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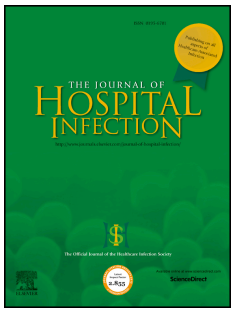

Evaluation of a modified cleaning procedure in the prevention of carbapenem-resistant A*cinetobacter baumannii* clonal spread in a burn intensive care unit using a high sensitivity luminometer

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Title: Evaluation of a modified cleaning procedure in the prevention of carbapenem-resistant A*cinetobacter baumannii* clonal spread in a burn intensive care unit using a high sensitivity luminometer.

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Running title: Validation of a modified cleaning procedure

Keywords: Carbapenem-resistant *Acinetobacter baumannii,* Burn intensive Care Unit, cleaning schedule, surfaces, ATP assay, PFGE.

Summary

Background: Enhanced environmental cleaning practices are one of the most accepted measures for controlling the spread of carbapenem-resistant *Acinetobacter baumannii* (CR-*Ab*).

Aim: To evaluate the impact of heightened cleaning on an ongoing CR-*Ab* outbreak in a Burn Intensive Care Unit (BICU) of an Italian teaching hospital, where chlorhexidine-60% isopropyl alcohol was applied as a complementary disinfectant on high touch surfaces.

Methods: The compliance with the microbial limit proposed for BICU by the AFNOR-NF-S90-351 (20 CFU/100cm^2) was assessed with plate count and compared with the results obtained with the intracellular Adenosine Triphosphate (ATP) detection. Genotyping was performed using pulsed-field gel electrophoresis.

Findings: During the standard cleaning regimen, 3 out of 23 samples (13%) gave results over the AFNOR limit and 5 (21.7%) showed unacceptable ATP levels with 100 RLU/100cm^2 as benchmark limit (sensibility 86,4%, specificity 92,2%). Following the improvement of the cleaning procedure, only 2 samples out of 50 (4%) did not satisfy microbiological criteria and 7 (14%) exceeded the ATP limit. 30 samples collected during suboptimal cleaning showed 27% unacceptable results (8/30).

Conclusions: Adding chlorhexidine-60% isopropyl alcohol as complementary disinfectant proved effective in reducing the environmental microbial contamination as well as ATP levels and *CR-Ab* infections/colonizations in patients admitted in the BICU. Real-time monitoring by ATP assay was useful in managing the cleaning schedule and in reducing hospital infections, although the calculated values must be interpreted as cleanliness indicators instead of risk indicators.

INTRODUCTION

Transmission of healthcare-associated pathogens most frequently occurs via the transiently contaminated hands of healthcare workers.[1,2] However, environmental surfaces such as medical equipment and electronic devices and high touch surfaces may also contribute to the spread of pathogens.[1, 3-4] Both Gram-positive (e.g. MRSA and VRE) and Gram-negative multidrug-resistant bacteria have been documented to survive on environmental surfaces.[1,3] In particular *Acinetobacter baumannii*, a pathogen capable of colonizing the patients and causing multiple healthcare-associated infections with fatality rate up to 40%, was commonly associated with moist and dry environmental sources.[5-8]

Hand hygiene, contact precaution, active patient screening and improved environmental cleaning practices are the most accepted measures for controlling the dissemination of MDR *A. baumannii*.[8] It is widely accepted that improved methods of disinfecting the hospital environment are needed since routine cleaning of housekeeping items does not always remove pathogens from contaminated surfaces.[9-10] Although routine sampling of environmental surfaces in healthcare environments is not usually indicated, it may be required in order to identify an environmental source of infection/contamination and to demonstrate efficacy of disinfection or cleaning procedures in endemic or epidemic situations.[11]

There is no standard method for measuring cleanliness of surfaces or the achievement of certain cleaning parameters or for defining the level of microbial contamination that correlates with good or poor environmental cleaning practices. As reported in the CDC Guidance,[12] the methods used so far include direct practice observation, swab cultures, agar slide cultures, application of fluorescent markers on surface and the detection of Adenosine Triphosphate (ATP) bioluminescence.[13] Since visual assessment can overestimate cleanliness,[14] proposals for bacteriological standards have been suggested as a more effective means of hygiene monitoring.[10]

