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Uncovering the high prevalence of bacterial burden in surgical site wounds with point-of-care fluorescence imaging

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Abstract

Detection of bacterial burden within or near surgical wounds is critical to reducing the occurrence of surgical site infection (SSI). A distinct lack of reliable methods to identify postoperative bioburden has forced reliance on clinical signs and symptoms of infection (CSS). As a result, infection management has been reactive, rather than proactive. Fluorescence imaging of bacterial burden (FL) is positioned to potentially flip that paradigm. This post hoc analysis evaluated 58 imaged and biopsied surgical site wounds from the multicentre fluorescence imaging assessment and guidance clinical trial. Diagnostic accuracy measures of CSS and FL were evaluated. A reader study investigated the impact of advanced image interpretation experience on imaging sensitivity. Forty-four of fifty-eight surgical site wounds (75.8%) had bacterial loads $>10^4$ CFU/g (median $= 3.11 \times 10^5$ CFU/g); however, only 3 of 44 were CSS positive (sensitivity of 6.8%). FL improved sensitivity of bacterial detection by 5.7-fold compared with CSS alone ($P = .0005$). Sensitivity improved by 11.3-fold over CSS among clinicians highly experienced with FL interpretation ($P < .0001$). Surgical sites that reach the stage of referral to a wound specialist frequently harbour asymptomatic high bacterial loads that delay healing and increase infection risk. Advanced imaging of pathological bacterial burden improves surgical site monitoring and may reduce the rate of SSIs.

KEYWORDS

bacteria, diagnosis, fluorescence imaging, MolecuLight, surgical site infection

Key Messages

- surgical sites that reach the stage of referral to a wound specialist are highly likely to harbour high bacterial loads, but unlikely to exhibit signs and symptoms of infection
- clinical signs and symptoms assessment has poor sensitivity (6.8%) for detecting high bacterial loads in surgical wounds

- point-of-care fluorescence imaging for high bacterial loads improved sensitivity by 5.7-fold compared with signs and symptoms alone
- advanced image interpretation training, including hands-on experience with this imaging modality, further increases sensitivity of fluorescence imaging up to 11.3-fold compared with clinical signs and symptoms alone

1 | INTRODUCTION

The incidence of surgical wound complications, including surgical site infections (SSIs), and wound dehiscence continue to rise despite advances in surgical technique, intraoperative practice, and a growing assortment of advanced wound care dressings. Development of an SSI is associated with a marked increase in morbidity, a 2- to 11-fold increase in rate of mortality, and prolonged hospital stays.[1] This places considerable economic burden on health systems. In Australia, costs exceed $268 million per year, while in the United Kingdom and United States, it can cost up to $10 billion per year.[2-5] These costs include extended stays in hospitals, readmissions, more frequent access to community nursing services for clinical management, and more resources required to manage complications. Approximately 2% to 5% of surgical wounds in the United States, Canada, and Australia develop a SSI,[6-8] while in Southeast Asia and Singapore, the incidence of SSI is as high as 7.8%[9] (Table 1). Surgical wound complications such as infection or dehiscence are often caused by a combination of factors during the preoperative, intraoperative, and postoperative phases of a patients' surgical journey.[10,11]

Early detection of surgical wound complications, including high bacterial levels on and near the incision site, may be critical to reducing the likelihood of an SSI. However, reliable and consistent methods to identify bacterial-associated complications such as SSIs in both the acute care and post-discharge setting have been lacking. Contemporary diagnosis relies upon the assessment of clinical signs and symptoms of infection (CSS) primarily, and reporting is based upon meeting Centres for Disease Control (CDC)[12] criteria. According to CDC criteria, a superficial incisional SSI is one that: occurs within 30 days of the operative procedure, involves only the skin or subcutaneous tissue of the incision, and has at least one of the following: diagnosis of a superficial incisional SSI is performed by a physician or physician designee; purulent drainage from the superficial incision; organisms isolated from an aseptically obtained culture of fluid or tissue from the superficial incision; superficial incision that is deliberately opened by a surgeon, unless culture of incision is negative, and at least one of the following signs or symptoms of infection present: pain or tenderness, localised swelling, redness or heat.[13] Other more objective wound infection scoring systems exist, including the Southampton Wound Assessment Scale and the ASEPSIS.[14,15] These assessments provide a numeric value to indicate the severity of infection but were developed for use following specific types of surgeries, therefore limiting their widespread utility. Like the CDC definition, these scoring systems rely heavily on the presentation of signs and symptoms of infection. However, in many instances, the signs of infection are absent or subtle. They may also be mistaken for the typical inflammatory response.[16]

