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# Multicenter Evaluation of Loop-Mediated Isothermal Amplification (LAMP) for Rapid Environmental Monitoring of Nosocomial Pathogens in Italian Hospitals

**Biagio Santella[1,†,*], Antonio Donato[2,†], Vittoria Satriani[2], Laura Grumiro[3,4], Stefano Stracquadanio[5], Emanuela Santoro[2], Luigi Fortino[2], Claudia Colosimo[3,4], Alice Nicolosi[6], Maddalena Calvo[7], Graziella Manciagli[7], Nunziata Zirone[8], Agata Silvana Rifilò[8], Maria Giuseppina Pecora[8], Giuseppe Arcoleo[9], Monica Cricca[3,4], Stefania Stefani[6,7], Vittorio Sambri[3,4], Giovanni Boccia[2,10,*]**

[1] Department of Chemistry and Biology, University of Salerno, Salerno, Italy;
[2] Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University of Salerno, Italy;
[3] Department of Medical and Surgical Sciences (DIMEC), University of Bologna, Bologna, Italy;
[4] Laboratory and Transfusion Medicine, Laboratory of the Greater Romagna Hub, Pievesestina (FC), Italy;
[5] Department of Medicine and Surgery, "Kore" University of Enna, Enna, Italy;
[6] Department of Biomedical and Biotechnological Sciences, University of Catania, Catania, Italy;
[7] University Hospital Policlinico-San Marco, University of Catania, Catania, Italy;
[8] ARNAS "GARIBALDI" Catania, Catania, Italy;
[9] Enbiotech s.r.l., Palermo, Italy.
[10] Department of Health Hygiene and Evaluative Medicine, A.O.U. San Giovanni di Dio e Ruggi d'Aragona, Salerno, Italy.

† These authors contributed equally to this work.
* Correspondence: bsantella@unisa.it; gboccia@unisa.it.

## Abstract

**Background:** Healthcare-associated infections are partly sustained by environmental contamination of hospital surfaces, particularly in high-risk clinical areas. Conventional culture-based monitoring remains essential but is time-consuming and may underestimate low-level microbial contamination. This multicentre study evaluated the performance and operational feasibility of loop-mediated isothermal amplification (LAMP) for rapid detection of major nosocomial pathogens on hospital surfaces. **Methods:** A multicentre observational method-comparison study was conducted in three Italian hospital centres. Overall, 299 environmental surface samples were collected from clinical areas included in local infection-prevention monitoring plans. For each sampling point, LAMP swab sampling was paired with adjacent contact-plate culture. LAMP assays targeted *Staphylococcus aureus*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. Manual and automated nucleic acid extraction workflows were analysed separately. Diagnostic performance, agreement metrics, and discordant paired results were assessed using contact-plate culture as the microbiological comparator. **Results:** No LAMP-negative/contact plate-positive results were observed across target pathogens and extraction workflows. Relative sensitivity percent agreement and negative predictive value were therefore 100% in all evaluable comparisons. Relative specificity ranged from 90.9% to 98.6%, while overall accuracy ranged from 93.8% to 98.6%. Discordant results were exclusively LAMP-positive/contact plate-negative and were more frequent for *S. aureus* and *P. aeruginosa*. Selective culture verification supported several LAMP-positive findings not recovered by routine contact plates. **Conclusions:** LAMP showed high negative agreement, high specificity, and rapid target-specific detection of selected nosocomial pathogens on hospital surfaces. It may complement

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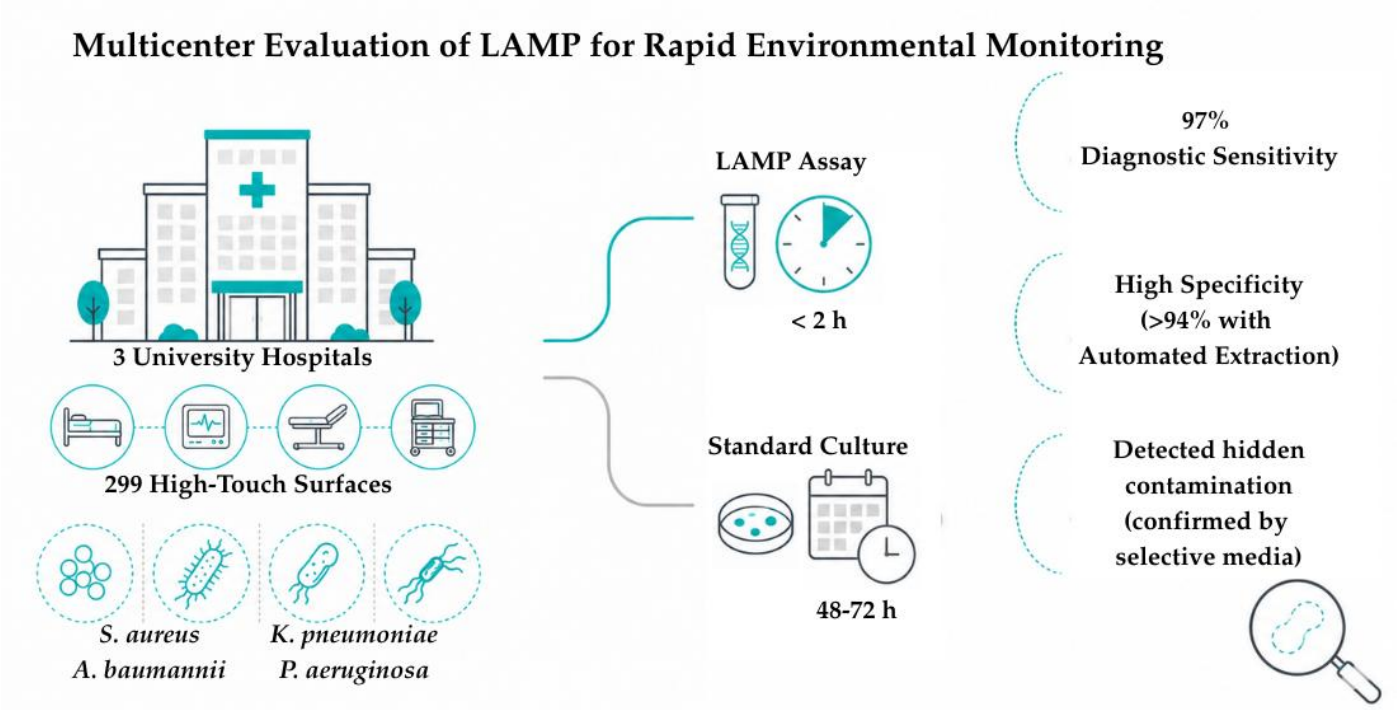
conventional culture in environmental surveillance, particularly when timely feedback is needed to guide infection-prevention and sanitation-monitoring strategies.