Since the 1980s, agar slide cultures have been used, first in the food, and then in the pharmaceutical industry, as a standard method to evaluate hygienic status.[15] This method has some limitations, such as the prolonged incubation periods required and the inability to detect viable but nonculturable microorganisms. In response to the need for alternative methods that are faster and more accurate the ATP testing of healthcare environmental surfaces and clean-room facilities was developed.[14, 15-17]

In Italy, a guideline for definition of microbial safety standards and environmental hygiene of operating rooms exists,[18] but environmental safety settings for other clinical settings are not standardised, with the exception of pharmacy clean rooms for which Good Manufacturing Standard are required. By contrast, probably the most stringent standard within Europe relating to healthcare premises is the French norm AFNOR NF-S90- 351;[19] this classifies four zones based upon the level of risk of infection a patient is exposed to.

In this study we evaluated the impact on an ongoing CR-*Ab* outbreak in a Burn Intensive Care Unit (BICU) of an Italian teaching hospital where chlorhexidine-60% isopropyl alcohol was applied as a complementary disinfectant on high touch surfaces. The efficacy of the modified cleaning process in the prevention of *CR-Ab* environmental spread was assessed by plate count and a rapid and sensitive bioluminescence test.

METHODS

SETTING: The study was carried out between April and October 2014 in a 7-bed regional Burn Center in the Azienda Ospedaliero-Universitaria Pisana (AOUP) a tertiary-care teaching hospital in Pisa, Italy. An increasing number of patients were acquiring nosocomial CR-*Ab* infection, In 2012, 13 out of 123 (10.6%) of admitted patients acquired CR-*Ab* infection associated with a positive blood culture; in 2013 19 out of 94 (20.2%) patients had this infection. Moreover, reduced carbapenem susceptibility was observed, the MIC_{50} values for imipenem and meropenem being 24 mg/L (range 0.25 to >32 mg/L) and >32 mg/L (range

4 to >32 mg/L), respectively. An infection prevention and control management plan was instituted, which included a bundle of practices such as screening at admission and thereafter twice weekly, strict application of contact precaution, isolation or cohorting of colonized or infected patients, dedicated equipment for single-patient use where possible. Moreover, the cleaning procedures were revised and audited and an antimicrobial stewardship program started. In particular, standard operating procedures for environmental cleaning and monitoring of critical surfaces were improved and evaluated by a cross over trial.

Standard cleaning procedure was performed at 8 am and 5 pm by an external contractor, whereas cleaning of surfaces in the immediate surrounding patient's area and surfaces regularly touched by healthcare workers (high-touch surfaces) was performed by hospital hostess staff. Both the external contractor staff and the hospital hostess staff took part in training courses on the adoption of proper cleaning procedures. The existing protocol involved disinfection by application of a manually prepared aqueous solution of 1400 mg/L of available chlorine sodium hypochlorite, with reusable cotton cloths (disinfected by the external contractor after use). Based on the results obtained during the first phase of the monitoring, the protocol was modified by supplementing the standard cleaning procedure with twice-daily wiping of all high-touch surfaces surrounding the patient and regularly touched by healthcare workers (e.g. mobile and office phones, tablets, keyboards and mouse, touch screen monitors, bed rails, patient tables); the same wiping was used on the stainless steel surfaces of the hydrotherapy tub on which residual chlorine showed a significant corrosion. Wiping was performed using disposable clothes moistened with a ready-for-use solution of 0.5% chlorhexidine-60% isopropyl alcohol.

SAMPLE COLLECTION: In each room, environmental surfaces were selected for monitoring on the base of their potential significance in the transmission of infection and according to the CDC definition of high touch surfaces.[12] All sites were sampled in the late morning, after cleaning, once a week.

Between April 2014 and October 2014, without notice to the BICU's operators, 103 sites were monitored by swab sampling to assess microbial contamination and ATP bioluminescence. We used three sterile, Ringer's solution moistened swabs, joined together, to sample an area of 100 cm^2 simultaneously. Immediately after sampling, the swabs were brought to the laboratory in a refrigerated container at a temperature of approximately 4°C, where one was used for ATP detection, and the remaining two were streaked on agar plates for total microbial count.

ATP DETECTION: The presence of intracellular ATP was assessed by the Pallchek™ Rapid Microbiology System (detection limit: 0.01 fmol of ATP). The bioluminescence released during D-Luciferin oxidation was measured in RLU/100cm^2 (Relative light units). Briefly, the cotton swab was eluted with 50 ml of sterile-ATP free Ringer's solution (Biokar Diagnostics, Beauvais, France), then filtered on 0.45 µm funnel cellulose-nitrate membrane. The membrane was rinsed with 100 ml of Ringer's solution to eliminate all the residual products. According to the manufacturing protocol, extraction reagent was added directly on membrane surface and after the addition of Luciferase's reagent the intracellular ATP was read as RLU.