Presence of high bacterial loads is a significant risk factor for the development of an SSI and delayed wound closure. A prospective observational study of 100 surgical wounds after lower limb vascular surgery found that high bacterial loads ($>10^5$ CFU/swab) on postoperative day 2 independently increased the risk of an SSI.[17] Similarly, other studies have shown that high peri- and postoperative bacterial loads in surgical sites are significantly correlated with greater risk of postoperative complications.[18,19] These findings are consistent with studies in chronic wounds that observed delayed healing associated with the presence of high bacterial loads.[20-22]

Notwithstanding the advancing antimicrobial resistance age, and a narrowing of the drug pipeline for antibiotics,[23] novel methods for detecting bacterial burden beyond CSS and traditional swabbing of wounds for infection have not been forthcoming. However, a relatively new point-of-care diagnostic imaging technology has recently amassed a compelling body of evidence demonstrating detection of the presence and location of bacterial at loads of clinical concern, within wounds and their surrounding tissue.[24-31] This contrast-free imaging

TABLE 1 Incidence of surgical site infection

Country/region	Surgical domain	Incidence
India	C-section	13%-38%[44,45]
Korea	Pooled	2%-9%[46]
Australia	CABG	3%[8]
USA	Pooled	2%-4%[6]
Canada	Pooled	2%-5%[7]
Southeast Asia and Singapore	Pooled	7.8%[9]

technology provides an opportunity to overcome challenges in early detection of bacterial burden in wounds by harnessing endogenous fluorophores from bacteria to create of map of high bacterial burden in and around wounds. The 350-patient fluorescence imaging assessment and guidance (FLAAG) clinical trial validated the diagnostic accuracy of fluorescence imaging to detect bacteria in chronic wounds, including SSIs, diabetic foot ulcers, venous leg ulcers, and pressure ulcers. Sensitivity of fluorescence imaging to detect wounds with higher bacterial burden was 4-fold higher compared with standard of care assessment of CSS,[24] leading to earlier detection of these burdened wounds, improved hygiene strategies, and more objective prescribing practices.[32] Although fluorescence imaging produced improved sensitivity across all wound types included in the FLAAG trial, sensitivity of imaging for SSIs was still lower than other wound aetiologies evaluated (eg, diabetic foot ulcers, venous leg ulcers, and pressure ulcers).[24] Of note, the clinicians participating in the FLAAG clinical trial were using the imaging modality for the first time. Given that all medical imaging modalities involving image acquisition and interpretation have a learning curve, it is unclear whether more experience with fluorescence image interpretation could lead to an increased sensitivity of fluorescence imaging. The present post hoc analysis evaluated (a) the prevalence of high bacterial burden in surgical wounds, (b) utility of fluorescence imaging for detection of bacterial burden in surgical wounds, and (c) the impact of image interpretation experience on sensitivity of fluorescence imaging to detect high bacterial burden.

2 | METHODS

2.1 | Study design and population

This post hoc analysis of the prospective, single-blind, multi-centre cross-sectional FLAAG clinical trial (clinical trials.gov #NCT03540004) evaluates 58 surgical site wounds that were part of a larger trial of 350 patient wounds (60 surgical sites, 138 diabetic foot ulcers, 106 venous leg ulcers, 22 pressure ulcers, and 24 of other wound types). The goal of the trial was to determine whether fluorescence imaging of bacterial loads would have superior sensitivity and non-inferior specificity to CSS assessment alone, and to understand the potential impact this would have on treatment planning. Detailed information on study design was reported by Le et al.[24] In brief, patients were recruited from 14 U.S. outpatient advanced wound care centres by 20 clinicians (12 podiatrists, 4 surgeons, 1 emergency room physician, 1 wound care physician, and 2 nurse practitioners). There were minimal exclusion criteria: treatment with an investigational drug within the last month, wound biopsy in the last 30 days, unable to consent, any contraindications to routine wound care and/or monitoring, or any wound that could not be imaged because of anatomical location. Only 1.1% of patients screened were excluded from the trial, making this highly representative of the real-world status of wound bacterial burden and its assessment.[24] An independent third party (Ironstone Product Development, Toronto, ON) was used to control for bias and ensure appropriate blinding. The study was conducted in accordance with Health Insurance Portability and Accountability Act guidelines, adhered to tenets of the International Conference on Harmonisation E6 Good Clinical Practice (ICH GCP) and the Declaration of Helsinki, and received ethics approval by an external institutional review board (Approval Number 16247, Veritas IRB, Montreal, Canada).

