Utility of MolecuLight i:X for Managing Bacterial Burden in Pediatric Burns

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Pediatric burn injuries are vulnerable to severe complications, most often infection, making prompt and precise diagnosis of bacterial bioburden vital to preventing detrimental consequences and optimizing patients' outcomes. Currently, burn wounds are assessed for infection via examining the clinical signs and symptoms of infection, which can be confirmed by swab culture analysis. While the former approach is subjective and experience-dependant, the latter technique is susceptible to missing subsurface, biofilm-associated colonization, and any peripheral bacterial burden, and also delays confirmation by up to 5 days. The MolecuLight i:X is a handheld, noncontact fluorescence imaging device, which can reveal real-time information about clinically significant levels of bacteria and their biodistribution in surface and subsurface burn wound tissues. We conducted a single-center observational study to assess the device efficacy in identifying critical bacterial levels in pediatric burn wounds and to test the children's compliance and the overall feasibility of the device integration into the current diagnostic practice. Ten patients with 16 wounds were recruited and assessed for the presence or absence of clinical signs and symptoms of infection and the presence or absence of bacterial fluorescence on images, with swabs taken to confirm findings. Results demonstrate the device's ability to visualize clinically significant bacterial burden and to localize distribution of pathogens. All clinicians agreed on the high compliance with the device and high feasibility of incorporating the device into routine wound assessments. The results of this study may pave the way toward including bacterial fluorescence imaging into the standard diagnostic algorithm for pediatric burn population.

There is an urgent demand for prompt and precise diagnostic methods in burn wounds to aid in identification of bacterial loads and infection. Infection is the most common burn wound complication, accounting for 75% of mortalities in burn patients.[1,2] Burned patients are more susceptible to antibiotic-resistant microorganisms, resulting in significantly longer admission periods, wound healing delay, and a higher mortality rate.[3] The current standard of care consists of clinician evaluation of clinical signs and symptoms (CSS) of infection and microbiological examination using swab cultures. Bedside visual examination to detect CSS of wound infection includes assessment for heat, pain, erythema, purulent exudate, foul odor, friable granulation tissue, and wound breakdown.[4–6] However, scientific literature exhibits uncertainty about the ability of (CSS) in determining the presence or absence of a clinically significant bacterial bioburden in wounds with a high degree of certainty,[7,8] attributed to their subjectivity, high variability among patients,[7,8] and the significant number of patients with critical bacterial bioburden or local infection which are asymptomatic.[9,10]

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Burn patients present an even greater challenge, as the loss of their primary barrier to bacterial invasion leads to constant exposure to pathogens and a more extensive inflammatory responses,[11] known as systemic inflammatory response syndrome (SIRS). Symptoms of SIRS include temperature elevation and white blood cell count alternation,[11] which are also widely accepted signs of infection.[2] The high prevalence of SIRS in the burn wound population, in addition to the known limitations of CSS, forces burn clinicians to search for other infection cues in the burn wound population.[11]

Swab cultures provide information about bacterial species present and antibiotic sensitivity; however, the swab culture is often called in question due to its inability to identify subsurface infection, microorganisms hiding in a biofilm, and due to its vulnerability to various technical-related limitations.[12,13] Additionally, it usually deploys the suboptimal Levine technique as a standard sampling method which samples only the center of the wound bed and spares the edges where pathogens may colonize.[14] Furthermore, 2 to 5 days are usually required for releasing swab results during which the bacterial bioburden may be altered and clinicians may be compelled to commence empirical antimicrobial therapy, hence, aggravating the issue of antibiotic resistance.[15]

Pediatric burn patients present additional challenges, as they have a higher incidence of infection as compared to adults[16] with an increased likelihood of developing toxic shock syndrome (TSS). TSS is an exotoxin-mediated, life-threatening condition caused mainly by toxin-producing strains of *Staphylococcus aureus*; it accounts for as high as 50% mortality rate if left untreated.[17,18] Diagnostic difficulties are often blamed for this high mortality, due to the nonspecific CSS of TSS mimicking other pediatric illnesses.[17,18] Owing

to these issues, preventing infection is of particular concern while managing pediatric burned patients. The early and correct identification of pediatric burn wound bacterial burden is of a paramount importance to prevent that cascade of detrimental events.[3]

The MolecuLight i:X fluorescence imaging device is a novel, noncontact technology enabling real-time visualization of regions with significant bacterial burden within and around wounds.[15–19] The device illuminates a wound with safe violet light via its two light-emitting diodes. In response to that illumination, the wound tissue components (eg, elastin and collagen) emit a green fluorescence,[20] most bacteria release endogenous red-fluorescing porphyrins, and *Pseudomonas aeruginosa* emits endogenous cyan (blue-green) fluorescing pyoverdins.[15] Optical filters housed within the device remove all noninformative color ranges and the resulting images are displayed on the device screen in real time. As a result, fluorescence images provide an immediate clue at the point of care regarding the presence or absence of significant bacterial loads,[14–19,21] without constraining the treatment plan or being forced to prescribe antimicrobial agents.[15–19]

The utility of this device in identifying clinically significant bacteria in burn wounds has previously been reported.[14–22] Literature generally states that a bacterial load of $\geq 10^5$ CFU/g is an infection indicator.[13–25] Multisite clinical trials with this device showed efficacy in detecting a bacterial levels of $\geq 10^4$ CFU/g (moderate to heavy loads),[22] emphasizing its ability for early identification of bacterial colonization/infection that can be translated into a rapid action to adjust wound management. A recent clinical trial reports that fluorescence information from the device increased sensitivity of detecting moderate to heavy bacterial loads by 3-fold, compared to CSS alone.[26] The device can also detect biofilm-associated bacterial colonization,[27] which are often missed by swab culturing.[22–25,27,28] However, the use of this device in diagnosing pediatric burn infection has not previously been tested.

The objectives of this work were: 1) to evaluate the efficacy of the MolecuLight i:X bacterial fluorescence imaging device in identifying pediatric burn wounds with clinically significant bacterial loads, including wounds with subtle infection, subsurface colonization, and/or infection; 2) to evaluate the ability of the MolecuLight i:X device in improving swabbing practices by providing a better guidance as compared to the CSS and in reducing the diagnosis time; 3) to determine the compliance of pediatric patients toward using this device for their burn wounds and the feasibility of integrating the device into the routine diagnostic practice.