MICROBIOLOGICAL ANALYSES: Total aerobic colony counts were determined by streaking one swab on Tryptic Soy Agar (TSA, Oxoid Ltd., Basingstoke, UK), incubated at 35°C for 72 hours and the other onto Sabouraud Dextrose Agar (SDA, Oxoid Ltd., Basingstoke, UK), incubated at 22°C for 120 hours for total moulds and yeasts count. Suspect *Acinetobacter* spp. growing on TSA were subcultured onto Violet Red Bile Agar, and lactose non-fermenting colonies were confirmed with **API**®/ID32 Strep Miniature System, (bioMérieux, Marcy l'Etoile, France).

MOLECULAR TYPING: A total of 20 *A. baumannii* clinical and environmental strains (11 and 9 respectively) were chosen to be genotyped in order to assess the source of nosocomial infection. Molecular typing was performed by comparing the genomic profile according to the PFGE Typing Protocol Recommended by ARPAC for *A. baumannii* as

described in details elsewhere.[20] In an attempt to improve inter-gel comparisons Gel Doc 2000 (Bio-Rad), was used to analyse the PFGE profiles. The patterns were analyzed by means of Diversity Database Software, V. 0.2 (Bio-Rad), using the Dice band-based similarity coefficient and the UPGAMA as clustering methods. The isolated strains were assigned to the same pulsetype if they had similarity coefficients of ≥ 0.95.

RESULTS

During the environmental monitoring phase, ATP values were determined on 103 surface samples, and showed a considerable variation of the light signal: RLU/100 cm^2 values ranged from 7.7 to 7200, with a mean value of 192.35 ± 0.44 RLU/100 cm^2.

High ATP values were generally associated with high microbiological loads, that exceeded the benchmark value of 20 CFU/100 cm^2: 13 out of 103 sites (12.6%) were unacceptable according to the French AFNOR recommendation, since the total microbial counts ranged from 20 to 950 CFU/100 cm^2, exceeding the limit of 20 CFU/100 cm^2.

Samples collected during the standard hypochlorite disinfection regimen showed 3 out of 23 (13%) values out of limit. In the first phase of the improved cleaning regimen, when a solution of 0.5% chlorhexidine-60% isopropyl alcohol was added as an auxiliary disinfectant, only 2 out of 50 samples (4%) had unacceptable test results; moreover, no clinical cases were observed during this phase. In a successive phase, a heavier surface contamination was detected (8 out of 30 samples, 27%, resulted unacceptable). We think that the high intensity of care in the BICU can place a heavy workload on staff and speculate that this may be why they fail to apply the enhanced disinfection protocol, that was perceived as non-mandatory and only experimental.

Sensitivity and specificity of the ATP method were calculated as reported elsewhere.[21] An ATP benchmark value of 100 RLU/100cm^2 offered the closest correlation with microbial

growth level of 20 CFU/100cm^2, providing a sensibility and specificity respectively of 11/13 (86.4%) and 83/90 (92,2%).

Comparing with aerobic colony counts, seven ATP positive samples gave false positive results, whilst only two were false negative (respectively 41 and 68 CFU/100 cm^2) (Figure 1). False positive results are possible because ATP bioluminescence analysis detects the bioburden present and not just the level of bacterial contamination, as also observed in previous studies.[10,11] Moreover, several classes of inhibitory substances interfere with the activity of luciferase enzyme activity thus determining false negative results[22] .

The Positive Predictive value was 11/18 (61.1%) and the Negative Predictive value was 83/85 (97.6%). All false positive results were found on rough plastic surfaces rather than on flat metal surfaces, highlighting how the rough surfaces are more difficult to clean.

The results of the environmental investigation are shown in Figure 2. Strict adherence to the new cleaning protocol by the nursing staff provided a remarkable hygienic improvement in the care areas: only 2 samples out of 50 showed a microbial count over the AFNOR limit (20 CFU/cm^2) and CR-*Ab* was not detected in the environmental sites, even if the pathogen was isolated in clinical samples obtained from hospitalized patients. The microbiological and luminometer results were fed back to cleaning staff in order to increase the perception of the risk and to obtain a more rigorous adherence to the new cleaning protocol. Once the new cleaning protocol ceased to be correctly applied anymore, we observed 8 unacceptable samples out of 30 (27%) according to the AFNOR limit, with 6 of those associated with the presence of CR-*Ab*.