**Keywords:** Hospital environmental monitoring; healthcare-associated infections; LAMP; nosocomial pathogens; Staphylococcus aureus; Pseudomonas aeruginosa; Klebsiella pneumoniae; Acinetobacter baumannii

## GRAPHICAL ABSTRACT




## 1. Introduction

Healthcare-associated infections (HAIs) remain a major challenge for modern health systems, contributing significantly to morbidity, mortality, and resource consumption worldwide [1,2]. The World Health Organization estimates that around 7% of hospitalised individuals in affluent nations and as many as 15% in low- and middle-income nations are expected to acquire at least one hospital-associated infection during hospitalization. Microbiologically contaminated surfaces serve as reservoirs for bacteria responsible for HAIs, particularly in high-risk areas such as intensive care units, operating theatres, hematology and oncology wards, and infectious diseases units [3,4].

High-touch surfaces in the near-patient environment, including bed rails, infusion pumps, monitors, and worktops, can harbor pathogenic bacteria capable of surviving for prolonged periods, especially under suboptimal cleaning and disinfection practices. This contamination acts as a reservoir from which microorganisms may be transferred via healthcare workers' hands or medical devices [5,6].

Among the most relevant nosocomial pathogens, capable of resisting environmental decontamination, are *Staphylococcus aureus*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. These species are frequently multidrug-resistant, capable of forming biofilms, and particularly adept at persisting on dry surfaces [7]. Environmental

microbiological monitoring, therefore, constitutes a fundamental pillar of HAI prevention and control, necessary both to evaluate decontamination and sanitization procedures and to identify resistant species that may require specific, appropriate interventions [8,9].

Currently, the culture method is considered the gold standard for detecting microorganisms on surfaces, such as contact plates or swabs plated on non-selective media, followed by incubation and identification of colonies by biochemical methods or MALDI-TOF mass spectrometry [10,11]. This method, while thorough, is unfortunately time-consuming, requiring 48 to 72 hours for the observation and identification of bacterial species on the plate. Moreover, it involves a complex microbiological process that demands significant effort. As a result, it is not suitable for non-culturable microorganisms or those present in very low load. Recent scoping work on cleaning-monitoring strategies has highlighted both the central role of microbiological methods and the need for faster, more sensitive tools integrated within multimodal surveillance frameworks [12,13].

Loop-mediated isothermal amplification (LAMP) is a nucleic acid amplification technique that operates at a constant temperature (typically 60–65°C), using a set of four to six primers recognizing multiple regions of the target gene and generating large amounts of DNA within a short time [14,15]. LAMP offers several advantages for environmental monitoring: high analytical sensitivity and specificity, rapid turnaround (often ≤1 h), relatively simple workflows, and the possibility of visual or instrument-based readout [16,17].

LAMP assays have been developed for a wide range of microorganisms and applied in water safety, food safety, clinical diagnostics, and, more recently, environmental surveillance of *SARS-CoV-2* and other pathogens [18].

A recent experimental study optimized LAMP for nosocomial pathogens on artificially contaminated surfaces, showing high sensitivity and specificity versus contact-plate culture after short pre-incubation periods [19]. However, evidence from real hospital environments remains limited, especially for multicenter studies using routine high-touch surfaces in wards with differing risk levels and sanitation protocols.

The present multicenter study was designed to address this gap by evaluating the performance and practical feasibility of LAMP-based assays for the detection of *S. aureus*, *A. baumannii*, *K. pneumoniae*, and *P. aeruginosa* on hospital surfaces. Environmental samples were collected from three Italian hospitals across different regions and risk settings and analyzed in parallel by LAMP and RODAC contact-plate culture method, with a selective plating protocol used to verify LAMP-positive findings. The aim was to characterize sensitivity, specificity, and turnaround time of LAMP and to explore its added value for environmental surveillance within infection prevention programs.

## 2. Results

### *2.1. Environmental sampling*

During the study period, 299 environmental surface samples were collected from three Italian regions: 132 samples in Campania, 93 in Emilia-Romagna, and 74 in Sicily. Sampling was performed on hospital surfaces selected according to local environmental monitoring plans and infection-risk profiles, according to local monitoring plans. For each sampling point, LAMP swab sampling was paired with an adjacent RODAC/contact-plate culture, allowing direct comparison between the molecular and culture-based approaches under real-world hospital conditions.

The final analytic-performance dataset included 153 valid paired comparisons per target pathogen for manual extraction and 145 valid paired comparisons per target pathogen for automated extraction. Results were analysed separately for *S. aureus*, *A. baumannii*, *K. pneumoniae*, and *P. aeruginosa*, and classified as true positives (TP), false positives (FP), true negatives (TN), and false negatives (FN) using RODAC contact-plate culture as the microbiological comparator.