METHODS

Patient Recruitment

This was a single-center observational study conducted over a 2-month period. All patients admitted as inpatients or seen in the outpatient clinic in the Burn Centre of the hospital were considered for this study. Patients were recruited by employing a nonprobability convenience sampling recruitment process. Any patient (under 16 years of age) with acute or chronic burn(s) of any size, depth, and TBSA met the inclusion criteria. Patients were excluded only if a written consent form could not be obtained. A parental consent for the acquisition of photos and for de-identified photo publication was granted for all recruited patients. The images were transferred to Clinical Photography & Design Service Department in the hospital and deleted from the device at the end of each day to guarantee patient confidentiality, as per hospital guidelines.

Study Checklist

For the purpose of the study, a brief history regarding the date, duration, and the mechanism of injury was obtained and a separate general physical examination and burn wound assessment (burn site, size [including depth], TBSA, and etiology) were recorded on the patient's de-identified study checklist. The presence of classical (overt) signs of infection were also assessed and recorded including the presence or absence of edema, warmth, erythema in the uninjured skin, purulent exudate, malodor, history of increasing pain, and delayed wound healing.

Routine Burn Wound Assessment

Recruited patients underwent a routine wound assessment by clinicians as per the hospital standards of care and the clinicians determined whether or not a swab was indicated based on CSS or any other suspicious findings. That was done in a separate manner to eliminate the interobserver influence. When obtaining a swab was indicated based on clinician's judgment from the visual (nonfluorescence) wound assessment, the clinician used the Levine swabbing method. In brief, this identifies an ~1 cm^2 area of a clean, viable tissue, typically from the center of a wound bed, and then rotates and presses a swab over the identified area to express the wound fluid. The clinician was blinded to the bacterial fluorescence status of the wound when taking the swab.

Fluorescence Imaging

The noncontact MolecuLight i:X device illuminates a wound with safe 405 nm violet light, exciting the bacteria and tissues in the region, to emit relevant fluorescence that can be captured and displayed on the display screen in real time (Figure 1). Bacterial fluorescence appears red (most species) or cyan (*P. aeruginosa*) while most tissues fluoresce green. Fluorescence imaging requires darkness; therefore, the room lights were switched off, door and curtains were closed (if applicable), and the device's ambient room light status indicator was monitored to ensure sufficient darkness for optimal imaging conditions. If sufficient darkness could not be obtained, surgical drapes were used to surround the device and enclose the wound acting as a light shield. Fluorescence images were acquired with the MolecuLight i:X positioned at an optimal imaging distance (8–10 cm from the wound) as indicated by the device's built-in range finder. After fluorescence imaging acquisition, the lights were turned on and a standard (white light) image was obtained by the device. Figure 2 outlines the sampling method decision tree that was followed to determine whether a conventional or a fluorescence-targeted sample should be acquired. When a swab was not indicated based on standard assessment, and the fluorescence images

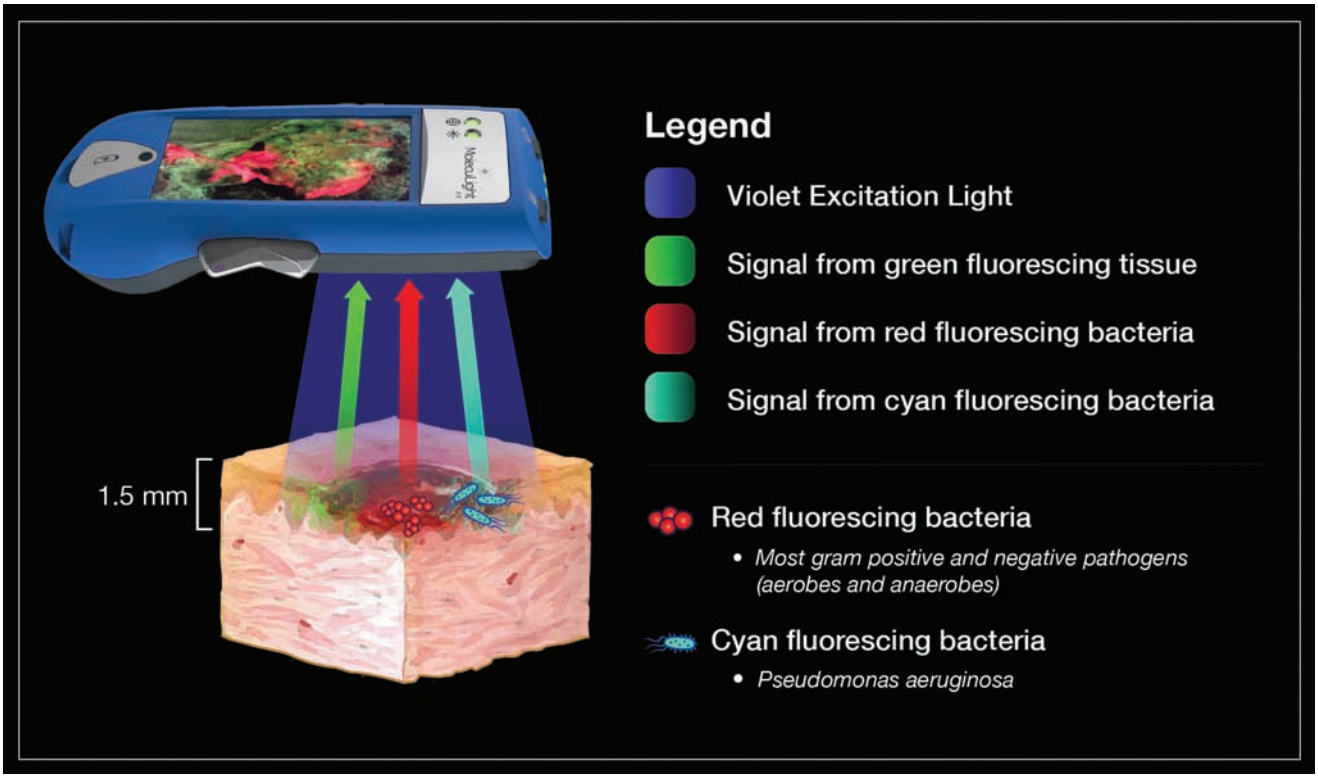

Figure 1. Schematic of the MolecuLight i:X imaging device in fluorescence mode.

