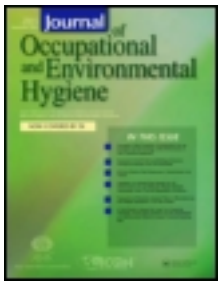

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Emanuele Amodio [a], Lucia Cannova [a b], Maria Rosaria Villafrate [a b], Anna Maria Merendino [a b], Luigi Aprea [a] & Giuseppe Calamusa [a b]

[a] Department of Sciences for Health Promotion "G. D'Alessandro", University of Palermo, Italy

[b] Sanitary management of the Azienda Ospedaliera Universitaria "P. Giaccone" of Palermo

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Emanuele Amodio[a], Lucia Cannova[a,b], Maria Rosaria Villafrate[a,b], Anna Maria Merendino[a,b], Luigi Aprea[a], Giuseppe Calamusa[a,b]

[a] Department of Sciences for Health Promotion "G. D'Alessandro", University of Palermo, Italy

[b] Sanitary management of the Azienda Ospedaliera Universitaria "P. Giaccone" of Palermo

Corresponding author:

Emanuele Amodio

Department of Sciences for Health Promotion "G. D'Alessandro," University of Palermo, Italy, Via Del Vespro n°133, cap 90127

Telephone number: 0039 338 97 20 919

Email: emanuele.amodio@unipa.it

Running title: Bioluminescesce for assessing hospital cleanliness

ABSTRACT

Contaminated hospital surfaces have been demonstrated to be an important environmental reservoir of microorganisms that can contribute to increase the risk of nosocomial infection in exposed patients. As a consequence, cleaning and disinfecting hospital environments play an important role among strategies for preventing healthcare-associated colonization and infections. Aim of the present study was to evaluate if adenosine triphosphate (ATP) presence, measured by bioluminescence methods, can predict microbiological contamination of hospital surfaces. The study was carried out between September and December 2012 at the University Hospital "P. Giaccone" of Palermo.

A total of 193 randomly selected surfaces (tables, lockers, furnishings) were sampled and analyzed in order to assess ATP levels (expressed as relative light units or RLU) and aerobic colony count (ACC) or presence of *S. aureus*. ACC had median values of 1.85 cfu/cm^2 (interquartile range = 4.16) whereas ATP median was 44.6 RLU/cm^2 (interquartile range = 92.3). Overall, 85 (44.0%) surfaces exceeded the established microbial benchmark: 73 (37.8%) exceeded the 2.5 cfu/cm^2 ACC standard, 5 (2.6%) surfaces were positive for *S. aureus* and 7 (3.6%) showed both the presence of *S. aureus* and an ACC of more than 2.5 cfu/cm^2. ACC and bioluminescence showed significant differences in the different surface sites ($p<0.001$). A significant correlation was found between ACC and RLU values (p-value<0.001; $R^2 = 0.29$) and increasing RLU values were significantly associated with a higher risk of failing the benchmark ($p<0.001$).

Our data suggest that bioluminescence could help in measuring hygienic quality of hospital surfaces using a quick and sensitive test that can be an useful proxy of microbial contamination, however further analysis will be necessary to assess the cost-efficacy of this methodology before requiring incorporation in hospital procedures.

Keywords: ATP bioluminescence, hospital, surfaces, aerobic bacterial counts.

INTRODUCTION

Contaminated hospital surfaces have been demonstrated to be an important environmental reservoir of microrganisms that can contribute to increase the risk of nosocomial infection in exposed patients.[1] As a consequence, cleaning and disinfecting hospital environments play an important role among strategies for preventing healthcare-associated colonization and infections. [2] However, a major problem in defining these recommendations is to classify when a surface can be considered "clean" and "acceptable". Although, a large number of international guidelines adopt visual surface inspection for measuring cleanliness, [3] however, this method cannot be considered an accurate measure of surface cleanliness or of microbial contamination and previous studies have indicated that it may overestimate cleaning efficacy [4] failing to predict the risk of infection for patients.[5] To this purpose, several other tools and methods including microbiological methods (e.g. aerobic bacterial counts, culture of indicator organisms) and bioluminescence tests have been proposed for assessing cleanliness on a more scientific basis. Microbiological cultures are commonly thought to adequately describe the infectious risk attributable to contaminated surfaces but they are time-consuming needing a minimum of 24 hours.