### *2.2. Analytical performance of LAMP*

Overall, no LAMP-negative/RODAC-positive results were observed for any target pathogen or extraction method. Relative sensitivity percent agreement was therefore 100% in all evaluable pathogen-extraction subgroups. Negative predictive value was also 100% across all comparisons, indicating that LAMP did not miss any culture-positive sample in the available dataset. Because several targets had a low number of comparator-positive samples, particularly *A. baumannii* and *K. pneumoniae*, sensitivity estimates should be interpreted together with their confidence intervals and with caution.

Relative specificity percent agreement remained high across the study, ranging from 90.9% to 98.6%. Overall accuracy ranged from 93.8% to 98.6%, while balanced accuracy remained consistently high, ranging from 95.5% to 99.3%. The complete diagnostic performance and agreement metrics according to pathogen and extraction workflow are reported in Table 1.

**Table 1**: Analytical performance and agreement analysis of LAMP according to target pathogen and extraction method.

| Extraction | Target | TP | FP | TN | FN | Sensitivity | Specificity | Accuracy | PPV | NPV | F1 | Balanced accuracy | κ | PABAK | McNemar *p* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Manual | *S. aureus* | 54 | 9 | 90 | 0 | 100% | 90.9% | 94.1% | 85.7% | 100% | 92.3% | 95.5% | 0.876 | 0.882 | 0.004 |
| Manual | *A. baumannii* | 5 | 3 | 145 | 0 | 100% | 98.0% | 98.0% | 62.5% | 100% | 76.9% | 99.0% | 0.760 | 0.961 | 0.250 |
| Manual | *K. pneumoniae* | 7 | 4 | 142 | 0 | 100% | 97.3% | 97.4% | 63.6% | 100% | 77.8% | 98.6% | 0.765 | 0.948 | 0.125 |
| Manual | *P. aeruginosa* | 20 | 7 | 126 | 0 | 100% | 94.7% | 95.4% | 74.1% | 100% | 85.1% | 97.4% | 0.825 | 0.908 | 0.016 |
| Automated | *S. aureus* | 6 | 9 | 130 | 0 | 100% | 93.5% | 93.8% | 40.0% | 100% | 57.1% | 96.8% | 0.545 | 0.876 | 0.004 |
| Automated | *A. baumannii* | 2 | 2 | 141 | 0 | 100% | 98.6% | 98.6% | 50.0% | 100% | 66.7% | 99.3% | 0.660 | 0.972 | 0.500 |
| Automated | *K. pneumoniae* | 1 | 4 | 140 | 0 | 100% | 97.2% | 97.2% | 20.0% | 100% | 33.3% | 98.6% | 0.326 | 0.945 | 0.125 |
| Automated | *P. aeruginosa* | 1 | 3 | 141 | 0 | 100% | 97.9% | 97.9% | 25% | 100% | 40.0% | 99.0% | 0.393 | 0.959 | 0.250 |

Results are reported as true positives (TP), false positives (FP), true negatives (TN), and false negatives (FN), positive predictive value (PPV), negative predictive value (NPV), F1-score, balanced accuracy, Cohen's κ, prevalence-adjusted bias-adjusted kappa (PABAK), and McNemar exact test. Percentages refer to comparisons with RODAC/contact-plate culture as the microbiological comparator. FP denotes LAMP-positive/contact plate-negative results, whereas FN denotes LAMP-negative/contact plate-positive results. McNemar exact test was used to assess asymmetric discordance between paired results; $p < 0.05$ was considered statistically significant.

### *2.3. Performance according to extraction workflow*

With manual extraction, LAMP showed 100% relative sensitivity percent agreement for all four target pathogens. Specificity ranged from 90.9% for *S. aureus* to 98.0% for *A. baumannii*, and accuracy ranged from 94.1% to 98.0%. Agreement was good to excellent, with Cohen's κ values ranging from 0.760 to 0.876 and PABAK values from 0.882 to 0.961. The strongest agreement was observed for *S. aureus* (κ = 0.876) and *P. aeruginosa* (κ = 0.825), the two targets with the highest number of comparator-positive samples.

With automated extraction, relative sensitivity percent agreement remained 100% in all evaluable target subgroups, and specificity ranged from 93.5% for *S. aureus* to 98.6% for *A. baumannii*. Accuracy ranged from 93.8% to 98.6%, and PABAK remained high across targets (0.876-0.972), supporting substantial overall agreement after adjustment for prevalence imbalance. However, Cohen's κ was lower for low-prevalence targets, particularly *K. pneumoniae* (κ = 0.326) and *P. aeruginosa* (κ = 0.393), reflecting the limited number of comparator-positive samples rather than a high number of false-negative LAMP results.

### *2.4. Discordant-result analysis*

Discordant results were represented exclusively by LAMP-positive/RODAC-negative samples, whereas no LAMP-negative/RODAC-positive results were observed. This pattern indicates that LAMP did not fail to detect any culture-positive sample but yielded additional positive signals compared with routine contact-plate culture. These additional LAMP-positive findings resulted in higher positivity rates for LAMP than for contact plates across all targets and extraction workflows.

For manual extraction, the absolute increase in positivity rate for LAMP compared with contact plates was +5.9% for *S. aureus*, +2.0% for *A. baumannii*, +2.6% for *K. pneumoniae*, and +4.6 for *P. aeruginosa*. McNemar exact test showed significant asymmetric discordance for *S. aureus* (p = 0.004) and *P. aeruginosa* (p = 0.016), driven by LAMP-positive/contact-plate culture negative results. For automated extraction, the corresponding increase in positivity rate was +6.2% for *S. aureus*, +1.4% for *A. baumannii*, +2.8% for *K. pneumoniae*, and +2.1% for *P. aeruginosa*. Significant asymmetric discordance was observed only for *S. aureus* (p = 0.004), whereas the remaining automated-extraction comparisons were not statistically significant.

