DIPARTIMENTO DI SANITÀ
PUBBLICA E MALATTIE INFETTIVE

SAPIENZA
UNIVERSITÀ DI ROMA

OGGETTO: Attività antibatterica contro *Staphylococcus aureus* meticillina resistente (MRSA) del POP Polimero Ossigenato Plastificato (PVC), Pure-Health™, di Orion srl

OBJECT: Antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) of POP Polimero Ossigenato Plastificato (PVC), Pure-Health™ (Orion srl).

INTRODUCTION

The role of the environment in Hospital-Acquired Infection transmission

Contaminated surfaces play an important role in the transmission of certain pathogens responsible for Hospital-Acquired Infections (HAI). Indeed, *Clostridium difficile*, methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), norovirus and multidrug-resistant (MDR) gram-negative rods such as *Acinetobacter baumannii* and *Klebsiella pneumoniae*, share the ability to be shed from infected or colonized patients and staff, survive on dry surfaces for extended periods and be difficult to eradicate by cleaning and disinfection (Otter et al., 2013; Weber and Rutala, 2013). In general, persistence of these pathogens on environmental surfaces is particularly high (**Table 1**) (Kramer et al., 2006).

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Table 1. Estimated persistence of microorganisms responsible for Hospital-Acquired Infections on environmental surfaces.

Microorganism	Persistence
Clostridium difficile (spores)	5 months
Staphylococcus aureus, including MRSA	7 days – 7 months
Enterococcus spp. including VRE	5 days – 4 months
Acinetobacter spp.	3 days – 5 months
Klebsiella spp.	2 hours – 30 months
Escherichia coli	1.5 hours – 16 months
Norovirus and Calicivirus	8 hours – 7 days

Pathogen transfer from a colonized/infected patient to a susceptible host generally occurs via the hands of Healthcare Providers. However, contaminated hospital surfaces are involved in the transmission pathways. Indeed, Healthcare Providers' hands and gloves can be contaminated by touching contaminated surfaces, which, in turn, have been previously contaminated by colonized/infected patients or by other Healthcare Providers. The role of environmental surfaces in HAI transmission is deducible from different elements (**Table 2**), which suggest that the scientific evidence is strong (Otter et al., 2013; Weber and Rutala, 2013).

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Table 2. Elements that support the role of contaminated environmental surfaces in Hospital-Acquired Infection transmission.

1	The surface environment in rooms of colonized or infected patients is frequently contaminated with pathogens
2	Pathogens are capable of surviving on hospital surfaces for a prolonged period of time
3	Contact with hospital environmental surfaces by Healthcare Providers frequently leads to contamination of hands and/or gloves
4	The frequency with which environmental surfaces are contaminated correlates with the frequency of hand and/or glove contamination of Healthcare Providers
5	Clonal outbreaks of pathogens contaminating the environmental surfaces of infected/colonized patients are demonstrated to be due to person-to-person transmission
6	The patient admitted to a room previously occupied by a patient infected/colonized with a pathogen has an increased likelihood of developing colonization or infection with that pathogen
7	Improved terminal cleaning of rooms leads to a decreased rate of infections
8	Improved terminal disinfection leads to a decreased rate of infection in patients subsequently admitted to the room in which the prior occupant was colonized or infected

Methicillin-resistant *Staphylococcus aureus* (MRSA)

MRSA was first isolated in the United Kingdom in 1961, only two years after the introduction of methicillin into clinical practice. MRSA clones disseminated worldwide during the 70's and today Hospital-Associated MRSA (HA-MRSA) is responsible for the majority of nosocomial infections. HA-MRSA infections are estimated to affect more than 150,000 patients in the European Union and almost