2.2 | Clinical signs and symptoms assessment and fluorescence imaging procedure

Clinicians reviewed patient history and visually inspected wounds for CSS using the International Wound Infection Institute (IWII) Wound Infection Checklist.[33] Assessment of infection was based on clinician judgement. Per IWII guidelines, wounds with three or more criteria present were considered positive for bacterial loads of concern ($>10^4$ CFU/g), but if one overwhelming sign or symptom was present, clinicians had the discretion to deem the wound positive for CSS. Immediately following CSS assessment, the clinician captured standard and fluorescence images with the fluorescence imaging device (MolecuLight i:X, Toronto, Canada). Prior to study commencement, clinicians were provided with 4 hours of on-site and online training on the use of device, image interpretation, good clinical practice, and trial procedures. Clinicians were required to pass (>80%) a colour blindness and image interpretation test before enrolling participants. A minimum of two images, standard and fluorescence, must be compared during image interpretation to discern bacterial signals from the fluorescence signals from wound tissues. Presence of red or cyan fluorescence signals in images were indicative of elevated bacteria loads ($>10^4$ CFU/g).[25,26] Red fluorescence is emitted from porphyrins, endogenous fluorophores produced by bacterial species[34]; while cyan fluorescence signal is attributed to pyoverdines, which are uniquely produced by *Pseudomonas aeruginosa*.[25,35] These signals are produced from bacteria both in planktonic state and bacteria encased in biofilm.[29,34] The colour of red fluorescence is dependent on the depth of bacteria; blush and pink are a result of subsurface bacteria.

2.3 | Microbiological analysis

A detailed description of microbiological analysis of wound biopsies is reported by Le et al.[24] In brief, quantitative tissue cultures from punch biopsies were collected from each study wound to quantify total bacterial load and understand the species present. Per the protocol, up to three biopsies (6 mm diameter) could be obtained under local anaesthetic: a biopsy from the wound centre, or if applicable, a biopsy outside of the wound centre from a region of the wound positive for bacterial fluorescence, or a region positive for CSS. However, there were no surgical sites where clinicians chose to biopsy outside of the wound centre based on a region positive for CSS. In wounds where red or cyan (bacterial[25,26]) fluorescence was observed, clinicians were directed to collect a biopsy from the region of the wound that was brightest for bacterial fluorescence. In two surgical wounds, fluorescence signals were detected in the periwound region, but a biopsy was only collected from the wound centre. These wounds were excluded from this post hoc analysis. To restrict bacterial contents to the penetration depth of imaging device, each biopsy sample was cut to a depth of 2 mm and transported to a central laboratory (Eurofins Central Laboratory, Lancaster, Pennsylvania) for microbiological culture analysis. The laboratory was blinded to the CSS and FL call of the wounds. Species were identified through MALDI-TOF mass spectrometry, as previously described.[36] Total microbial load (CFU/g) was determined through serial dilutions and quantitative cultures as described in detail by Serena et al.[37] Semi-quantitative cultures (eg, scant, light, moderate, or heavy loads) were also performed. However, given their demonstrated lack of reliability for depicting bacterial load[37] only quantitative bacterial load data are reported herein.

2.4 | Impact of image interpretation experience

Clinicians were selected to participate in the fluorescence imaging reader study if they completed didactic and hands-on training and had performed the imaging procedure at least 200 times. It was thought that this would have provided both experience and confidence in interpreting more challenging images. Three "expert reader" wound clinicians (1 MD [surgeon], 1 DPM, 1 LPN) from three different clinical centres participated in a reader study. Each had used fluorescence imaging to acquire and interpret images indicating wound bacterial burden presence, location, and load routinely for 6 months or longer, outside of the clinical trial setting, when medically indicated.[38] Prior to reviewing the images, the three expert clinical readers were required to pass an advanced image interpretation test with a score of >80% (score range: 83%-100%). Each reader's image analysis was performed independent of other readers. Readers first reviewed each standard image and the accompanying fluorescence image on the fluorescence imaging device screen then scored the images as either positive or negative for red fluorescence and cyan fluorescence. In instances where consensus could not be reached on the presence or absence of red or cyan fluorescence in images, an additional tie-breaking read was provided. Readers reviewed each of the 58 surgical wound images in duplicate to establish intrareader reliability of image interpretation. Reads were made solely based on the readers' interpretation of the fluorescence images; readers were blinded to the microbiology, CSS positive or negative call, and the original study clinician's interpretation of the fluorescence images.