These findings should not be interpreted as evidence of diagnostic equivalence between methods. Rather, they show that discordance was directional and mostly reflected additional LAMP-positive findings. In environmental monitoring of surfaces, results may stem from low-level contamination, diminished cultivation capacity, uneven microbial distribution on surfaces, or the presence of stressed cells.

### *2.5. Target distribution across centres*

The distribution of target pathogens varied across centres and extraction workflows. In the manual-extraction dataset, valid comparisons were available for 47 samples from Cesena, 32 from Catania, and 74 from Salerno for each target. In the automated-extraction dataset, valid comparisons were available for 46, 41, and 58 samples, respectively. *S. aureus* was the most frequently detected target overall, particularly in the manual-extraction dataset, followed by *P. aeruginosa*, *A. baumannii* and *K. pneumoniae* were detected less frequently and were characterized by a low number of comparator-positive samples.

Because sampling was performed according to centre-specific monitoring needs and included different wards, surfaces, and sanitation conditions, centre-level results were interpreted descriptively. Therefore, differences in target distribution across centres should be regarded as local detection patterns rather than direct prevalence comparisons between hospitals.

*2.6. Selective verification of LAMP-positive findings*

Selective plating of enrichment broths corresponding to LAMP-positive samples was used as a microbiological verification step, particularly for LAMP-positive/RODAC-negative results. This verification approach was useful when routine non-selective contact plates showed no target-pathogen recovery or when target colonies could have been masked by non-target environmental flora. Available verification results supported several LAMP-positive findings and reinforced the interpretation of LAMP-positive/ contact-plate culture negative samples as biologically relevant discordant results rather than automatic analytical false positives.

Overall, the Results indicate that LAMP provides rapid, pathogen-specific detection with high negative agreement against contact-plate culture and the ability to identify additional target-positive signals not recovered by routine surface culture. These findings support its role as a complementary molecular tool for hospital environmental surveillance, particularly in settings where rapid feedback is needed for infection prevention and sanitation monitoring.

## 3. Discussion

*3.1. Principal findings*

This multicentre real-world study evaluated the performance of a LAMP-based workflow for the rapid detection of four clinically relevant nosocomial pathogens on hospital environmental surfaces. The main finding was the absence of LAMP-negative/contact plate-positive results across all target pathogens and extraction workflows. Accordingly, relative sensitivity percent agreement was 100% in all evaluable comparisons. This result indicates that, within the limits of the culture-based comparator used in this study, LAMP did not miss any culture-positive sample. At the same time, relative specificity percent agreement remained high, ranging from 90.9% to 98.6%, and negative predictive value was 100% for all targets and extraction methods. These findings support the potential role of LAMP as a rapid screening tool in environmental microbiological surveillance, particularly when the priority is the early exclusion of contamination by selected high-risk pathogens [17,20].

A second key finding was the systematic direction of discordance. Discordant results were represented by LAMP-positive/contact plate-negative samples, whereas no LAMP-negative/contact plate-positive results were observed. This pattern is relevant from both a statistical and microbiological perspective. Statistically, it explains the significant McNemar tests observed for *S. aureus* with manual extraction, *P. aeruginosa* with manual extraction, and *S. aureus* with automated extraction. Microbiologically, it suggests that LAMP may identify target-specific signals that are not recovered by routine contact-plate culture [21]. Such results should therefore be interpreted as discordant findings rather than simply as false-positive molecular results, especially when supported by selective culture verification [22].

*3.2. Interpretation of diagnostic performance and agreement metrics*

The diagnostic performance profile observed in this study is typical of a rapid molecular assay applied to complex environmental matrices. Sensitivity (PPA) was uniformly high because no false-negative results were recorded. However, several target-pathogen subgroups included only a small number of culture-positive samples, particularly *A. baumannii* and *K. pneumoniae*, and the automated-extraction subgroups for *K. pneumoniae* and *P. aeruginosa*. Consequently, sensitivity estimates of 100% should be interpreted together with the underlying denominators and confidence intervals. In these low-prevalence subgroups, a single positive sample strongly affects performance estimates, and wider uncertainty is expected.

Specificity (NPA) remained consistently high across targets, but positive predictive value varied substantially according to pathogen prevalence and extraction workflow.

This was particularly evident for *K. pneumoniae* and *P. aeruginosa* with automated extraction, where PPV values were low despite high specificity and high overall accuracy. This apparent discrepancy reflects the low number of culture-positive samples and the predominance of concordant negatives. In this context, PPV should not be interpreted as an intrinsic limitation of the LAMP assay alone, because the contact-plate comparator may fail to recover low-level, unevenly distributed, stressed, or overgrown target organisms from environmental surfaces.

Agreement analysis helped to clarify this issue. Cohen's kappa ranged from moderate to excellent in the manual-extraction subgroup and was lower in some automated-extraction comparisons, especially when target prevalence was very low. This behavior is expected because Cohen's kappa is sensitive to prevalence imbalance and may underestimate agreement when most samples are negative. For this reason, PABAK was also reported. PABAK remained high across all comparisons, supporting substantial overall agreement after accounting for the imbalance between positive and negative results. Therefore, Cohen's kappa and PABAK should be interpreted jointly rather than in isolation.

The McNemar exact test provided additional information by assessing the symmetry of paired discordant results. Significant p-values did not demonstrate equivalence or non-equivalence between methods; rather, they indicated that discordance was asymmetric and mainly driven by LAMP-positive/contact plate-negative results. Non-significant McNemar tests, particularly in subgroups with few discordant pairs, should not be interpreted as evidence of equivalence. Overall, the statistical analysis shows that LAMP and contact-plate culture are not simply interchangeable methods, but complementary approaches that capture partially different microbiological information.