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400,000 in the United States annually, resulting in attributable extra costs to healthcare systems of € 380 million in the EU and $ 14.5 billion in the US (Petti and Polimeni, 2011). As anticipated, the role of environmental surfaces in HA-MRSA infection transmission is confirmed by overwhelming evidence (Dancer, 2008). For example, a prospective study conducted in an Intensive Care Unit showed that among patients who were colonized/infected by MRSA during hospitalization, there was strong evidence that 11.5% of them acquired MRSA from the contaminated environment (Hardy et al., 2006). Among the reasons for transmission, there is the MRSA ability to survive in dry environment. Indeed, MRSA strains are able to survive on smooth surfaces for at least four months in an environment where humidity is relatively low (23-47%) (Petti et al., 2012). Studies that attempted to control MRSA infections through enhanced cleaning reported a drop in incidence rates from 3% to 1.5% in an Intensive Care Unit (Datta et al., 2011) and from 3.5% to 0.5% in a Surgical Ward (Rampling et al., 2001). These good results are partly in disagreement with perspective cross-over trials, which showed contrasting results, as one study on two wards in the same hospital reported that during enhanced cleaning the number of HA-MRSA infections dropped from nine to four (Dancer et al., 2009), while another study on two hospitals found that with similar measures the number of HA-MRSA infections passed from seven to nine (Wilson et al., 2011). Other environmental control measures such as pulsed xenon UV device (Simmons et al., 2013) and hydrogen peroxide vapor (Mitchell et al, 2014) were able to halve HA-MRSA infection rates.

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An alternative surface decontamination strategy is the use of thin-film coatings with photocatalytic activity that produce Reactive Oxygen Species (ROS). ROS production by titanium dioxide, with formula TiO_2 (titania), is induced through absorption of high-energy photons and subsequent electron excitation. During this photoexcitation, highly reactive hydroxyl radicals (·OH), superoxide anion ($\cdot O_2^-$) and hydrogen peroxide (H_2O_2) are produced from water. These products result in killing of microorganisms on the surface and up to 50 µm distance from the surface. The antibacterial activity of photoactivated TiO_2 is essentially due to membrane and cell-wall damage and the list of bacteria, fungi, protozoa, algae and viruses that are killed by photoactivated TiO_2 is endless. Nano-TiO_2 in anatase phase has been shown as the most potent form of TiO_2 to produce ROS (Foster et al., 2011; Joost et al., 2015).

According to a study performed in a tertiary hospital, some surfaces were coated with nano-TiO_2-based film. In areas exposed to MRSA carriers, these microorganisms and/or Gram-negative bacteria were detected in 12% untreated surfaces and in 4% treated surfaces. Thus, the fact that results regarding MRSA were merged with those regarding Gram-negative bacteria clouded the true effectiveness of this substrate against MRSA (Chow et al., 2013).

Aim of the study

The aim of the present study was, therefore, to investigate the activity against MRSA of nano-TiO_2-based coating applied on a typical environmental surface.

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MATERIALS AND METHODS

MRSA strain

A sporadic MRSA strain, which resulted long-term persisting in the environment (Petti et al., 2012) was used. This strain was isolated through samples collected from nasal swabs of hospitalized patients. The swabs were placed in saline solution (9 g/L NaCl, 4°C), plated onto Mannitol Salt Agar (MSA) and incubated aerobically (37°C, 48 h). Colonies with typical *S. aureus* morphology were subcultured in Trypticase Soy Broth (TSB; 37°C, 24 hours). The strains were Gram's stained and tested for coagulase. Coagulase-positive strains were presumptively identified using VITEK-2 "Gram-Positive Identification" and "Antibiotic Susceptibility Testing" cards (BioMérieux, Italia; Bagno a Ripoli, Italy). Following CDC criteria, MRSA were considered those *S. aureus* strains showing oxacillin resistance, that is, Minimum Inhibitory Concentration (MIC) >4 µg/mL, which implied the presence of the staphylococcal cassette chromosome mec (SCCmec) (Kuehnert et al., 2006).

Clinical Contact Surface

A thermoplastic synthetic resin was used as environmental surface. Specifically, polyvinyl chloride (PVC) made of 57% chlorine and 43% carbon. This material is frequently used in hospitals for flooring and surface covering because it is smooth and tough and, therefore, it is easy to clean and prevents the accumulation of dirt and microorganisms. Two PVC surfaces of equal area (70x70 cm) were used. One surface (Test) was coated with nano-TiO_2-based thin film (Polimero Ossigenato

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Plastificato, POP, Pure-Health™, Orion srl, Calenzano, Italy), responsible for photocatalytic disinfection activated by visible-band fluorescence light. The lamp was switched on three hours before surface contamination to let photocatalysis start. The other surface (Control) was left untreated.

To simulate real-life conditions, the Test and Control surfaces were put on a table into an annex of the Department of Public Health and Infectious Diseases of the "Sapienza" University of Rome. The annex had natural and artificial light, the walls were transparent and partly open at the top. The area of the annex was roughly 10 m^2. Air speed, temperature and humidity, measured during all the study, performed between May and July 2014, ranged between 0.00 and 0.03 m/sec, 24.5°C and 29.3°C, and 51.4% and 63.7%, respectively.