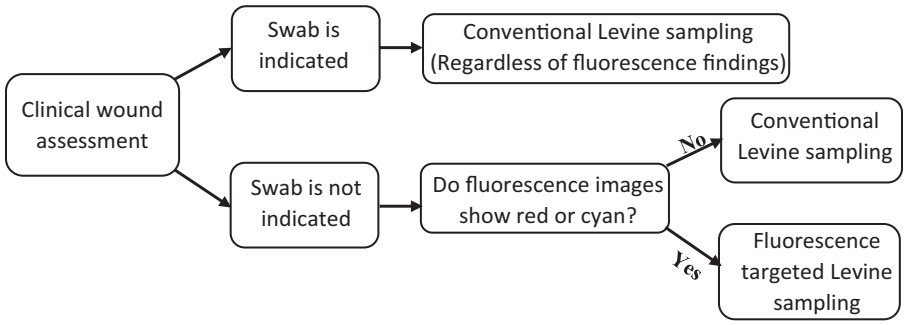

Figure 2. Process followed to determine sampling method for a given burn. Levine swabs were obtained in all cases, but the majority were not targeted to regions of fluorescence.

showed no red/cyan signal, the nurse performed a conventional swab. When a swab was not indicated based on standard assessment but a red/cyan fluorescence signal was observed, the nurse was shown the fluorescence image and asked to perform a fluorescence-guided Levine swab, targeting a region of suspected bacterial fluorescence.

Microbiology

Standard microbiological culture and sensitivity testing was performed, including gram staining, identifying the species present in the wound along with a semiquantitative scale of bacterial load (no growth = no pathogen isolated or a growth of nonpathogenic strains only, light: ≤10 CFU/plate, moderate: 10–100 CFU/plate, and heavy: ≥ 100 CFU/plate). Microbiological technicians were blinded to the CSS and Fluorescent Light (FL) status of the wound being tested.

Clinician Questionnaire

Immediately after imaging, attending clinicians were asked to complete a questionnaire which evaluated patient's fear of the device and/or the darkness required for fluorescence imaging, overall patient's compliance with the imaging process, and the practicality of using the MolecuLight i:X imaging device as part of routine practice. Each was rated on a scale from 0 (absent/lowest) to 4 (highest). The questionnaire also assessed the occurrence of any adverse events (if applicable) and the average time spent acquiring a fluorescence image.

RESULTS

Patient Population

Ten pediatric burn patients (eight males, two females) with 15 wounds, and 16 observations (one wound was examined twice over a 15-day period) were assessed during the study period.

The median patient age was 2 years and 3 months. Eight of the 15 wounds (53%) were on upper extremities, five (33%) on lower limbs, and two (13%) on trunk. A Lund & Browder Chart used to assess the extent and size of burn wounds revealed a median of 1.25% TBSA. Nine wounds (60%) were partial thickness while the remaining 40% were full-thickness burns. Contact burns were the most prevalent (60% of cases), followed by scald (30%) and flame (10%) burns. No patients were excluded from the study and no adverse events were reported.

Fluorescence Images

Eight wounds (50%) from six patients exhibited red or cyan (bacterial) fluorescence on MolecuLight i:X images, indicative of bacteria at clinically significant loads. From those eight wounds, 62.5% exhibited bacterial fluorescence in the wound center and peripheries (Figure 3A), and only a single wound exhibited bacterial fluorescence solely in the wound center, the most likely location for sampling based on current standard practice.[29]

The presence or absence of bacterial fluorescence on images was consistent with CSS of infection in 87.5% of cases (14/16). However, in the remaining two cases MolecuLight i:X images detected red (bacterial) fluorescence in the absence of any signs or symptoms; both these cases were confirmed to be bacterial positive (moderate growth of *S. aureus*) via culture of fluorescence-targeted swabs.

The presence or absence of bacterial fluorescence on images was consistent with culture analysis in 81% of cases (13/16), and no false negatives were detected. The remaining three wounds (4, 5a, and 5b in Table 1) were fluorescence and CSS positive in both observations (according to the researcher and attending clinician's findings), and were therefore swabbed using the conventional Levine method, that is, the swab did not target the region(s) of bacterial fluorescence, so it likely missed the region of bacteria. Figure 3B summarizes the findings. The time between collection of the wound swabs and the receipt of the samples by the Microbiology Department ranged from a same-day delivery (0 day) up to 3 days. Surprisingly, *S. aureus* was the only pathogen reported from microbiological cultures in this patient population.

Clinician Questionnaire

Twenty-seven responses from healthcare staff rating patient response to the imaging device and overall practicality were collected immediately following fluorescence image acquisitions. Overall, patient's fear of required darkness was entirely absent, patient's compliance with imaging procedure was entirely positive, and patient trepidation toward the imaging device itself was minimal (Figure 4). The reported time required for taking fluorescence images ranged from 25 to 45 seconds (mean of 35 seconds). No adverse events have been reported.

CASE REPORTS

The records of five patients (seven wounds) are described below, demonstrating cases where: 1) fluorescence images identified wounds positive for pathogens that were missed by routine assessment, 2) nontargeted swabs failed to locate pathogens in wounds positive for bacterial fluorescence and CSS, 3) fluorescence images guided swabbing to a region of the wound that would otherwise not have been sampled, resulting in positive culture reports, and 4) images were able to spectrally discern *P. aeruginosa* by visualizing its characteristic cyan fluorescence signal.

Patient 1

A 22-month-old male patient presented with a 3.5% TBSA, partial-thickness scald burn involving the plantar aspect of the left foot and the upper half of right foot's dorsal aspect (wounds 1a and 1b in Table 1). The patient was presenting for checkups and dressing changes at 11 and 15 days postburn. At the day 11 checkup, dressings were removed (a silver-based antimicrobial dressing) and both wounds were cleaned with normal saline and a sterile gauze. The examining nurse conducted a visual examination and stated that there was no need to take swabs from either the wounds. No clinical signs of local infection were noticed in wound 1b. However, the region adjacent to wound 1a was tender, warm, edematous, and erythematous. Fluorescence images supported these findings; no red or cyan fluorescence signal was detected in wound 1b, while a red fluorescence signal was noticed in wound 1a's peripheries (Figure 5B). A nurse was asked to

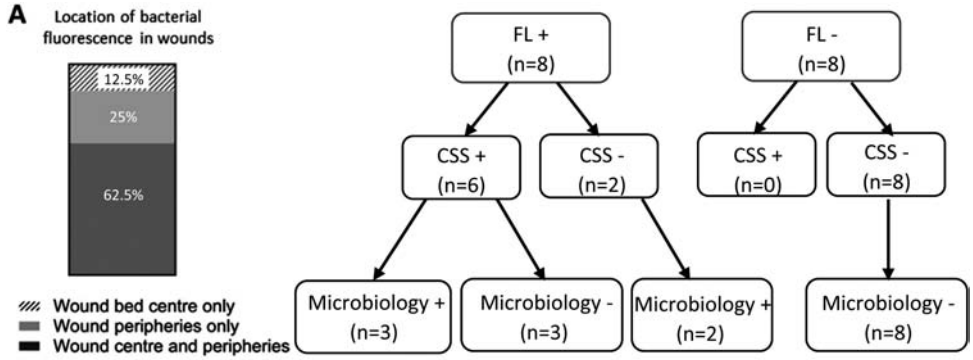

Figure 3. Bacterial fluorescence (red or cyan) was observed in eight study wounds. (A) The location of bacterial fluorescence in these eight wounds, shown as percentages. (B) Flow chart of clinical signs and symptoms (CSS) and microbiological culture results (Microbiology) in both bacterial fluorescence positive (FL+) and bacterial fluorescence negative (FL–) study wounds.