### *3.3. Manual versus automated extraction workflow*

The stratified analysis by extraction workflow showed high performance for both manual and automated extraction. Manual extraction produced high accuracy, high NPV, and good-to-excellent agreement for all four targets, with Cohen's kappa values ranging from 0.760 to 0.876 and PABAK values from 0.882 to 0.961. Automated extraction also showed high specificity and NPV, but agreement metrics and PPV were lower for some pathogens because the number of culture-positive samples was very small. The updated *P. aeruginosa* automated-extraction data showed one true-positive result, three LAMP-positive/contact plate-negative discordant results, and no false negatives, making sensitivity estimable in this subgroup.

These findings should not be used to infer the superiority of one extraction approach over the other, because the study was not designed as a direct paired comparison of manual and automated extraction on the same individual samples. Rather, the data indicate that both workflows are operationally feasible and can be adapted to different laboratory settings. Automated extraction may improve standardization, reproducibility, and contamination control, whereas manual extraction remains a practical option where automation is not available or where sample volumes are limited. Future studies specifically designed to compare extraction methods in a paired format would be useful to define the most efficient implementation strategy.

### *3.4. Comparison with previous literature*

The present findings are consistent with previous LAMP studies showing high performance after pre-enrichment. Controlled experiments demonstrated target-dependent effects of incubation time, while later hospital studies reported sensitivity of 1.00 and specificity generally above 0.90 [19]. The current study adds a larger multicenter sample, includes four clinically relevant target species, and directly examines manual and automated extraction workflows. Its novelty therefore lies primarily in pragmatic

implementation rather than in first demonstrating the analytical feasibility of LAMP on surfaces.

In the broader literature, LAMP has been applied to environmental monitoring of water and air, where it has been shown to provide rapid and sensitive detection of microbial indicators and specific pathogens [23,24]. Notably, recent work has used LAMP for *SARS-CoV-2* surface monitoring and comparison with RT-qPCR and droplet digital PCR, highlighting its suitability for point-of-care or on-site environmental testing [25]. Our study contributes novel data on bacterial nosocomial pathogens, demonstrating that LAMP can maintain high performance in complex hospital environments and across multiple institutions.

From an infection-control standpoint, several reviews have emphasized the need for multimodal environmental monitoring, combining visual inspection, ATP bioluminescence, and microbiological methods [8,26-28]. The slow turnaround of traditional culture has been identified as a major limitation, potentially delaying feedback to cleaning staff and the implementation of corrective measures. Our data support the integration of LAMP into these multimodal strategies as a rapid microbiological component, capable of providing actionable results during or shortly after cleaning activities.

### *3.5. Implications for environmental monitoring and infection prevention*

The findings have direct implications for hospital hygiene programs and infection prevention practice. Conventional environmental culture requires 48-72 h before colonies can be counted and identified, which delays feedback to cleaning teams and infection-control personnel. In contrast, after the pre-incubation step, the LAMP analytical phase can provide pathogen-specific results within a shortened workflow. This may allow earlier identification of residual contamination, faster reinforcement of cleaning procedures, targeted re-cleaning of high-touch surfaces, and more timely evaluation of sanitation effectiveness.

The clinical value of such a workflow is particularly relevant in very high- and high-risk areas, including intensive care units, neonatal intensive care units, infectious diseases wards, hematology, oncology, surgical areas, and other settings caring for vulnerable patients. In these environments, even low-level contamination by pathogens such as *S. aureus*, *A. baumannii*, *K. pneumoniae*, or *P. aeruginosa* may be epidemiologically relevant. Rapid, target-specific environmental screening could therefore function as an early-warning tool within risk-based cleaning and outbreak-prevention strategies.

The detection of LAMP-positive/contact plate-negative results is one of the most important practical findings of this study. These discordant results may reflect bacterial loads below the recovery threshold of contact plates, uneven distribution of contamination on surfaces, reduced culturability after exposure to disinfectants, microbial stress, masking by non-target flora, or molecular detection of residual DNA. The selective verification protocol strengthened interpretation by confirming several LAMP-positive findings on targeted media, especially when routine non-selective culture was negative or difficult to interpret. In practice, this suggests that LAMP could be used as a rapid screening method, with selective culture reserved for positive or epidemiologically relevant samples.

The proposed workflow is therefore best understood as complementary to existing environmental monitoring frameworks rather than as a complete replacement for culture. Culture remains necessary when quantitative CFU data, viable isolates, susceptibility testing, or molecular typing are required. Conversely, LAMP may be most useful when rapid yes/no information is needed to guide immediate infection-control decisions. Integrating both approaches may provide a more complete assessment of environmental risk than either method alone.

### *3.6. Strengths and limitations*

This study has several strengths. It was conducted in three real hospital settings rather than under exclusively experimental conditions, included a relatively large number of surface samples, and focused on clinically relevant pathogens with established roles in healthcare-associated infections. The use of paired LAMP swabs and adjacent RODAC/contact plates enabled direct comparison between molecular and culture-based methods under the same environmental conditions. The stratified performance analysis by pathogen and extraction workflow, together with agreement metrics, PABAK, McNemar testing, and descriptive discordant-result analysis, provides a more complete statistical framework than simple sensitivity and specificity estimate alone.

The study also incorporated a selective verification protocol for LAMP-positive samples. This is an important methodological feature because contact-plate culture is an imperfect comparator for environmental surveillance. Contact plates sample a limited area, may be affected by surface geometry and residual disinfectants, and may fail to recover stressed or low-abundance organisms. Selective culture of enrichment broths allowed additional microbiological interpretation of LAMP-positive/contact plate-negative findings and helped distinguish statistical discordance from potentially meaningful biological detection.