Before each experimental event, Test and Control surfaces were cleaned, disinfected and rinsed to remove residual environmental microorganisms and particles that could interfere with the experimental procedures. For every surface a washable cloth (32x32 cm - Softtronic; Vermop Italia Professional Cleaning Systems, Milan, Italy. Composition: 75% polyester microfiber, 25% polyamide microfiber) was soaked with tap water and 1-3 mL commercially available soap (5-10% sodium lauryl sulphate). The cloth was gently passed on the surface with rotational movements for two minutes. Another similar cloth was soaked with 10-20 mL of a 1:10 dilution of 5.25% to 6.15% sodium hypochlorite and was used with similar movements and for the same time on the surface. The solution remained in contact with the surface for 7 minutes. In order to remove the residual disinfectant and

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soap, another similar cloth was soaked with sterilized hard (i.e., tap) water and was passed with the same movements and modalities for 3 minutes on the surface.

Natural-like Experimental Surface Contamination

The present study simulated the case of a nasal MRSA carrier responsible for airborne spread of these microorganisms in the environment shedding skin particles with adherent MRSA, touching objects and surfaces, coughing, sneezing and talking, thus resulting in a slow and continuous deposition of microorganisms on the environmental surfaces (Dancer, 2008) with a plausible MRSA cell density which could be defined moderate, that is, 12-40 colony-forming units (cfu)/cm^2 or high that is, >40 cfu/cm^2 (Mulvey et al., 2011).

The day before the test, the MRSA strain was subcultured in Trypticase Soy Broth (37°C, 24 h). The day of the test, the bacterial suspension was centrifuged, resuspended and diluted by 1:40 in saline solution. Then it was placed into a 2-ounce fingertip mist sprayer (Tolco Corporation, Toledo, OH) and was vortexed for 1 minute. For each of the two surfaces, the sprayer was positioned 1 m above the tested environmental surface at a 45-degree inclination. The suspension was then sprayed twice to produce the natural-like MRSA aerosol above the surface (Petti et al., 2013).

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Environmental Surface Sampling

Environmental surface samples were performed using 24-cm^2 Rodac plates containing MSA, which were pressed gently onto the surface for 10 seconds at a pressure of approximately 25 g/cm^2 without rotation or lateral movements. For each sampling, four plates were used and material was collected from different sites of the surface. Contact plates were preferred to other sampling methods because they provided the most reliable results for the recovery of MRSA on environmental surfaces (Obee et al., 2007).

At each experimental event and for each surface a pre-experiment sample was performed before surface contamination with MRSA suspensions and after cleaning/disinfection, to verify that staphylococci and micrococci, which could grow on MSA and cloud the results of the study, had been correctly removed from the surfaces. Then, environmental samples were performed at various intervals to assess the MRSA cell density. These intervals were 15, 30, 45, 60, 90, 120, 150, 180 minutes after contamination.

Plates were incubated at 37°C for 48 h in aerobiosis and colonies were counted. Five experimental events were performed for both Test and Control surfaces.

In order to minimize the sources of contamination that may obscure the results of the study, at every experimental event the microbiologists wore disposable white coats, gloves and masks and remained into the annex throughout the experiment duration leaving the door closed. In addition, at every experimental event and for every surface two-three colonies were picked from the MSA plates

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after incubation and were submitted to the above mentioned isolation and identification procedures.

Statistical Analysis

For each surface, experimental event and sampling time the number of MRSA cfu obtained from the four Rodac plates was summed and divided by 96, thus obtaining the cell density expressed in cfu/cm^2.

For each surface and sampling time median and range of the five experimental events were assessed. Then, for each sampling time the difference between Test and Control surfaces was assessed with the Mann-Whitney U-test for independent samples, the most powerful nonparametric alternative to the t-test for independent samples.