Otherwise, bioluminescence tests, assessing the adenosine triphosphate (ATP) presence, provide a quick and objective feedback on surface cleanliness, reporting the presence of organic materials including microbiological contamination. Few studies have evaluated ATP bioluminescence methods for monitoring cleanliness in hospitals but the relationship between bioluminescence and microbiological results remains still poorly characterized. [4,6-8] In this

topic, the aim of the present study was to evaluate if ATP presence, measured by bioluminescence methods, can predict microbial contamination of hospital surfaces.

METHODS

Study design

The study was carried out between September and December 2012 at the University Hospital "P. Giaccone" of Palermo that includes 72 hospital Units accounting for over 500 beds.

Surfaces to sample were randomly selected proportionally to the size of the each Unit. Sampling was performed during the morning (7.00 to 11.00 am) about 2 hours after sanitization. Surface sites included tables, lockers and furnishings (e.g. bed, chairs) in patient and healthcare worker rooms. Each site was sampled in order to measure aerobic colony count (ACC), ATP and *Staphylococcus aureus* presence.

Bioluminescence and microbiological analyses

ATP levels were measured using Lumicontrol II (PBI International, Italy) and expressed as relative light units (RLU). A swab for ATP revelation in a 10x10 cm surface sampled by a close zig-zag pattern according to the manufacturer's guidelines.

Microbiological assessment was based upon growth on slides pre-coated by the manufacturer (OXOID, Cambridge, UK) with Plate count agar and Mannitol Salt Agar with addition of germicide inhibitors. Each slide (55 mm) was pressed for 15 seconds onto the surface adjacent to

that where ATP was determined and then incubated aerobically at 37°C for 48 hours. Colony density was determined by visual count according to the manufacturer's standards. Each colony present in Mannitol Salt Agar and suggestive for *S. aureus* was then isolated in Diagnostic Sensitivity Test Agar and then identified and confirmed by detection of bound coagulase/protein A and biochemical specific test for bacterial identification (Api Staph – BIOMERIEUX, Florence, Italy).

Surfaces were considered at risk for determining infection when they have an ACC of at least 2.5 cfu/cm^2 or presence of *S. aureus,* as indicator organism of pathogen contamination. [5,9-11]

Statistical analyses

Data were analyzed using the R statistical software package.[12] Statistical significance was defined as $p \leq 0.05$, two-tailed. Absolute and relative frequencies were calculated for qualitative variables. Quantitative variables were summarized as median (interquartile range). Frequencies were analyzed by Chi-square test whereas medians were compared by using the Mann Whitney Rank sum test. Pearson correlation coefficient and linear regression model were used to describe the relationship between logarithmic transformed ACC and ATP values. Finally, ACC>2.5 cfu/cm^2 or presence of *S. aureus* were considered as standard microbial benchmark indicating significant environmental contamination.

RESULTS

During the study period, a total of 193 surfaces were analyzed: 89 tables (46.1%), 50 lockers (25.9%) and 54 furnishings (28%). ACC had median values of 1.85 cfu/cm^2 (interquartile range = 4.16) whereas ATP median was 44.6 RLU/cm^2 (interquartile range = 92.3).

Overall, 85 (44.0%) surfaces exceeded the established microbial benchmark: 73 (37.8%) exceeded the 2.5 cfu/cm^2 ACC standard, 5 (2.6%) surfaces were positive for *S. aureus* and 7 (3.6%) showed both the presence of *S. aureus* and an ACC of more than 2.5 cfu/cm^2. Although surfaces contaminated with *S. aureus* showed higher ACC and ATP medians (3.5 vs. 2 cfu/cm^2 and 51.3 vs 43.5 RLU/cm^2, respectively), these associations were not statistically significant (p=0.057 and p=0.36, respectively).

As reported in figure 1, both ACC and bioluminescence showed significant differences in the three surface sites (p<0.001 in both cases). In particular, lockers and tables were more contaminated with microbiological (median ACC = 5.5 and 2.5 cfu/cm^2, respectively) and organic (median ATP = 61.3 and 46.1 RLU/cm^2, respectively) materials.