Table 1. Summary of patients' results

No.	Age/Gender	Aetiology	Bacterial fluorescence	Sampling technique	Microbiology results	Signs of local infection
1a	1 y 10 m M	Scald	Red signal present	Targeted	Moderate growth *Staphylococcus aureus*	Erythema, edema, warmth, pain
1b	=	=	Absent	Conventional	No pathogen isolated	Absent
1a	=	=	Absent	Conventional	No growth	Absent
2	1 y 3 m M	Scald	Red/cyan signal present	Conventional	Heavy growth *Staphylococcus aureus*	Erythema, edema, pain, purulent excaudate, foul odor
3a	2 y M	Contact	Red signal present	Targeted	Moderate growth *Staphylococcus aureus*	Absent
3b	=	=	Red signal present	Targeted	Moderate growth *Staphylococcus aureus*	Absent
4	2 y 8 m F	Contact	Red signal present	Conventional	No pathogen isolated	Edema, erythema, warmth
5a	7 y 4 m M	Flame	Red signal present	Conventional	No pathogen isolated	Erythema, pain, purulent exudate, foul odor
5b	=	=	Red signal present	Conventional	No pathogen isolated	Erythema, pain
6a	1 y 3 m M	Contact	Absent	Conventional	No growth	Absent
6b	=	=	Absent	Conventional	No growth	Absent
7a	3 y F	Contact	Red signal present	Conventional	Light growth *Staphylococcus aureus*	Erythema, warmth, pain
7b	=	=	Absent	Conventional	No growth	Absent
8	1 y 4 m M	Contact	Absent	Conventional	No growth	Absent
9	13 y 9 m M	Scald	Absent	Conventional	No growth	Absent
10	7 y M	Contact	Absent	Conventional	No growth	Absent

F, female; *M*, male; *m*, month; *y*, year; =, similar to above.

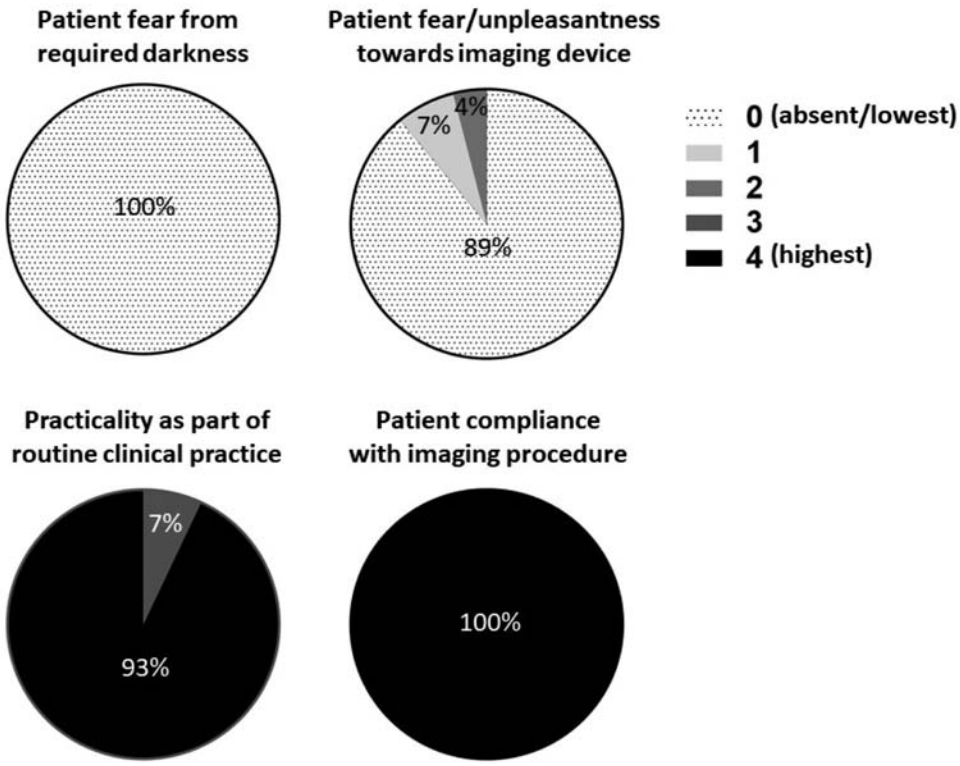

Figure 4. Results of clinician questionnaire assessing the practicality of using this fluorescence imaging device on the pediatric population.