MRSA cell densities were log transformed and the mean log cell density at each sampling time was assessed for both the Test and the Control surfaces. These values were used to estimate the time required to reach the microbiological threshold for hospital surface hygiene of less than 1 MRSA per cm^2 (Dancer, 2004). For both Control and Test surfaces the linear regression analysis was made with "time since contamination" as independent variable (*x* variable, expressed in minutes), and "MRSA log cell density" as dependent variable (*y* variable, expressed in log cfu/cm^2). Then slope ("b" in the regression line) and intercept ("a" in the regression line) were estimated with 95% confidence intervals. Robustness of the overall regression model and of intercept/slope estimates were assessed through

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adjusted R^2 and t-ratio, respectively. The time required to reach the threshold for hospital surface hygiene was assessed as follows:

From the formula of the regression line ***y* = a + b*x***

The threshold for hospital surface hygiene was ***y* = 0** (i.e., 0 log cfu/cm^2, corresponding to 1 cfu/cm^2)

The value of x for y = 0 was given by the formula ***x* = (-a)/b**.

The 95% confidence intervals were assessed substituting the lowest and the highest 95% confidence limits to the point estimates for “a” and “b”.

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RESULTS

Three hundred-sixty Rodac plates were necessary to perform the experiments. In addition, a series of pilot studies were performed to assess the most appropriate time intervals between sampling occasions, the duration of the experiment and the number of sprayings necessary to obtain a plausible and natural-like MRSA cell density on the surfaces. All these pilot studies resulted in a number of plates and experimental events comparable to those that were actually used for the present analysis.

The cleaning and disinfection procedures resulted in a contamination level (0.03 cfu/cm^2 – **Table 3**) that was low enough not to interfere with the results of the experiments. MRSA cell density on both Test and Control surfaces was moderate 15 minutes after spraying MRSA suspension (median, 27.5 cfu/cm^2), increased slightly for 30 minutes (medians, 43-46 cfu/cm^2) and then decreased homogeneously on the Test and Control surfaces for 15 minutes (medians, 35-36 cfu/cm^2) (**Figure 1a**).

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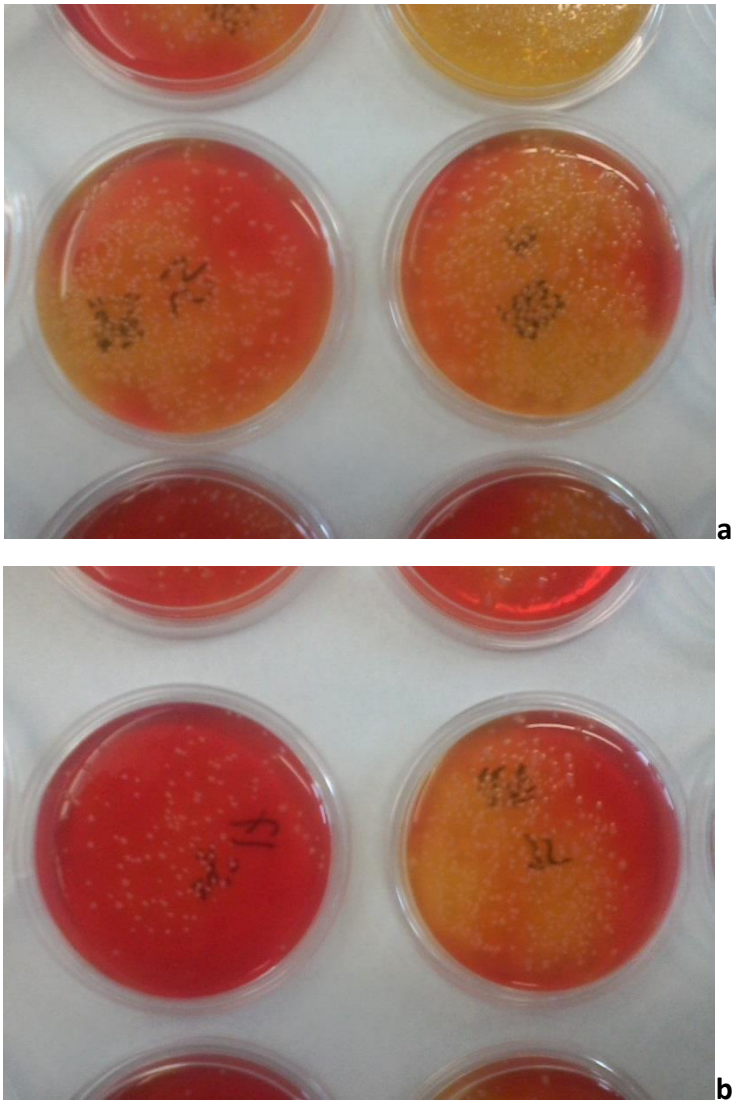
Figure 1. MSA Rodac plates after incubation detected on the Test (left) and Control (right) surfaces one (**a**) and two hours (**b**) after airborne contamination. MRSA cell density decrease is perceptible in the Test and is not appreciable in the Control.