The Apa I macrorestriction profiles of the genomic DNA identied seven different PFGE patterns, arbitrarily named Pt1-Pt7 (Figure 3).

According to Tenover criteria, five of them (Pt2-Pt5, Pt7) were possibly related to each other, but, having been recorded only once during the surveillance, they were considered sporadic strains. Pattern Pt2 accounted for a single environmental isolate, never isolated from infected or colonized patients. The two major PFGE groups (Pt1 and Pt6 profiles, observed

respectively for 58% and 37% of the isolates) resulted closely related and so part of the same outbreak.

The dendrogram of the PFGE patterns demonstrated a greater similarity (95%) between epidemic Pt1 and Pt6 and a high similarity with sporadic Pt7 and Pt4 (73% and 68% respectively) suggesting the evolution of molecular variants from a single clone.

DISCUSSION

Recently a direct correlation was found between the number of *A. baumannii* isolates obtained during environmental monitoring and the number of patients who were colonized/infected with the same strain; moreover, an effective surface disinfection in the immediate environment of patients was described to reduce acquisition of *A. baumannii.*[23-24] Hospitals are encouraged to develop programs to optimize the thoroughness of high-touch surface cleaning in high-risk areas, in particular during *A. baumannii* outbreaks.

Several guidance documents on the control of MDR microorganisms support interventions based on a combination of two to seven infection control measures, and provide good evidence for the effectiveness of environmental surfaces decontamination in eradicating non-fermenting gram-negative rods, such as *A. baumannii.*[25] However dedicated resources are needed to implement monitoring programs to assess the efficacy of cleaning.[9]

The BICU environment may well act as a significant reservoir for potential pathogens because of the severe fluid loss by the hospitalized patients and for the high humidity and temperature of these environments. Several studies showed that patients admitted to rooms previously occupied by individuals infected or colonized with *A. baumannii* were at significant risk of acquiring these organisms from previously contaminated environmental sites.[7, 26]

The data obtained in our study suggest that an inadequate cleaning procedure was in place in the BICU since we observed 13% of values exceeded the AFNOR recommended threshold of 20 CFU/100 cm^2; by contrast, only 4% were out of limit after the improvement of the

cleaning protocol. The sodium hypochlorite solution was prepared in house by the nursing staff in non-sterile containers with non-sterile water, while the 0.5% chlorhexidine-60% isopropyl alcohol solution was ready-to-use and proved effective in reducing the microbial contamination and ATP values detected on the same surface samples. The RLU values observed during the monitoring correlated well with colony counts, except for seven false positives and two false negative results out of 103 samples obtained with the benchmark value of 100 RLU/100 cm^2 (8,7% incorrect results).

The same benchmark value (100 RLU/100 cm^2), and a hygiene standard (250 CFU/100 cm^2) similar but higher than the limit chosen in our study (20 CFU/100 cm^2), were reported by Mulvey *et al* to assess the efficiency of cleaning in several hospital environments (ICU's, reception, etc.) regardless of the level of risk.[21] The French recommendation AFNOR NF-S-90-351 classifies areas most sensitive to contamination (operating theatres, intensive care units, etc.), as higher risk class, whilst administration areas (corridors, waiting areas) in the lower risk class. The limit reported by Mulvey *et al* may be considered to be insufficiently precautionary in high-risk areas, because high microbial growth values means high probability to find pathogens such as *A. baumannii* on surfaces.[21] Likewise, they may be too precautionary in administration areas where the risk is low. Choosing the limit of 250 CFU/100 cm^2 we would not have had false positive and negative results, but the level of risk would have been underestimated. We believe it is important to increase the perception of risk in the cleaning staff so that procedures are carried out more rigorously in high-risk areas.

According to our results, the false positive results by the ATP method were obtained on rough plastic material and this could be explained by a higher rate of retention of microorganisms along with the occurrence of microscopic irregularities caused by abrasion or impact damage which may influence the cleanability of a surface[27]. Unlike the standard cultural method, ATP measures residual surface organic soil, which may include micro-organisms with variable amounts of ATP based on cell size and metabolic activity. Typically, only 33% of the ATP from hospital hand contact surfaces is likely to be of microbial origin[28]

and microbial cells may be present in a viable but nonculturable (VBNC) state, a condition gained by the bacteria in response to stress such as the disinfectant exposition. Therefore, current cleanliness verification protocols based on cultural technique are not able to detect VBNC cells as well as different species that require different media or environments for growth.[29] In conclusion, conventional microbial assay techniques may underestimate the level of risk while in high-risk areas we believe it is important to increase the perception of risk in the cleaning staff so that procedures are carried out more rigorously.