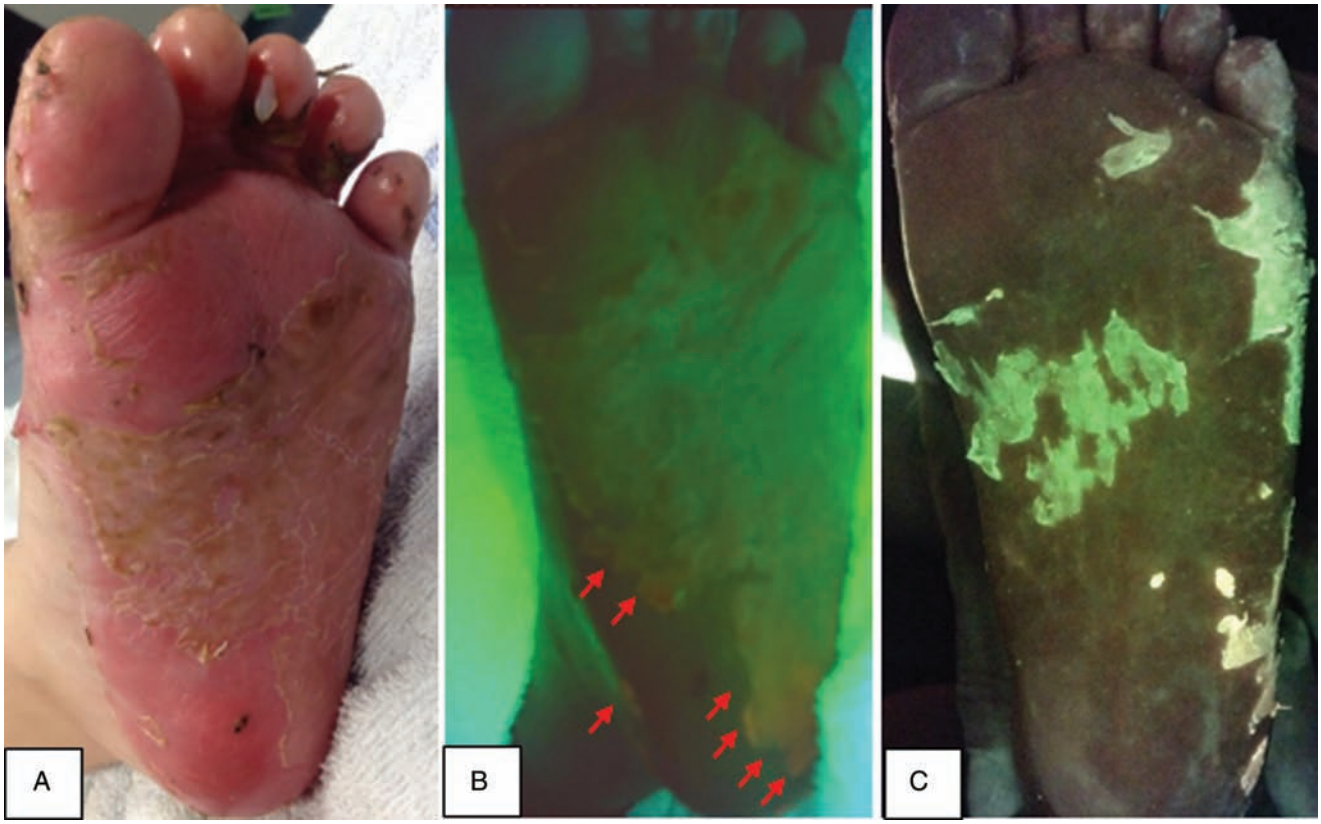

Figure 5. Wound 1 "a." (A) White light image, 11 days postburn. (B) Fluorescence light image, 11 days postburn. ➔ Red fluorescence signals. (C) Fluorescence light image, 15 days postburn showed no red/cyan fluorescence signal.

perform a targeted Levine swab based on the fluorescence image for wound 1a and a conventional swab for wound 1b; the swabs were collected and delivered to the laboratory a day after. Microbiology results revealed a moderate growth of *S. aureus* bacteria for wound 1a, while the swab from wound 1b isolated no pathogen.

Four days later, the same patient came to the outpatient clinic for a dressing change. Inspection of wound 1a showed notable healing with no signs of infection, which made the examining nurse to decide discharging the patient with no need for further dressings. No red/cyan fluorescent signals were detected by the fluorescence imaging device (Figure 5C). Nevertheless, a conventional swab was taken from the wound and negative microbiological results were yielded.

Patient 2

A 15-month-old male patient was recruited to the study during a dressing change session in the burn wards. The patient presented with full-thickness burns involving the abdomen, chest, and part of the neck due to a scald injury. His previous silver-based dressings were removed, wound cleansing was performed, and the visual examination revealed an edematous, erythematous wounds with copious amount of a bluish-green, thick exudate and a characteristic malodor that suggested *P. aeruginosa* infection.[30] As per the examining nurse assessment, a swab was indicated. In spite of being relatively large (≈7% TBSA), the wound was sampled by one swab, taken from the center of the wound using a conventional, nontargeted Levine technique. This swab was delivered to the lab 1 day after collection. Fluorescence images displayed a characteristic cyan color indicating the presence of *P. aeruginosa*, consistent with the CSS, with red fluorescence positive signals also emitting from various regions all over the wound, suggesting the presence of additional bacterial species (Figure 6B and D). However, microbiological cultures revealed only a heavy growth of *S. aureus* bacteria, without isolating *P. aeruginosa* species.

Patient 3

A 2-year-old male patient was recruited to the study 6 days post-injury. He suffered from 1% to 1.5% TBSA burns caused by a direct contact to a hot object involving the dorsal radial aspect of the left distal forearm and thumb (wound 3a) and the ventral aspect of the middle third of the right forearm (wound 3b). Clinicians decided to manage his two deep-dermal thickness burns via wound excision and skin grafting. The patient had previously undergone a vigorous debridement. Both wounds exhibited no clinical signs of infection and the attending nurse stated the unnecessity of obtaining swabs from either wound. Fluorescence images revealed red fluorescent signals in both wounds, in the center and peripheries (Figure 7, wound 3 "a," "b"). Hence, targeted swabs focusing on those areas were obtained and culture analysis identified a moderate growth of *S. aureus*.

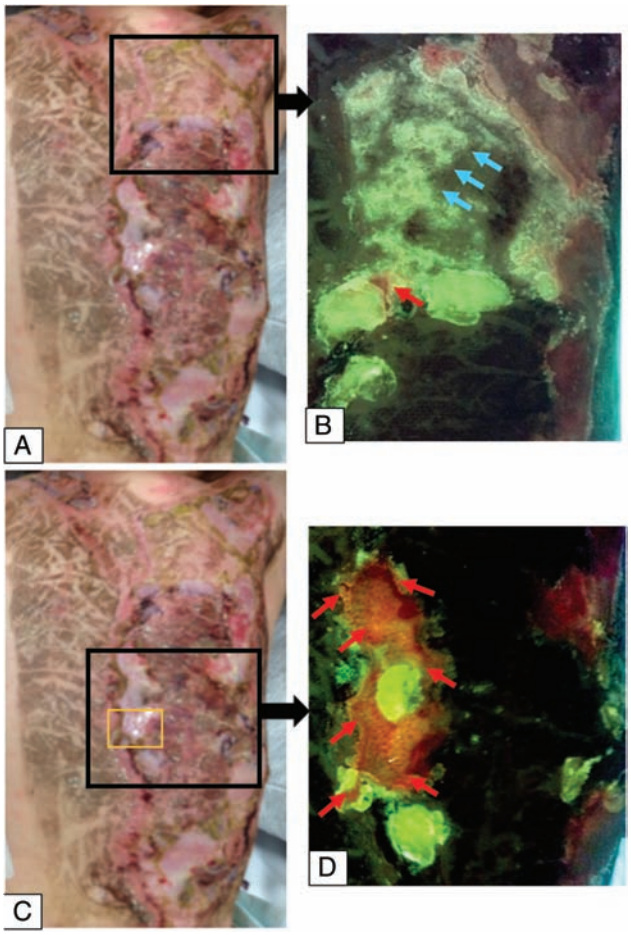

Figure 6. Wound 2. (A, C) White light images for the whole burn. (B, D) Fluorescence light images for specific sections. → Red fluorescence signals, → cyan fluorescence signals, ▭ the place from which the swab was taken.