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Since then, cell density decrease became more marked on the Test surface and the difference between densities became statistically significant (medians, 19.5 vs. 7.4 cfu/cm^2 120 minutes after contamination) (**Figure 1b**). At the end of the experiment, cell density on the Test surface was lower than the threshold for hospital surface hygiene (median, 0.7 cfu/cm^2), while on the Control surface it was far from this limit (median, 10.1 cfu/cm^2).

Out of the five experimental events, the lowest density detectable 180 minutes after contamination on the Control surface was 8 cfu/cm^2, while on the Test surface density resulted always lower than the threshold excluding in one event (1.3 cfu/cm^2).

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Table 3. MRSA cell density on the two PVC surfaces artificially contaminated through aerosol. The Test surface was coated with nano-TiO_2-based thin film (Polimero Ossigenato Plastico, POP, Pure-Health^{TM}, Orion, Calenzano, Italy), responsible for photocatalytic disinfection activated by visible-band fluorescence light. Medians and ranges are expressed as cfu/cm^2.

Time	Control Surface		Test Surface		Mann-Whitney U-test
	Median	Range	Median	Range	p-value
Before MRSA contamination	0.03	0.02-0.05	0.03	0.01-0.05	0.67
15 minutes	27.6	19.1-30.2	27.5	14.9-42.3	0.91
30 minutes	35.1	19.0-40.3	34.3	16.7-51.1	0.91
45 minutes	46.3	31.4-76.1	43.1	29.4-60.8	0.75
60 minutes	35.1	13.3-52.7	36.5	25.6-46.8	0.91
90 minutes	**22.2**	14.4-37.6	**12.7**	5.2-16.5	**0.01**
120 minutes	**19.5**	13.2-26.6	**7.4**	5.7-10.4	**0.009**
150 minutes	**16.8**	14.3-18.6	**1.3**	0.8-2.9	**0.009**
180 minutes	**10.1**	8.3-16.2	**0.7**	0.4-1.2	**0.009**

In red bold character statistically significant differences between Test and Control surface cell densities are shown

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The MRSA cell density trend is displayed in **Figure 2**. The quick rise occurred soon after contamination –between 0 and 15 minutes, as well as the steadily high density –observed for 45 minutes, were overlapping in both Test and Control lines. Then, the two lines started diverging, with MRSA cell density on the Control surface remaining over the level of 1 log cfu/cm^2, corresponding to 10 cfu/cm^2, throughout the duration of the experiment, while on the Test surfaces it dropped below the threshold of hospital surface hygiene (green line) within two hours.

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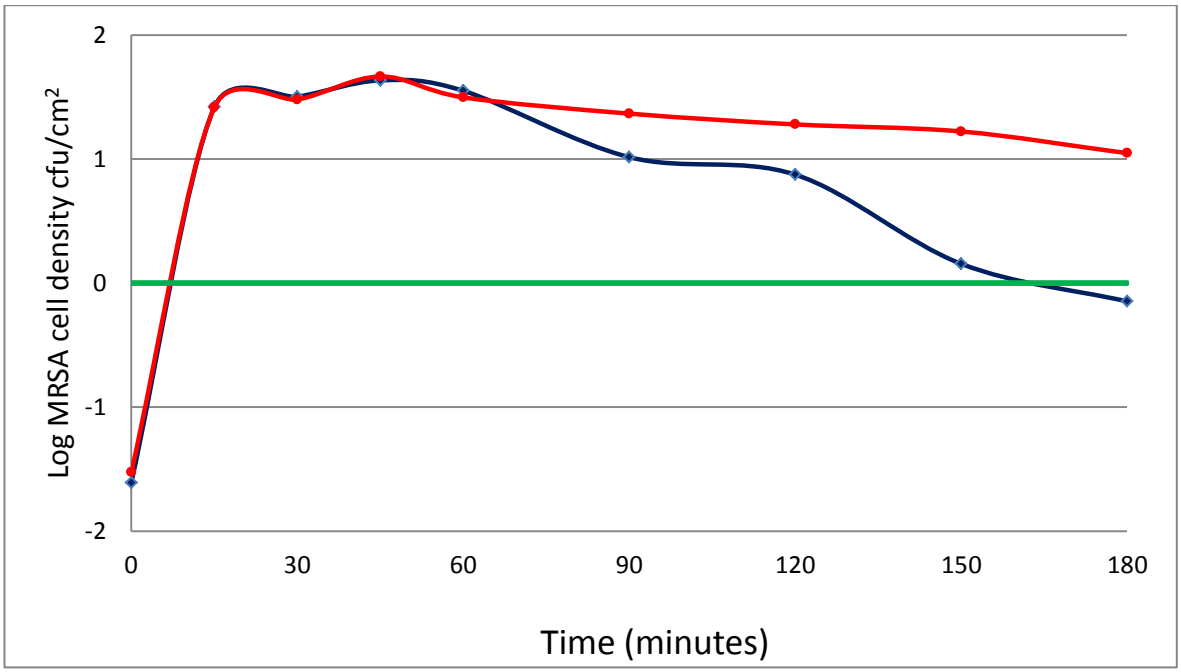
Figure 2. Trend of log MRSA cell density on the Test (blue line) and the Control (red line) surfaces. The green line is the threshold for standard hospital surface hygiene (0 log cfu/cm^2, corresponding to 1 cfu/cm^2).



