Appl Biomater 2008;85:453—60.
[52] Tian Y, Cao H, Qiao Y, Meng F, Liu X. Antibacterial activity and cytocompatibility of titanium oxide coating modified by iron ion implantation. Acta Biomater 2014;10:4505—17.