Figure 2 shows the correlation between ACC and logarithmic transformed RLU values (F-statistic=80.3; p-value<0.001, with a R^2 value of 0.29).

Finally, figure 3 presents the distribution of samples that failed the established microbial benchmark (ACC>2.5 cfu/cm^2 or presence of *S. aureus*) in relation to their ATP values.

Increasing RLU values were significantly associated with a higher risk of failing the benchmark (p<0.001). The totality of samples with RLU values <100 passed the benchmark whereas more than 40% of samples with RLU >1,000 failed the standard.

DISCUSSION

Although hygiene of hospital surfaces is internationally advocated as necessary to control nosocomial infections, to date there are still several concern about the methods to prefer for assessing environmental cleanliness. As previously reported, visual assessment is probably the less accurate measure of surface cleanliness [4,5] whereas microbiological assessments could be considered as a reference method, being able to well predict, at least theoretically, the risk of infection for patients. In the last decades several authors have purposed detection of ATP bioluminescence for quickly monitoring and having a standardized sensitive measure of hospital cleanliness. However, among the few studies that compared this method with microbiological cultures, a large majority found no correlation between RLU values and ACC.[13-15] Otherwise, a minority of authors reported a good agreement or a weak relationship between the two methods.[16-17] Our results confirm these last findings, suggesting that surfaces with low microbial contamination had also low bioluminescence and that RLU values could explain about one third of the ACC variability. According to this observation, Griffith et al found that about 33% of the ATP from hand contact surfaces is likely to be of microbial origin with the remainder non-microbial.[4]

The awareness that a part of measured bioluminescence is usually due to microbial presence could be useful to set a minimum ATP value that could be suggestive of low microbial contamination. Furthermore, benchmark values of RLU 100, [17] 250 [9] and 500 RLU [3,4,14] for ATP testing have been proposed for identify surfaces with a significant bacterial contamination, as recommended by international guidelines. The present study shows that a certain number of surfaces with ATP values ≤500 RLU could have a microbial contamination higher than 2.5 cfu/cm^2 or presence

of *S. aureus* and, consequently, considers that the use of such a benchmark could be questionable. Otherwise, our experiences suggest that, using Lumicontrol II, an ATP value of 100 RLU could be a better bioluminescence benchmark, exposing to a very low risk (<5%) of considering as "clean" a surface significantly contaminated.

All these considerations could be, at least potentially, affected by some limitations. As first point, the culture plates used are a selective filter and only indicate presence of organisms that may be culturable on such a media whereas no information are provided about those that are not culturable (e.g. anaerobic organisms and viable but non-culturable organisms). Secondly, our study has not considered standardized experimental contamination but included surfaces cleaned by various personnel and highly contaminated by patients, personnel and visitors. This last point can also be considered a strength since it describes the real surface situation in hospital environment. In conclusion, our data suggest that bioluminescence could help in measuring hygienic quality of hospital surfaces using a quick and sensitive test that can be an useful proxy of microbial contamination, however further analysis will be necessary to assess the cost-efficacy of this methodology before requiring incorporation in hospital procedures.