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The two regression lines with “time since contamination” and “MRSA cell log density” as variables (**Table 4**) showed rather high goodness of fit. Indeed, high adjusted R^2 values (0.7 and 0.8 for Control and Test, respectively) and statistically significant intercept and slope estimates were found. The estimates of time required to reach the threshold for hospital surface hygiene were, therefore, robust enough. Such an estimate for the Test surface was ranging between 1 hour and 46 minutes and 6 hours and 29 minutes, with 95% probability, while for the Control surface was ranging between 6 hours and 27 minutes and 26 hours and 54 minutes, with 95% probability. In other terms, the time necessary to reach the threshold for hospital surface hygiene was between 3 and 5 times higher in the Control surface than in the Test surface.

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Table 4. Regression analysis of the log MRSA cell density trend-lines for the Test and Control surfaces. Intercept, Slope, Value of *x* for *y*=0, corresponding to the estimated time required to reach the standard for hospital surface hygiene (1 cfu/cm^2 corresponding to 0 log cfu/cm^2) are shown (95% confidence interval between parentheses).

Surface	Intercept	**Slope**	**Time required for *y*=0**	Adjusted R^2
Control	1.6084	**-0.0027**	**595.7**	0.696
	(1.4413 - 1.7756)*	**(-0.0044 - -0.0011)***	**(327.6 – 1614.2)**	
Test	1.9256	**-0.0107**	**180.0**	0.874
	(1.5432 – 2.3080)*	**(-0.0145 - -0.0070)***	**(106.4 – 329.7)**	

*t-ratio tests. P<0.05

In red bold character statistically significant differences between Test and Control are shown

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The two regression trend-lines are displayed in **Figure 3**. The difference between the two lines lied principally in the slope, so that the Test trend-line was highly inclined and the intercept with the *x*-axis was relatively close to the *y*-axis, while the Control trend-line was almost flat and the intercept with the *x*-axis was far from the plot.