Patient 4

A 2-and-a-half-year-old female presented for a scheduled examination and dressing change 9 days after a partial-thickness, contact burn involving ≈2% TBSA of the plantar aspect of her right foot. The previous dressing was removed, the wound was cleaned with normal saline, and a standard examination revealed classical signs of local wound infection including edema, erythema, and warmth in the surrounding tissues. Her previous blood investigation revealed a leukocytosis. A consultant's opinion was sought for this case and a decision to take a conventional swab was made. Fluorescence imaging displayed a large red fluorescence area involving the center and peripheries of the wound (Figure 7, wound 4). The swab was delivered to microbiology 1 day after collection and culture results attained 3 days later isolated no pathogen.

Patient 5

A 7-year-old male presented with mixed depth (superficial and deep) burns from a direct flame injury representing 1% to 1.5% TBSA and involving the right axillary region and the upper right part of the chest (wounds 5a and 5b in Table 1). Patient was attending clinic for a dressing change (silver-based antimicrobial). Visual examination of wound 5a revealed an erythematous area with a moderate amount of purulent excaudate of a foul-smelling odor and tissue sloughing. The wound was vigorously cleaned and a swab was taken from a viable area adjacent to that manifesting CSS of infections. Interestingly, fluorescence images localized a red fluorescence to an area opposite to that of clinical interest (Figure 7, wound 5 "a"). Nevertheless, the swab was obtained from the clinically relevant area based on the nurse's judgment and as per routine practice. Wound 5b had erythema and a history of increasing pain. The examining nurse recommended a conventional swab for his wound. Fluorescence images revealed red fluorescence in the wound center and peripheries (Figure 7, wound 5 "b"). Despite agreement between fluorescence images and CSS, swab results were negative for both wounds.

DISCUSSION

The results of this study demonstrate the efficacy of the MolecuLight i:X bacterial fluorescence imaging device in identifying pediatric burn wounds with clinically significant bacterial loads, including wounds with clinically subtle infection, those with subsurface colonization, and/or infection. The presence of red or cyan fluorescence mapped regions of bacteria in the wound, which were found in the wound center and often in the wound peripheries, where a standard swab would typically not assess.[29] All bacterial fluorescence negative wounds resulted in negative microbiological cultures. We observed that swabs acquired without fluorescence guidance tended to miss regions of bacteria, resulting in negative cultures, and one swab revealed only *S. aureus* and missed the presence of *Pseudomonas*, which may have required a treatment plan change.[31,32] Pediatric patients demonstrated strong compliance and overall comfort toward incorporation of this device into their care and clinicians reported high feasibility of integrating this device into routine care in the pediatric burn population.

Several studies have evaluated the effectiveness of this device by comparing bacterial fluorescence imaging results with that of swab culturing.[14–25,27–33] However, mounting evidence suggests that the swab culturing technique may be suboptimal for image validation. For example, Hoeflok et al[33] interpreted two observations from a total of 48 as "false positives" when fluorescence images suggested significant bacterial loads while culture analysis results revealed no growth. Similarly, both Blumenthal and Jeffery[15] and Blackshaw and Jeffery[28] each identified one wound from a total of 20 and 17 respective wounds with an image positive for bacterial fluorescence and a negative culture result. One of these "false positives" also exhibited CSS of infection. In the current study, fluorescence positive wounds yielded microbiological positive cultures in only 66% of samples, despite these wounds being both bacterial fluorescence and CSS positive. Undetectable bacteria by swab culturing does not necessarily imply a "false positive" from the fluorescence images, as the lack of cultured bacteria can be attributed to the failure of swab cultures in detecting 1) subsurface or biofilm-associated bacteria which the device is able to visualize,[20–25,27] 2) bacterial presence in a wound's peripheries that was not specifically swabbed, 3) anaerobic/fastidious bacteria that are not typically cultured for in a clinical microbiology laboratory,[34,35] 4) or as an impact