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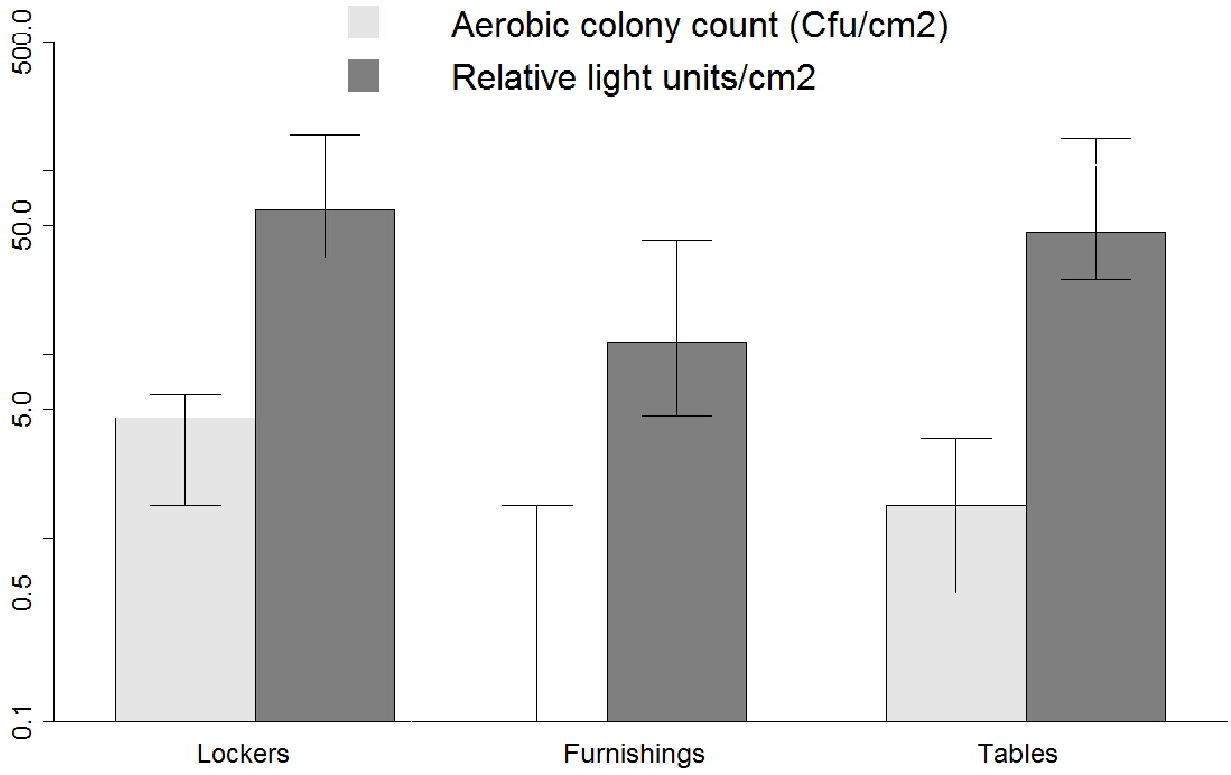

Figure 1: ACC and bioluminescence (median with 25th and 75th percentile) measured in different surface sites.

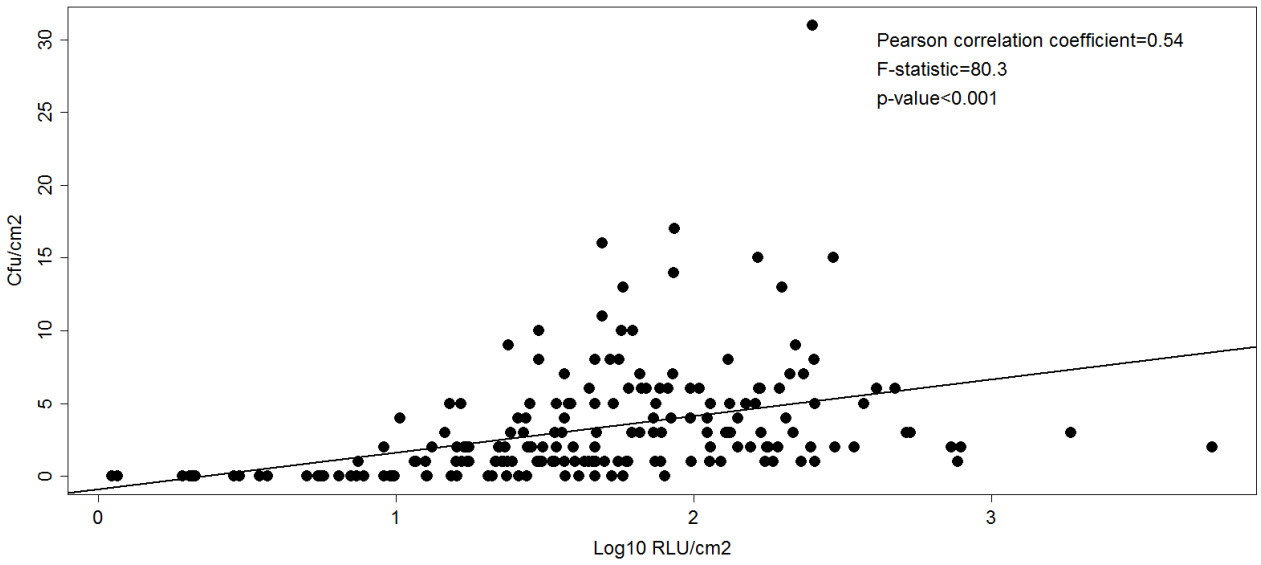

Figure 2: Correlation between ACC/cm^2 and logarithmic transformed RLU/cm^2 values.