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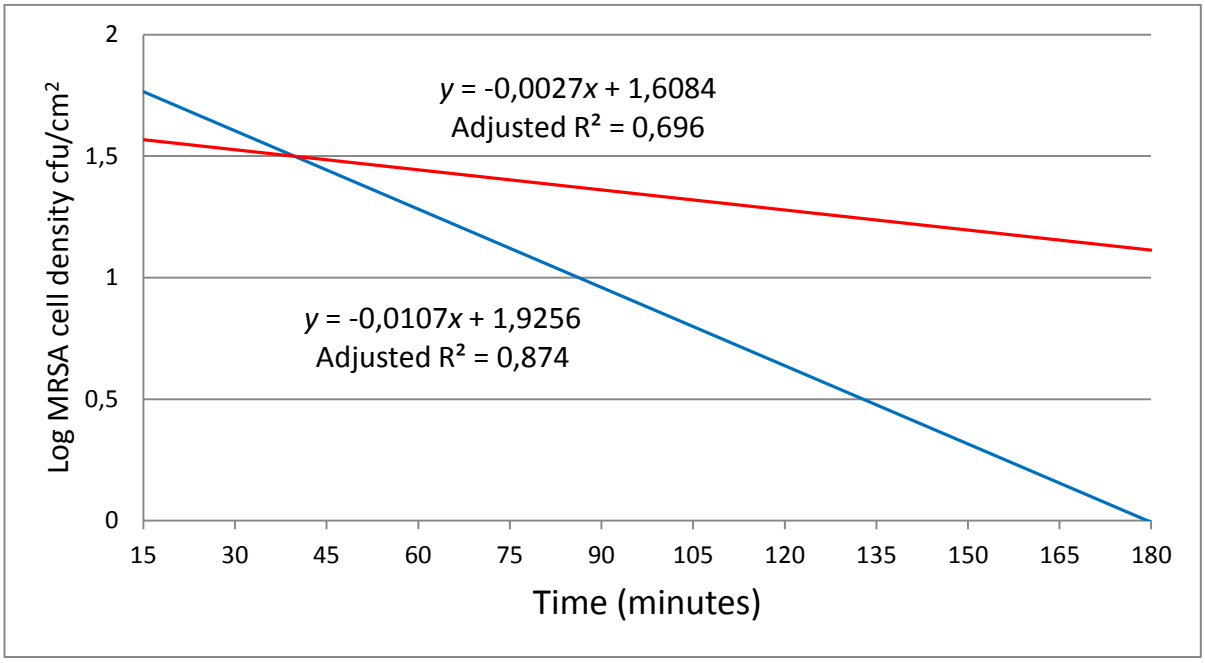
Figure 3. Log MRSA cell density trend-lines in the Test (blue line) and the Control (red line) surfaces assessed through regression analysis. The values of *x* for *y*=0 are 595.7 for the Control, corresponding to 9 hours and 56 minutes 180 for the Test, corresponding to 3 hours.



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DISCUSSION

The problem of MRSA survival in hospital environment is serious, because infected individuals are often immune-depressed and, therefore, unlike Community-Acquired MRSA (CA-MRSA) infections, which generally affect otherwise healthy subjects, HA-MRSA infections often yield high case-fatality rates. Indeed, the annual number of deaths from MRSA infections in US is higher than the number of deaths from HIV and Hepatitis virus infections (Boucher and Corey, 2008), while more than 5,000 deaths are annually attributable to MRSA infections in EU (Köck et al., 2010). In addition, the infectious dose necessary to be colonized by MRSA may be as low as five cells (Dancer, 2014). For these reasons the antibacterial activity of photocatalysis was tested aagainst MRSA in the present study. Another reason was that Gram-positive bacteria are the most resistant to photocatalytic killing and, therefore, MRSA represents a valid test of effectiveness (Foster et al., 2011).

This study simulated real-life conditions of MRSA infected patients who spread these microorganisms in the hospital environment shedding skin particles with adherent MRSA, touching objects and surfaces, coughing, sneezing and talking. These activities result in contaminated aerosol and surfaces. In such real-life conditions, the density of surface contamination is relatively low. Indeed, studies on infected patients, who yield high microbial loads, show that MRSA cell density on hospital surfaces and floor are usually ranging between <1 to 50 cfu/cm^2 (Rutala et al., 1983; Boyce et al., 2007; Otter et al., 2011).

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Although several studies sought to assess the antimicrobial activity of nano-TiO_2-based films against MRSA, this is the first with practical implications for healthcare settings because such an antibacterial activity was assessed in real-life hospital conditions. Indeed, according to the aforementioned study performed in a tertiary hospital it was not possible to assess whether the disinfectant activity of nano-TiO_2-based film was directed against MRSA or Gram-negative bacteria (Chow et al., 2013). Laboratory studies reported contrasting results. One of them used dual layer TiO_2/CuO films, which combine antimicrobial activities of photocatalysis and metal ions. MRSA level showed a limited decrease within two hours, passing from $\log_{10}$ 6 to >$\log_{10}$ 5 cfu (antibacterial activity was, therefore, <1 $\log_{10}$ cfu), while total killing required as many as 24 hours. The tested MRSA density was also not plausible for hospital environmental contamination (Foster et al., 2012). Another laboratory study, investigating the activity of silver-modified photocatalysis, used nano-TiO_2-based films as control and showed similar results. Indeed, a decrease in MRSA level of less than 1 $\log_{10}$ cfu, passing from $\log_{10}$ 5 to >$\log_{10}$ 4 cfu after two hours of exposure was found. Once again, the tested MRSA load was too high. Very interestingly, this study reported the total degradation of bacterial cell wall, which is particularly thick among Gram-positive bacteria like MRSA, due to the process of photocatalysis (Tallósy et al., 2014). Finally, another study that investigated the activity of silver-titanate thin films found that TiO_2-based films, used as control, had no antibacterial activity against MRSA at a density level of 10^5 cfu (Inoue et al., 2010).