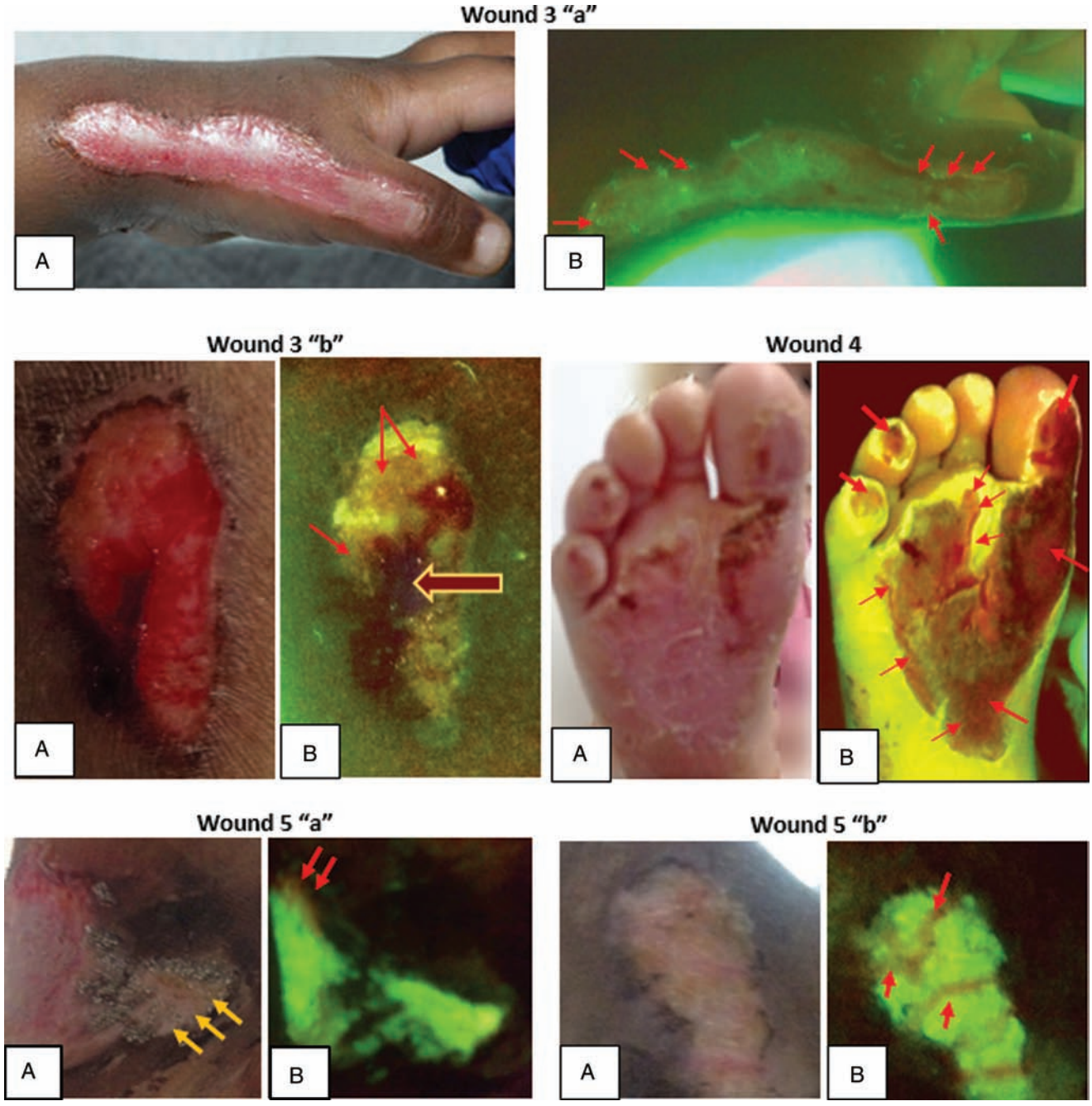

Figure 7. Wound 3, 4, 5. (A) White light image. (B) Fluorescence light image. ⟹ Red fluorescence signals, {inline_image} blood within wound's vasculature, → area with Clinical signs of infection.

of antimicrobial usage which leads to false-negative culture results.[12–25,27–36]

Subsurface bacterial colonization can go unnoticed during standard wound assessment and is often associated with wound chronicity, if left untreated.[6–25,27–37] Previous studies have demonstrated the ability of the MolecuLight i:X to capture subsurface bacterial colonization, to a depth of ~1.5 mm.[21–25,27–38] Note that overlying tissues cause optical scattering of fluorescence signals resulting in an attenuated fluorescence intensity[20–22] and a lighter red color (pink/bluish) on images.[19,22] A recent multi-center, 60-patient clinical trial was conducted to assess the positive predictive value of red fluorescence on images.[21] This study used fluorescence-guided biopsy and curettage sampling methods, both of which assess surface and subsurface bacterial load. Strikingly, no false-positive cases were obtained from the 60 red-fluorescing wounds (some of which fluoresced pink/blush) recruited for the trial, resulting in a Positive Predictive Value (PPV) of 100% of red/pink/blush fluorescence on MolecuLight i:X images for detecting bacteria.[21] An ongoing trial sampling cyan-fluorescing regions via curettage and culture-based analysis reported similar interim results, again with a PPV of 100%.[39] These studies suggest that fluorescence-guided curettage or biopsy sampling must be used when evaluating bacterial fluorescence

effectiveness to prevent instances of false-negative microbiology due to surface-only sampling. The ability of the device in detecting subsurface bacterial fluorescence in this study is highlighted by patient 4, who exhibited various shades of red on the MolecuLight i:X image as well as local signs of infection and a leukocytosis in a previous blood count; yet the swab from this patient yielded no pathogens.

This study and others also attest to the importance of targeting sampling to regions of fluorescence.[14–21] Fluorescence-guided swabs have previously been shown to significantly improve accuracy over swabbing guided by CSS alone.[14–21] From the eight bacterial fluorescence (red or cyan) positive images (eight wounds) in this study, the majority of wounds exhibited bacterial fluorescence either solely in the wound's peripheries (two wounds) or in the wound's center and peripheries (five wounds). This colonization pattern has a high potential to be missed by the routine swab. All swabs targeted to regions of bacterial fluorescence in this study yielded microbiologically positive cultures, despite that some of these wounds being even CSS negative. In contrast, only 40% (2/5) of wounds that were swabbed by a conventional, nonfluorescent targeted method, yielded microbiological positive cultures despite these wounds being both bacterial fluorescence and CSS positive. Furthermore, in one instance the wound swab revealed the presence of *S. aureus* but missed the presence of *Pseudomonas* when cyan fluorescence on the wound was not specifically targeted. Hence, the routine swab technique completely missed significant bacterial bioburdens in 60% (3/5) of observations. Those results are accordant with a prior clinical trial in which fluorescence images were able to detect significant bacterial loads in 67.1% of observations which had been overlooked by the Levine swabbing technique.[21] A recent case series ($n = 7$) found that swabs targeted to regions of bacterial fluorescence resulted in heavy bacterial loads on microbiological cultures in all cases.[19]

Patient 2 illustrates how the MolecuLight i:X device was able to spectrally discern *P. aeruginosa* by visualizing its characteristic cyan fluorescence signal.[39] Early identification of this pathogen is critical, as a unique management strategy is required to prevent severe outcomes and high rates of mortality in *Pseudomonas*-infected burn wounds.[31,32] The patient clinical evaluation was suggestive of *P. aeruginosa* presence.[30] However, the microbiological cultures did not isolate this species. The hospital's microbiology practice cultures burns swabs in blood, chocolate, and CLED (cystine–lactose–electrolyte-deficient) agars where *P. aeruginosa* is readily grown.[40] Therefore, failure of isolating this pathogen may be due to the sampling procedure, which did not target the cyan-emitting regions and instead sampled only the center from a relatively large wound (7% TBSA) or this could be attributed to the use of the topical antimicrobial dressing that may result in false-negative cultures.[41]

Real-time evidence of bacterial presence at loads of concern ($\geq 10^4$ CFU/g)[19] enabled immediate identification of subclinically infected patients that were not flagged by CSS alone. Determining whether the CSS of infection are present or absent is highly subjective and experience-reliant, prone to false negatives even for the most experienced clinicians,[9] and often associated with an inter-rater variance.[6–25,27–43] In this study, one wound was deemed to be uninfected by the nurse' judgment, yet presented CSS of infection from the researcher's point of view, resulting in an approximately 93% interobserver agreement which were consistent with microbiology results. However, wounds 3a and 3b were missed by both the researcher and the examining nurse, whereas fluorescence images immediately revealed a prominent red fluorescent signal (moderate growth of *S. aureus*). Previous studies have identified a role for MolecuLight i:X images in identifying subclinical (asymptomatic) infections in chronic foot ulcers patients,[37] venous leg and pressure ulcers,[19,26] and driveline infections,[44] which necessitated immediate treatment plan modifications involving antibiotics prescription and wider margins/more aggressive debridement sessions. Fluorescence-guided debridement was not part of the current study protocol; however, a study of 20 diabetic foot ulcer patients subjected to aggressive curettage debridement found that 17 cases (85%) were submitted to additional curettage debridement when fluorescence images revealed persistence of the red or cyan signal (likely subsurface bacteria).[38] Debridement targeting red or cyan regions based on MolecuLight i:X guidance also spared nonfluorescing, healthy tissue from being removed.[38] A study of 63 venous and lymphatic ulcers in which bacterial fluorescence was mapped with MolecuLight i:X found that 44% exhibited a persistent or increased red fluorescence signal post-debridement, heralding a deep compartment infection.[45] These studies highlight the role for MolecuLight i:X in evaluation of debridement effectiveness based on real-time evidence of bacterial presence and location at loads of clinical concern.