Several limitations must also be acknowledged. First, only four bacterial species were included; therefore, the findings cannot be extended to other relevant environmental organisms such as *Enterococcus spp.*, *Clostridioides difficile*, fungi, or respiratory viruses. Second, LAMP provides qualitative results and does not quantify microbial burden in CFU, which limits its use for threshold-based environmental standards. Third, LAMP does not provide antimicrobial susceptibility data or isolates for epidemiological typing. Fourth, molecular assays may detect DNA from non-viable organisms, especially after cleaning and disinfection. Although the pre-enrichment and selective verification steps reduce this concern, they do not eliminate it.

An additional limitation is the structure of the available dataset. The analysis was based on target-specific aggregated comparisons rather than a complete individual-level dataset including all clustering variables, such as centre, ward, surface type, sanitation phase, sampling date, and extraction workflow for each sample-target observation. For this reason, formal mixed-effects modelling was not performed, and centre-level or ward-level patterns were interpreted descriptively. Future studies using individual-level data will be needed to evaluate the independent effects of centre, ward risk class, sanitation status, surface type, and extraction method on LAMP positivity.

### *3.7. Future directions*

Future research should focus on prospective implementation studies designed to assess whether LAMP-based environmental surveillance improves infection-prevention outcomes. Longitudinal studies could evaluate whether rapid LAMP feedback reduces response time after detection of contamination, improves compliance with cleaning protocols, and contributes to reducing environmental reservoirs during outbreaks. Economic evaluations are also warranted to compare LAMP-based workflows with conventional culture, ATP monitoring, and combined multimodal surveillance strategies.

Further technological development should include broader LAMP panels targeting additional nosocomial pathogens and resistance markers, such as carbapenemase genes or methicillin-resistance determinants. Semi-quantitative LAMP outputs, time-to-positivity thresholds, or combined LAMP-culture algorithms may help define actionable contamination thresholds. Finally, integration of LAMP results into digital dashboards could support real-time visualization of environmental risk, traceability of corrective actions, and feedback to frontline staff. In this perspective, LAMP should be viewed not

only as a diagnostic test, but as a rapid operational tool within modern infection prevention and environmental hygiene programs.

## 4. Materials and Methods

### *4.1. Study design and setting*

This multicentre observational method-comparison study was designed to evaluate the diagnostic performance and operational feasibility of loop-mediated isothermal amplification (LAMP) for the rapid detection of selected nosocomial pathogens on hospital environmental surfaces. Environmental samples were analysed in parallel using the Enbiotech LAMP system and a conventional culture-based (contact-plate method), which was used as the microbiological comparator for the primary diagnostic-performance analysis. Sampling sites were selected according to local environmental monitoring plans and risk-based criteria derived from national hospital sanitation guidance and UNI EN 17141:2021 for microbiological control of controlled environments. The sampling campaign was conducted from January 2024 to December 2025.

Three Italian hospital and laboratory centres participated in the study, representing different geographical and organizational settings: ARNAS Garibaldi of Catania (Sicily center; 497-bed hospital; microbiological analyses performed at the Department of Biomedical and Biotechnological Sciences), "A.O.U. San Giovanni di Dio e Ruggi d'Aragona", Salerno (Campania center; 642-bed teaching hospital; environmental sampling and processing coordinated by the Hospital Hygiene and Epidemiology Unit), and AUSL Romagna - "DiMeLabeT" (Emilia-Romagna center; 3.350-bed hospital; laboratory work conducted at the Department of Laboratory Medicine and Transfusion). All centers followed the same procedure of sampling and laboratory protocol shared within the network, according to the manufacturer's guidelines.