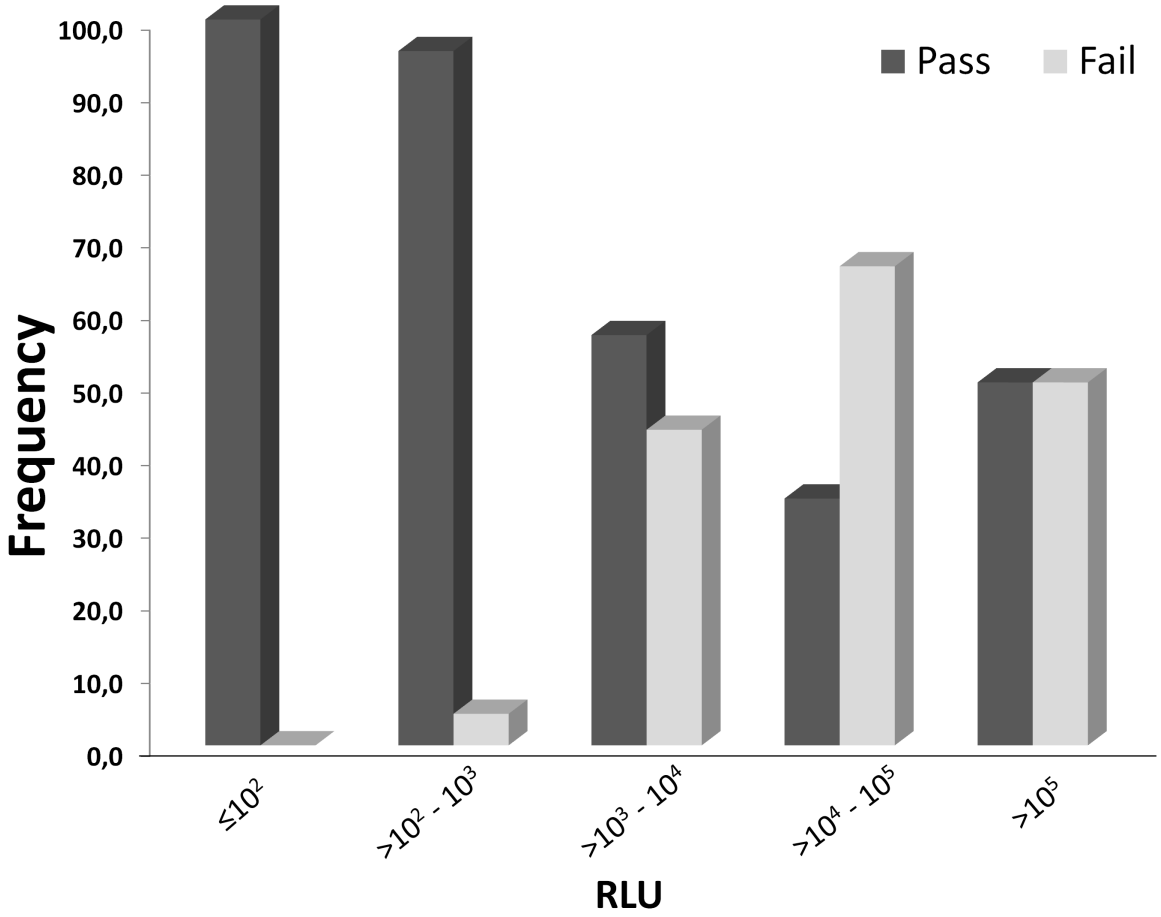

Figure 3: Distribution of samples that failed or passed the established microbial benchmark (ACC>2.5 cfu/cm^2 or presence of *S. aureus*) stratified for different ATP values.