Recently, Guerrero demonstrated that interventions including education and monitoring of cleaning performance with feedback to housekeepers were associated with reduced environmental contaminations and acquisition of nosocomial pathogens.[30] Knape also showed that feedback of ATP measurements served as a valuable educational tool for staff to improve cleaning procedures.[31]

ATP readings can provide real-time feedback to housekeepers regarding their performance, an advantage over the 24–48 hours required to obtain results using microbiological methods. In our study we applied a luminometer with high sensibility and specificity, able to detect very low amounts of ATP present on surfaces, and we chose a benchmark value very low (100 RLU/100 cm^2) in order to assess the efficacy of daily hospital cleaning practices with a higher level of environmental hygiene and patient safety in preventing healthcare-associated pathogens infection. However, the ATP values must be interpreted as cleanliness indicators more than as risk indicators.

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Potential conflicts of interest. This study was supported by Pall Medical, Division Life Sciences, Port Washington, NY, USA, which was not involved in study design, data analysis and data presentation.

All authors report no conflicts of interest relevant to this article.

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Figure 1

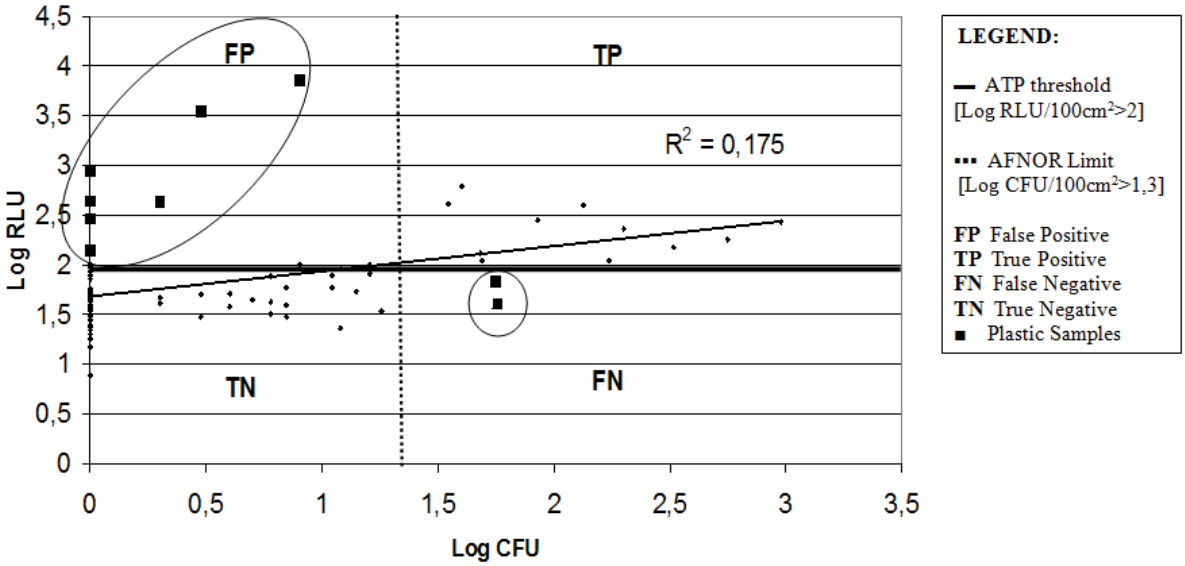

Figure 2

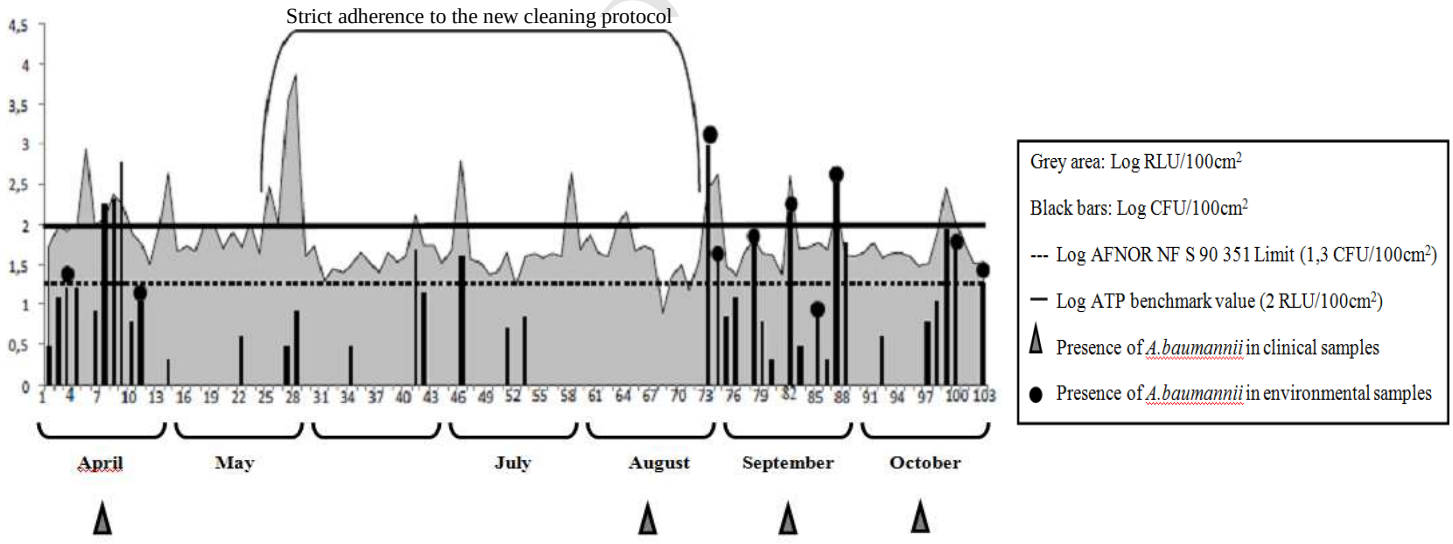

Figure 3

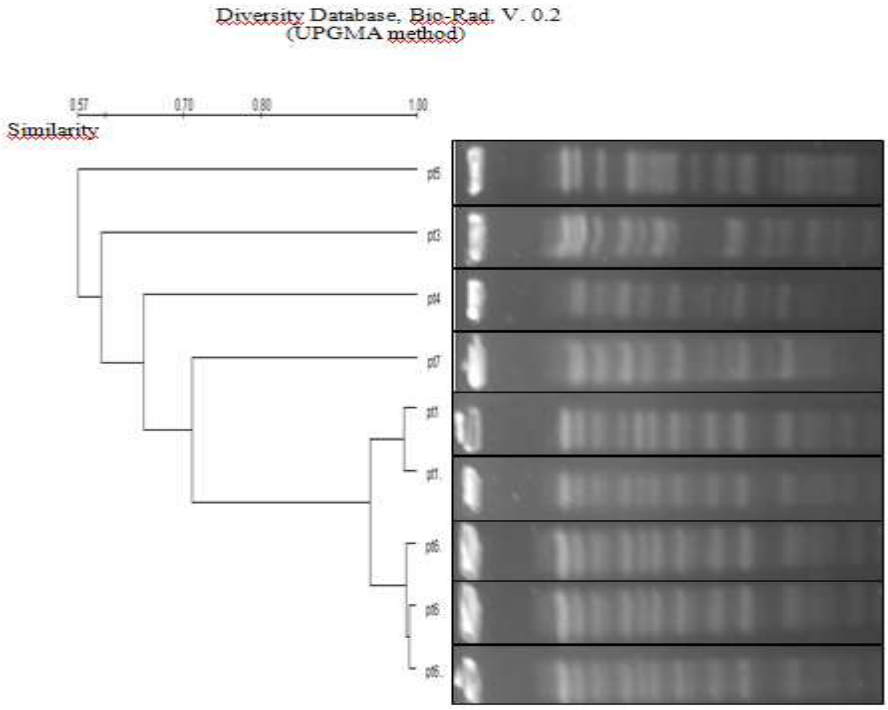

FIGURE LEGENDS

Figure 1. Correlation between the values of Agar Slide Cultures (log CFU) and ATP bioluminescence (log RLU) in environmental samples.

Figure 2. Results of the environmental investigation obtained on 103 surface samples.

Figure 3. Genotyping results by PFGE analysis.