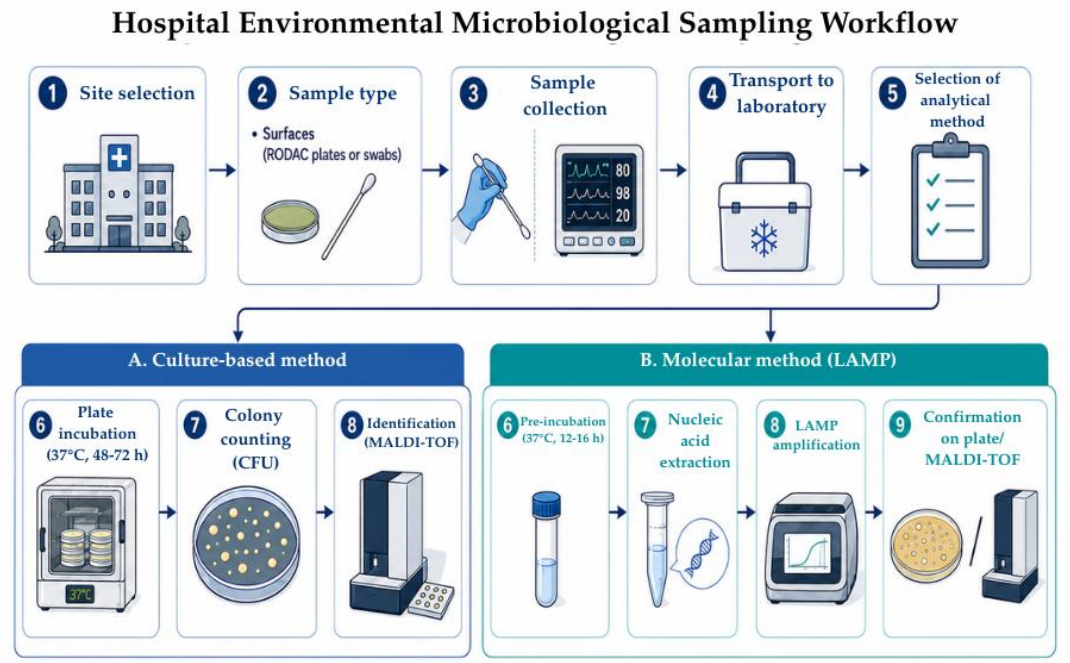



**Figure 1.** Hospital Environmental Microbiological Sampling Workflow

445 *4.2. Target pathogens and LAMP assays*

446 The study focused on four bacterial species selected for their clinical relevance in
447 healthcare-associated infections, environmental persistence, and potential involvement in
448 hospital outbreaks: *Staphylococcus aureus*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*,
449 and *Pseudomonas aeruginosa*. Pathogen-specific commercial LAMP kits supplied by
450 Enbiotech s.r.l. (Palermo, Italy) were used according to the manufacturer's instructions.
451 Each assay provided a qualitative positive/negative result for the corresponding target
452 pathogen. Positive and negative controls supplied with the kits were included in each
453 analytical session, and only runs with valid controls were considered interpretable.
454
455 *4.3. Environmental surface sampling*

456 A total of 299 environmental surface samples were collected during the study period,
457 including 132 samples from Campania, 93 from Emilia-Romagna, and 74 from Sicily.
458 Sampling was performed in hospital wards and clinical areas included in local infection-
459 prevention monitoring plans. Samples were collected either before sanitation or after
460 routine cleaning and disinfection, according to each centre's operational protocol. High-
461 touch and patient-near surfaces were prioritized when defined by the local monitoring
462 plan, because these surfaces are most relevant for environmental transmission risk and
463 for evaluating sanitation procedures.

464 For each sampling point, a paired environmental sampling strategy was applied. For
465 the molecular workflow, a flocked swab moistened with neutralizing solution was rubbed
466 over a 10 × 10 cm area of the selected surface using a standardized multidirectional
467 pattern. The swab was then placed into a tube containing enrichment medium. A second
468 dry flocked swab was passed over the same area and added to the same tube to increase
469 recovery from the sampled surface. Tubes were vortexed, labelled, transported to the
470 laboratory under controlled conditions, and incubated at 37 °C for 12–16 h before nucleic
471 acid extraction and LAMP analysis.

472 For the culture-based comparator, a RODAC/contact plate containing TSA-LTH
473 medium was applied to a 25 $cm^2$ area adjacent to the LAMP sampling site using uniform
474 pressure for 10 s. Contact plates were incubated at 37 °C for 48–72 h. After incubation,
475 colonies were counted and target colonies were identified to species level by MALDI-TOF
476 mass spectrometry. The use of adjacent swab and contact-plate sampling allowed a paired
477 comparison under the same environmental conditions, while minimizing interference
478 between the two sampling procedures.
479
480 *4.4. Culture-based comparator and definition of primary diagnostic outcomes*

481 The primary microbiological comparator consisted of conventional RODAC/contact-
482 plate culture followed by species identification. For the primary diagnostic-performance
483 analysis, each pathogen-specific comparison was classified using a 2 × 2 contingency table.
484 A true positive was defined as a LAMP-positive result in a sample that was also positive
485 for the same target pathogen by contact-plate culture. A false positive was defined, for the
486 primary comparison, as a LAMP-positive/contact plate-negative result. A true negative
487 was defined as concordant negativity by both methods, whereas a false negative was
488 defined as a LAMP-negative/contact plate-positive result. Because LAMP provides
489 qualitative results, the comparison was based on the presence or absence of each target
490 pathogen rather than on quantitative CFU thresholds.

491 Contact-plate culture also provided semi-quantitative information on surface
492 microbial burden and allowed recovery of viable isolates for identification. Therefore,
493 culture was retained as the reference comparator for conventional environmental
494 monitoring, even though it was not considered an absolute gold standard for all
495 biologically plausible contamination events. This distinction was important for
496 interpreting LAMP-positive/contact plate-negative discordant results.
497

*4.5. LAMP pre-enrichment, nucleic acid extraction and amplification*

After an overnight pre-enrichment culture, nucleic acid extraction from enrichment tubes was performed using either a manual or an automated workflow. Manual extraction was carried out according to the manufacturer's standardized protocol for nucleic acid isolation. Automated extraction was performed using automated platforms validated for use with the Enbiotech LAMP workflow. The extraction method used for each sample was recorded, enabling stratified analysis by manual and automated extraction. Because the allocation of samples to extraction workflows reflected the operational organization of the participating centres, manual and automated workflows were analysed descriptively and were not interpreted as fully paired analytical conditions.

Extracted nucleic acids were amplified using the corresponding pathogen-specific LAMP kit. Reactions were performed under isothermal conditions at the manufacturer-recommended temperature range of approximately 60–65 °C. Amplification was monitored in real time, and results were interpreted as positive or negative according to the presence of a target-specific amplification signal and the validity of the run controls. The LAMP workflow was therefore designed to provide a rapid qualitative result for each target pathogen, complementary to the slower culture-based workflow.

*4.6. Selective verification of LAMP-positive findings*

To support the microbiological interpretation of discordant results, a selective plating verification protocol was applied to enrichment broths corresponding to LAMP-positive samples, particularly when the paired contact plate did not recover the same target pathogen. Aliquots from the enrichment broths were plated onto target-selective media: Baird-Parker agar for *Staphylococcus aureus*, Acinetobacter selective medium (LAM) for *Acinetobacter baumannii*, MacConkey agar for *Klebsiella pneumoniae*, and P.A.CN selective agar for *Pseudomonas aeruginosa*. Plates were incubated at 37 °C for 24–48 h, and suspected target colonies were identified by MALDI-TOF mass spectrometry when required.
Selective verification was used as a secondary microbiological support tool for discordant-result interpretation. It was not used to replace the primary RODAC/contact-plate comparator in the diagnostic-performance table, but it provided additional evidence that some LAMP-positive/contact plate-negative results represented target organisms recoverable after enrichment and selective culture.

