Investigation of the Cleanliness of Hospital Environmental Surfaces by Adenosine Triphosphate Bioluminescence Assay

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Summary

This study evaluated the combined use of adenosine triphosphate (ATP) bioluminescence and microbiological assays for monitoring environmental surfaces in a teaching hospital to develop a method for rapid detection of microbial contamination that could constitute a health risk to patients. ATP bioluminescence assay and microbiological screening were performed of various surfaces of toilet facilities in outpatient wards. In each of the five sites screened, the ATP levels were significantly higher than on a cleaned, disinfected stainless steel surface, indicating that all screened surfaces were “high-touch” surfaces and the surfaces harbored significantly higher levels of certain organic matter. The microbiological assay confirmed that the microbiological contamination had spread throughout the screened sites. The ATP values of the samples positive for microbes occurred in a significantly higher range than those of the samples negative for microbes (p < 0.01). However, no linear relationship was established between the ATP values and aerobic colony counts of the screened sites. These results clearly imply that the use of ATP bioluminescence to measure the microbial contamination of an environment yields qualitative rather than quantitative data. In conclusion, ATP monitoring is a rapid and convenient method to assess environmental contamination and persistence of microbes and to monitor the effectiveness of current cleaning practices.

Key words: hospital environment, environmental surfaces, ATP bioluminescence, microbiological contamination, cleaning procedure

Introduction

The primary function of hospitals is to provide medical treatment, but inevitably these facilities also act as a gathering place for patients and their attendants, physicians, supporting staff, and visitors. Such a varied population will include not only the patients infected with diverse pathogens but also healthy carriers. Any of these individuals can act as vectors through contact with the hospital surfaces. Numerous studies have shown that hospital surfaces become contaminated by various pathogenic and nonpathogenic organisms, even in the absence of direct contact with the patients, and that various drug-resistant microorganisms inhabit hospital surfaces. Most infections occurring in hospital environments are known to result from transmission through touching with the hands. Therefore, any hospital surface that is frequently touched with the hands could pose a substantial risk for harboring and transmitting pathogens.

Environmental cleanliness is measured using methods such as adenosine triphosphate (ATP) bioluminescence assay and microbiological screening. These methods have both been modified to assess hospital cleanliness by generating values expressed in relative light units (RLU) or aerobic colony counts (ACC), respectively. The present study surveyed organic and microbio-
logical contamination on the surfaces of toilet facilities in the hospital environment, and evaluated the association between the level of contamination by organic matter and the level of microbiological contamination in order to determine the microbiological risks posed by the environmental surfaces.

Methods

1. Screening frequencies
   This study was performed at a teaching hospital in Tokushima, Japan. ATP bioluminescence assay and microbiological screening were each performed twice for each survey site examined.

2. Choice of screening sites
   The choice of screening sites is important, because clinical risk is associated with surfaces that are frequently touched.11) We selected the toilet facilities in the outpatient wards. The screened sites included a wall of the facility, the locking mechanism and the grab bar inside a toilet stall, the remote control unit for activating the toilet’s bidet and other functions, and the stainless steel cover of the toilet paper holder. All these surfaces were routinely cleaned by a professional cleaning service. The area targeted for screening was divided into two approximately equal parts so that one part could be used for the bioluminescence assay and one part for the microbiological screening. The exception was the remote-control units, in which the same surface areas of the buttons were sampled for both assays.

3. ATP bioluminescence assessment
   Organic matter at each site was measured using ATP bioluminescence. ATP levels were determined using LuciPac Pen swabs (Kikkoman Biochemifa Co., Tokyo, Japan) and a luminometer, Lumiitester PD-20 (Kikkoman Biochemifa Co.), in a close zigzag pattern and expressed as RLU according to the manufacturer’s instructions.

4. Quantitative and qualitative microbiological assessment
   Total ACC was performed in a separate half of each targeted area, so that the areas used for the ACC and ATP bioluminescence assessment did not overlap. We used a sterile cotton-tipped swab (Check Stick; Sansei Medical Co., Ltd., Kyoto, Japan) for microbial sampling. First, the area was rubbed using a pre-moistened swab with sterile 0.01 M phosphate-buffered saline (PBS, pH 7.5). Then, the cotton-tipped portion of the swab was snapped off and put into a test tube containing 2 ml of sterile PBS. The tube was vortexed for 1 minute. ACC was determined using Trypticase Soy Agar (BD Japan, Tokyo, Japan) plates for the sample solution and its 10-fold dilution (each 100 μl). The plates were incubated aerobically at 30°C for 4 days.

5. Data analysis
   Data are expressed as the median (1st–3rd quartile). The results were primarily analyzed using Bartlett’s test to test for homogeneity of variances. If the results did not show normal distribution, a non-parametric Kruskal-Wallis test was used to compare the groups in addition to the standard descriptive statistical calculations (means, standard deviations, medians, and interquartile ranges), and a post hoc Tukey-Kramer multiple comparison test (Steel-Dwass test) was utilized to compare subgroups. Statistical significance level was set at p < 0.05. All statistical analyses were performed with Statcel 3.