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Summarizing the data reported by previous studies, the antibacterial activity of TiO_2-based photocatalysis led to a decrease in MRSA load by less than 1 $\log_{10}$ cfu, corresponding to less than 90%, within the first two hours of exposure. The present study corroborated such an activity within two hours, as shown in **Figure 2**, but added further practical information. Indeed, starting from MRSA cell density levels similar to those usually encountered on highly contaminated hospital surfaces, it was possible to observe an antibacterial activity that exceeded 1 $\log_{10}$ cfu within three hours and, very importantly, MRSA cell density decreased to safe levels (i.e., $<\log_{10}$ 0 cfu/cm^2). Photocatalytic disinfectant activity could be, therefore, considered in addition to the conventional MRSA control methods in hospital and other healthcare settings where the basic infection control measures are not enough, such as Intensive Care Units, Burn Units, etc.

All the conventional environmental MRSA control methods show drawbacks. Surface disinfection is one of these. Indeed, 7-minute contact of bleach on smooth surfaces contaminated by light to moderate MRSA cell density (i.e., 10-14 cfu/cm^2) is effective in decreasing density to minimal values (i.e., 0.03 cfu/cm^2), lower than the standard for hospital surface hygiene (Petti et al., 2013). However, the use of disinfectants is limited by some surface characteristics, such as porosity, roughness, sensitivity to chemical agents, etc., that may decrease their effectiveness or prevent their use. Residual activity may expose Healthcare Providers and patients to the risk of inhalation of toxic and carcinogenic compounds. In addition, it seems that MRSA may carry biocide resistance genes, such as the plasmid-linked quaternary ammonium compound resistance and resistance to triclosan that evolves soon after

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exposure (Cimolai, 2008). MRSA survival on the cloths used for disinfection is another problem, since the reuse of these cloths allows for cross contamination of secondary environments during further wiping (Cheng et al., 2011).

Cleaning is another effective environmental MRSA control method, able to decrease cell density on smooth surfaces from light-moderate to lower than the standard for hospital surface hygiene. However, once again, surface characteristics are crucial (Petti et al., 2013). In addition, enhanced-cleaning effectiveness is based principally on the awareness of the staff and to human resources to devote to such cleaning measures, two conditions that are often difficult to meet (Dancer, 2014).

Other environmental MRSA control methods are not completely convincing. For example, the use of disposable barriers is effective but impractical, because expensive and time-taking and because disposable barrier materials create an important environmental impact (Petti et al., 2013). Pulsed xenon UV light is partly effective, but it is also expensive and its use is limited by environmental factors such as humidity, surface characteristics, etc. (Dancer, 2014; Jinadatha et al., 2014) Hydrogen peroxide vapors show a variable effect (Dancer, 2014).

These data suggest that the best solution to achieve complete MRSA eradication from the hospital environment probably lies in combining basic methods, such as disinfection, with supplementary methods. One of these is certainly the use of nano-TiO_2-based coatings. Indeed, this method has several merits. First, nano-TiO_2 is a catalyst and, therefore, its activity depends only on light activation and is inexhaustible. Second, surface disinfection by photocatalyzed reaction is less toxic/carcinogenic than chemical disinfectants. Third, the

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antimicrobial activity of nano-TiO_2-based coatings is nonselective, and prevents microorganisms from developing resistance to treatment. Fourth, antimicrobial activity is not affected by the characteristics of the surface because it occurs when microorganisms are in direct contact with the surface or close to it (Dancer, 2014; Foster et al., 2011; Joost et al., 2015).

CONCLUSION

The present analysis showed that three hours of exposure to photocatalytic disinfection due to nano-TiO_2-based thin film coating on PVC surfaces is enough to eradicate MRSA from high cell density level that is usually reported in contaminated hospital surfaces, to very low levels that minimize the risk for MRSA infection transmission through environmental surfaces. This disinfection method has important practical applications in the design of healthcare facilities.

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