*4.7. Statistical analysis*

All statistical analyses were performed using R software (R Foundation for Statistical Computing, Vienna, Austria). Qualitative LAMP results obtained with the Enbiotech system were compared with conventional RODAC/contact-plate culture, which was used as the microbiological comparator method. For each target pathogen and extraction method, results were arranged in 2 × 2 contingency tables and classified as true positives (TP), false positives (FP), true negatives (TN), and false negatives (FN). Analytical performance was assessed by calculating relative sensitivity/positive percent agreement (PPA), relative specificity/negative percent agreement (NPA), overall accuracy/overall percent agreement (OPA), positive predictive value (PPV), negative predictive value (NPV), F1-score, and balanced accuracy. Exact 95% confidence intervals for binomial proportions were calculated using the Clopper–Pearson method when appropriate.

Agreement between LAMP and contact-plate culture was evaluated using Cohen's κ coefficient. Because several pathogen-specific subgroups had a low prevalence of positive samples and highly unbalanced positive/negative distributions, prevalence-adjusted bias-adjusted kappa was also calculated. The exact McNemar test was used to assess whether discordant paired results were symmetrically distributed between LAMP-positive/contact plate-negative and LAMP-negative/contact plate-positive outcomes. A two-sided p-value < 0.05 was considered statistically significant. McNemar results were

interpreted as evidence of asymmetric discordance and not as evidence of analytic equivalence or non-equivalence between methods.

The absolute difference in positivity rates between LAMP and contact-plate culture was also calculated for each pathogen and extraction workflow. Discordant results were analysed descriptively, with particular attention to LAMP-positive/contact plate-negative samples and to the results of selective verification. Because the available dataset was based on aggregated target-specific contingency counts rather than complete individual-level observations including all clustering variables, formal mixed-effects modelling was not performed. Centre-level and ward-level patterns were therefore interpreted as exploratory descriptive analyses.

## 5. Conclusions

This multicentre real-world study shows that LAMP can be integrated into hospital environmental surveillance as a rapid, target-specific molecular tool for selected nosocomial pathogens. Across the available pathogen-extraction comparisons, no LAMP-negative/contact plate-positive results were observed, and relative sensitivity/positive percent agreement and negative predictive value were 100% in all evaluable subgroups. Specificity/negative percent agreement and overall accuracy were consistently high, supporting the reliability of the assay when interpreted against conventional RODAC/contact-plate culture.

The main operational value of LAMP lies in its ability to provide earlier qualitative information than culture and to identify additional LAMP-positive/contact plate-negative signals. These discordant results, partly supported by selective verification, are particularly relevant in high-risk areas and post-sanitation monitoring, where low-level or stressed contamination may not be readily recovered by routine contact plates. Therefore, LAMP should be regarded as an early-warning and screening tool that can guide timely corrective actions, targeted re-cleaning, and prioritization of confirmatory culture.

At the same time, LAMP should not be viewed as a complete replacement for culture-based monitoring. Conventional culture remains indispensable for CFU quantification, recovery of viable isolates, antimicrobial susceptibility testing, and epidemiological typing. The most appropriate implementation is therefore a complementary workflow in which LAMP provides rapid target-specific screening, while culture confirms positive or epidemiologically relevant results and provides isolate-based characterization.

Future prospective studies using individual-level data should assess the impact of LAMP-based environmental monitoring on cleaning performance, outbreak preparedness, turnaround time, cost-effectiveness, and HAI-related outcomes. Expansion of target panels to include additional pathogens and resistance markers could further strengthen its role in integrated infection prevention and environmental hygiene programmes.

**Author Contributions:** For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, B.S., G.B., G.A., S.S., V.Sa., S.Ste., L.G.; methodology, B.S., G.A., L.G., S.Str.; software, G.A., A.D., B.S.; validation, S.Str., G.A., V.Sa., G.B.; oginal draft preparation, B.S., A.D., L.G., V.S., L.F., C.C., A.N., M.C., G.M., S.S., G.B.; writing—review and editing, B.S., A.D., G.B., V.Sa., S.St., G.A., M.G.P., A.S.R., N.Z., G.M., M.C., A.N., C.C., L.F., E.S., S.S., L.G., V.S., X.X.; visualization, X.X.; supervision, G.B., V.Sa., S.St., M.G.P.; G.B., V.Sa., S.Ste.;. All authors have read and agreed to the published version of the manuscript." Please turn to the CRediT taxonomy for the term explanation. Authorship must be limited to those who have contributed substantially to the work reported.

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## Abbreviations

The following abbreviations are used in this manuscript:

| | |
|---|---|
| CFU | Colony-forming units |
| DNA | Deoxyribonucleic acid |
| FN | False negatives |
| FP | False positives |
| HAIs | Healthcare-associated infections |
| IPC | Infection prevention and control |
| LAMP | Loop-mediated isothermal amplification |
| MALDI-TOF MS | Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry |
| NPA | Negative percent agreement |
| NPV | Negative predictive value |
| OPA | Overall percent agreement |
| PABAK | Prevalence-adjusted bias-adjusted kappa |
| PCR | Polymerase chain reaction |
| PPA | Positive percent agreement |
| PPV | Positive predictive value |
| RODAC | Replicate organism detection and counting |
| PABAK | Prevalence-adjusted bias-adjusted kappa |
| TAT | Turnaround time |
| TN | True negatives |
| TP | True positives |
| TSA-LTH | Tryptic soy agar with lecithin, Tween 80 and histidine |
| WHO | World Health Organization |
| κ | Cohen's kappa coefficient |

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