Results

In the hospital environment, housekeeping surfaces are generally classified into surfaces with minimal hand contact (referred to as “low-touch” surfaces) and surfaces with frequent hand contact (“high-touch” surfaces). To clarify the characteristics of the sites chosen for screening, organic matter in 152 and 106 samples were screened for ATP assessments and microbiological detections, respectively.

The ATP levels of the 152 screened sites categorized according to the types of surfaces sampled are summarized in Fig. 1. In our preliminary experiments, a well-cleaned stainless steel vessel was au-
toclaved for 20 minutes at 121°C, and then its surface was examined for organic matter as a negative control. The ATP values were only 40–80 RLU (data not shown). In contrast, every ATP level detected in the present study was considerably higher. The ATP values were generally lower in the wall (median, 3,277 RLU; 1st–3rd quartile, 1,934–6,421 RLU) and the door lock samples (median, 3,775 RLU; 1st–3rd quartile, 1,896–9,445.5 RLU), and higher on the grab bar (median, 14,412 RLU; 1st–3rd quartile, 5,694–26,289 RLU), the remote control unit (median, 6,514 RLU; 1st–3rd quartile, 3,211.5–12,411 RLU), and the stainless steel cover samples (median, 8,180.5 RLU; 1st–3rd quartile, 3,425.2–20,578.8 RLU). Statistical analyses indicated significant differences between the wall and grab bar samples, between the wall and stainless steel cover samples, and between the grab bar and door lock samples. Except for these combinations of samples, no statistically significant differences were found between the types of surfaces sampled.

Figure 2 summarizes the AAC levels of the 106 screened sites classified into the five groups described above. No microbial growth was found in 21 samples, whereas some colonies formed in all others. No statistically significant differences were found between the wall and stainless steel cover samples, or between any of the pairs of grab bar, remote control unit, and door lock samples. On the other hand, significant differences were observed between each of the latter three sites compared to the wall or stainless steel cover sites.

In the present survey, samples with viable microbes were referred to as the positive microbial detection group, whereas samples with no viable microbes were referred to as the negative microbial detection group. The smallest ATP value within the positive microbial detection group was 849 RLU. ATP levels of the negative microbial detection group ranged from 522 to 10,865 (median, 3,274 RLU; 1st–3rd quartile, 1,900–4,367 RLU), and those of the positive microbial detection group ranged from 849 to 448,563 (median, 8,350 RLU; 1st–3rd quartile, 3,645–23,012 RLU) (Fig. 3).

Samples were classified according to previously published microbial growth criteria as follows: scanty growth (<2.5 cfu/cm², SG), light growth (2.5–12 cfu/cm², LG), moderate growth (12–40 cfu/cm², MG), heavy growth (>40 cfu/cm², HG), and no growth (NG).15) The ATP values within the MG and HG categories were combined, since there was only one data point within the HG category. Statistical analysis revealed significant differences between each of the three categories (SG, LG, and MG and HG) and the NG category. However, no significant differences were observed between any two pairings within the three categories (Fig. 4).

Table 1 shows the distribution of samples for each microbial growth category that exceeded four ATP values (1,000, 5,000, 10,000, and 50,000 RLU) for
Table 1. Proportionate samples according to four chosen relative light unit (RLU) values for each microbial growth category

<table>
<thead>
<tr>
<th>Microbial growth category</th>
<th>Total number of samples</th>
<th>ATP values within each microbial growth category that exceeded the ATP values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1,000 RLU</td>
</tr>
<tr>
<td>NG (&lt;2.5 cfu/cm²)</td>
<td>21</td>
<td>17 (81%)</td>
</tr>
<tr>
<td>SG (2.5–12 cfu/cm²)</td>
<td>52</td>
<td>51 (98%)</td>
</tr>
<tr>
<td>LG (12–40 cfu/cm²)</td>
<td>25</td>
<td>25 (100%)</td>
</tr>
<tr>
<td>MG (&gt;40 cfu/cm²)</td>
<td>7</td>
<td>7 (100%)</td>
</tr>
</tbody>
</table>

All microbial growth values are presented as aerobic colony counts per cm². NG, no growth; SG, scanty growth; LG, light growth; MG, moderate growth; HG, heavy growth.