Patients were very compliant to use of the device, as evidenced by clinician questionnaire responses and staff testimonials. A subset of the patients worried that the device would come into contact with their wounds and cause pain, but after switching on the violet light they viewed it as a fun toy. The iPod system housed within the device enabled download of cartoons, games, and videos, a capability which could be used in future studies to enhance patient acceptance and interest. No study patients were scared of the required darkness; older patients were told how the device works, so they would understand why the lights needed to be turned off, while younger patients were simply informed that we would be switching the lights off to show them something interesting. The device has been validated for its safety to be used, provided that the users are adherent to the safety instructions mentioned in the user guide.[46] No any adverse events were witnessed during imaging sessions and this proved its safety in the short term. An average time of 35 seconds to take an image is not unreasonable, and should not cause any delay or interference with the daily diagnostic workup, especially if it will reduce the time needed for confirming the presence of significant bacterial loads (on the order of days for culture results in this study). Overall, the MolecuLight i:X imaging device can be efficiently integrated into the standard of care wound assessment process, is well-accepted by children, and is safe and fast to use.

This observational study found that MolecuLight i:X was able to instantly detect the presence and distribution of significant bacterial loads over the wound area, regardless of whether patients had overt symptoms or subtle/asymptomatic bacterial burden. Prompt recognition of burn wound

bacterial colonization and/or infection will facilitate timely, evidence-based wound management, which is likely to have a beneficial effect on wound prognosis and healing.[21] Instant identification of a patient bacterial status could potentially aid antimicrobial stewardship, as has been shown by others,[19] providing real-time evidence for antibiotic decision making; this is a vital first step in addressing burgeoning world-wide antibiotic resistance.[41] Results of this study demonstrate how real-time confirmation of bacterial status could reduce the need for swabs (eg, real-time identification of *Pseudomonas*) and would prevent false-negative swabs by targeting a region of the wound with bacterial (red or cyan) fluorescence. We anticipate that this would translate to cost savings, attained from integrating the device into routine practice. In the United Kingdom, the swab test currently costs around £90, with an extra £5 for any additionally requested sensitivity test per microorganism.[47,48] Cost savings could also be attained through evidenced-based dressing selection. In this study, most patients were managed with silver-based dressings, some of which were unnecessary based on imaging and culture results. Silver-based dressings cost the UK National Health Service more than £25 million in 2010[49]. Incorporating the MolecuLight i:X device in routine wound assessment may palliate this financial strain, reducing the use of unwarranted silver and antimicrobial dressings. A thorough cost-benefit analysis comparing the use of MolecuLight i:X to the current practice is required for a better understanding of the device's cost-effectiveness.

Limitations

The primary aim of this observational, limited sample size (n = 16) study was to explore the utility of MolecuLight i:X in pediatric burns, as it is the first study to use the device on this patient population. Study results demonstrate the practicality of including MolecuLight i:X in routine pediatric burn management. However, a larger-scale, multi-center, randomized study would be required to fully understanding the device utility in pediatric burn assessment and wound management. Limitations of the device itself should be noted. Outside of *P. aeruginosa* the MolecuLight i:X cannot provide information about the species of microorganisms present or about the antibiotic susceptibility. Cultures or other microbiological methods are still required to obtain this information, ideally from a fluorescence-targeted, curettage or biopsy sample.[22] Note that the device will detect most culturable and nonculturable (fastidious) species. Clinicians should be aware that a small number of bacterial genera (eg, *Streptococcus*) do not fluoresce.[20] However, these species occur in conjunction with fluorescing pathogens in the vast majority of wounds (>99%),[50] so are likely to be detected regardless (eg, seen in[26]). The device does not indicate the specific bacterial load in a wound, only that the load is higher than 10^4 CFU/g, a level considered as the tipping point between requiring vigilance of the wound to requiring intervention to address the bacterial load.[6] Care should be taken to remove blood from the field of view prior to imaging,[27] as attenuation of the excitation light can occur by the presence of blood, resulting in a darker fluorescence area which could impede the detection of subsurface bacteria.[46] Wound 3b is an excellent example, where despite having been subjected to a preimage cleansing, the presence of blood within the wound vasculature appeared as a dark area in the fluorescence image. Fluorescence imaging requires complete darkness, which was not possible to be achieved in all hospital rooms. A "MolecuLight DarkDrape" is available specifically for this purpose, but was not used in this study. Rather, surgical drapes were used to surround the device to ameliorate the lighting conditions, when possible; though this created useable darkness it was not always possible to apply them when the child was moving. "DarkDrapes" are recommended for future studies, particularly those on the pediatric age group.

Other practice-related limitations were also faced. To guarantee optimal recovery of all bacteria, swab samples have to be transferred for microbiology analysis once collected, typically within 4 hours.[6–25,27–49,51] However, the average time samples have taken to be analyzed in this study was 1 day. Additionally, plates for anaerobic bacteria are not employed in the routine practice of culturing burn wound swabs at the parent hospital. Anaerobic bacteria represent a significant component of bacterial population[50] and are often associated with a delayed wound healing in acute and chronic wounds.[13] Molecular methods are more likely to detect anaerobes and other fastidious bacteria.[50] Additionally, most of patients recruited in this study were managed with topical antimicrobial agents before obtaining swabs that could result in false-negative cultures.[41] It is recommended to take these limiting factors into consideration for future studies.

CONCLUSION

As smart technology is encroaching on every aspect of healthcare practice, it is of no doubt that the future diagnostic techniques will be of a single-touch approach. The MolecuLight i:X imaging device is a portable, noninvasive diagnostic tool that provides real-time information about the presence and biodistribution of clinically significant bacterial loads at point of care. The study results revealed the device's ability to immediately detect a significant bacterial bioburden, including subsurface burden, and subclinical bacterial colonization, and/or infection, thus potentially avoiding unnecessary swabs and the delay associated with waiting for the results. This has the potential to financial savings along with improving outcomes. Results of a clinicians-targeted questionnaire revealed the potential of the device's successful integration into the routine diagnostic system. As an aid to the applied diagnostic modalities, this device can bridge the gaps of the current diagnostic deficits and may shape the future of wound care management.

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