Discussion
Routine cleaning of toilets and hand wash facilities is important to prevent the risk of spread of germs.16–19 However, cleaning in a crowded cluttered place is far more complex than cleaning offices. In the facility investigated by the present study, the cleanliness and sanitation of the hospital environment was managed by outsourcing the responsibility to a commercial cleaning service. Periodic general cleaning and sanitation were performed by company staff. However, wide variation in the ATP values was detected at the different sites examined, and every ATP level detected was considerably higher than that of the cleaned, disinfected stainless steel surface used as a control (40–80 RLU). Well cleaned surfaces with little organic matter yield <250 RLU, whereas poorly cleaned surfaces can yield >1,000 RLU.14 Therefore, it would be hard to conclude that the cleaning procedures used for the toilet facilities were adequate to maintain sanitary conditions. As with all professional activities, effective cleaning requires teaching and training, and never more so than in hospital.20

Figure 1 shows that the ATP values of the samples could be classified into two groups: a lower–ATP group consisting of the wall and the door lock samples, and a higher–ATP group consisting of the grab bar, the remote control unit, and the stainless steel cover samples. Significant differences were found between the wall and the grab bar samples, between the wall and the stainless steel cover samples, and between the grab bar and the door lock.
samples. No statistically significant differences were found at other combinations of sites. However, the highest median value was only 4.4 times higher than the lowest median value. These findings clearly demonstrate that all the surfaces screened in the present study were “high-touch” surfaces and harbored significantly higher levels of certain organic matter, probably including microbes.\(^\text{21,22}\)

The microbiological screening proved that microbiological contamination was spread throughout all the sites screened (Fig. 2). The AACS of the wall and the stainless steel cover samples were lower than those of the other sites screened. The latter finding was inconsistent with the high ATP level of the stainless steel cover samples (Fig. 1). Possibly the conventional procedure for cleaning and disinfection of the various surfaces had failed to remove persistent organic matter but did reduce the ACCs depending on the shapes and materials.\(^\text{21,22}\)

**Figure 3** clearly shows that the ATP values of the positive microbial detection group occurred within a significantly higher range than those of the negative microbial detection group (\(p < 0.01\)). In contrast to a previous study,\(^\text{23}\) we failed to show a linear relationship between the ATP values and ACCs of the screened sites (data not shown). On the other hand, we found no significant differences between any two of the microbial growth categories (SG, LG, and MG and HG) (Fig. 4). These results clearly imply that the assessment procedure using ATP bioluminescence for microbial contamination of the environment is only qualitative and not quantitative. Comparing the distributions of the number of ATP values for each microbial growth category that exceeded four ATP values (1,000, 5,000, 10,000, and 50,000 RLU) for each screening site clearly demonstrated that almost half of the samples of the positive microbial growth categories were present whereas few samples of the population of NG category remained (Fig. 5).

In conclusion, microbiological and ATP monitoring successfully revealed environmental contamination and the persistence of microbes, and quantified the effect of current cleaning practices within this hospital environment. The ward, toilet, and hand washing facilities must be shared by many people, raising the risk of touching common environmental surfaces.\(^\text{5-11}\) Transfer of microbes from contaminated hands to the environment is well known.\(^\text{9}\) Hand hygiene is considered to be the single most important measure that can be taken to prevent infection.\(^\text{24}\) Contaminated surfaces are a risk for microbial transmission through contamination of the hands of hospital staff or patients, and also throughout the community through the hands of discharged patients, visitors, and staff. Various bacteria can survive on the hands and surfaces for hours or even days after initial contamination.\(^\text{25-27}\)

Cleaning and disinfecting environmental surfaces may be a routine task, but requires professional approaches. Meticulous attention to cleaning and disinfection is necessary to prevent cross contamination. The ATP bioluminescence assay is an outstanding tool for the rapid and convenient assessment of microbial contamination on environmental surfaces.

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ATP 拭き取り調査による院内環境表面のモニタリング

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要 旨
病院環境表面のモニタリングについて、アデノシン三リン酸(ATP)生物発光および微生物学検査を組み合わせて使用することにより評価した。目的は、患者に対して危険をもたらしうる微生物汚染の迅速な検知方法を開発することである。この目的のために、私たちは、外来病棟におけるトイレ設備表面の ATP 生物発光分析および微生物学的検査を行なった。5か所のスクリーニング部位はすべて、清潔で消毒されたステンレス鋼表面よりも著しく高い ATP レベルを示し、高度接触表面であること、そして有意に高い濃度の有機物を保持していることがわかった。微生物学的検定により、微生物汚染がスクリーニング部位の至る所に広がっていることを確認した。微生物が検出されるサンプルの ATP 値は、微生物が検出されないサンプルの ATP 値より有意に高かった (p<0.01)。しかしながら、スクリーニング部位の ATP 値と好気性コロニー数との間の直線的関係は確立されなかった。これらの結果は、環境の微生物汚染の測定において ATP 生物発光を使用して得られるデータは、定量的ではなく定性的なものであることを明らかに示唆している。結論として、ATP モニタリングは、微生物による環境汚染およびそれが持続的な汚染状態にある可能性を把握し、院内環境に対する現在の清掃方法に警鐘を鳴らすうえで、迅速かつ簡便な優れた方法である。

Key words：院内環境、環境表面、ATP 生物発光、微生物汚染、クリーニング方法

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