2.5 | Statistical analysis

One-sided exact McNemar tests were used for comparisons of sensitivity, specificity, and accuracy of detecting bacterial loads $>10^4$ CFU/g. Fleiss' kappa statistic with corresponding 95% confidence intervals (CIs) was used to measure the degree of agreement in classification among the 3 clinical expert readers. Assessing for intra-user consistency, duplicate images were considered as new images such that there were 116 images in total analysed by the expert readers. Kappa values were interpreted according to Landis and Koch with the following levels of agreement: a κ value <0 was considered poor agreement or disagreement, 0.01 to 0.2 slight agreement, 0.21 to 0.4 fair agreement, 0.41 to 0.6 moderate agreement, 0.61 to 0.8 substantial agreement, 0.81 to 1 almost perfect agreement.[39]

3 | RESULTS

3.1 | Demographics

Basic demographic information along with wound duration and location are reported in Table 2. Mean age of participants was 57 years and 50% of participants were female. Wound duration exceeded 3 months in 63.7% of wounds; 70.7% of surgical wounds were on a lower extremity.

3.2 | Microbial load of surgical wounds

Microbiological analysis of wound biopsies revealed that study surgical site wounds (SS), which had reached the stage of referral to a wound specialist, were highly likely to harbour high bacterial loads. Of the 58 wounds

TABLE 2 Demographic characteristics of surgical wounds included in the fluorescence imaging assessment and guidance (FLAAG) trial

Characteristic	N (%)
Total number of surgical wounds	58
% Female	50.0
Average age (years)	57
Wound duration	
<3 months	21 (36.2)
3-6 months	14 (24.1)
6-12 months	12 (20.7)
>12 months	11 (18.9)
Wound location	
Upper leg	2 (3.4)
Lower leg	15 (25.9)
Foot	24 (41.4)
Torso	10 (17.2)
Other	7 (12.1)

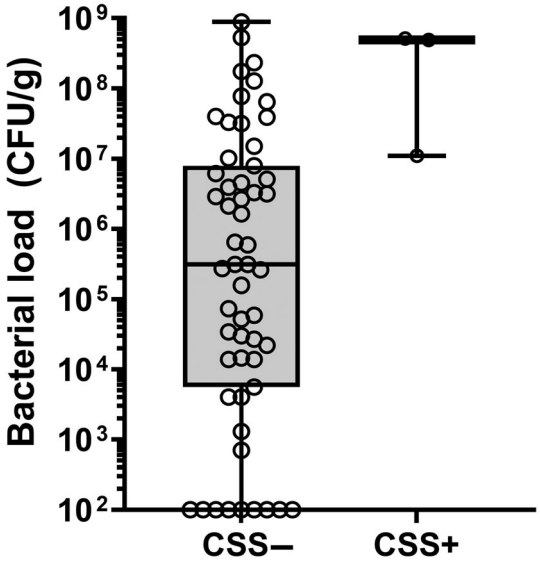

FIGURE 1 Bacterial load of surgical site wounds identified as negative (CSS−) or positive (CSS+) for clinical signs and symptoms of infection. A total of three wounds were identified as CSS+ while 55 wounds were identified as CSS−. Within each category (CSS− or +), each circle represents biopsy findings from a wound (n = 58 across both categories). Boxes contain the 25th to 75th percentiles of the dataset while the centre line indicates median bacterial load. Black whiskers represent minimum and maximum bacterial load values. CSS, clinical signs and symptoms of infection

included in the study, 75.9% (44/58) had bacterial loads $>10^4$ CFU/g, and 46.6% (27/58) had bacterial loads $>10^6$ CFU/g (Figure 1). The most prevalent bacterial species present were *Corynebacterium species* (34.5%), *Staphylococcus aureus* (31.0%), and *Enterococcus faecalis* (19.0%). The average number of bacterial species per surgical wound was 2.05 (range 0-9).

3.3 | Evaluation of clinical signs and symptoms

According to IWII guidelines, to be considered positive for clinical signs and symptoms of infection, three or more signs or symptoms of infection or one overwhelming symptom must be present.[33] Interestingly, despite the high prevalence of bacterial burden, only three wounds were identified as positive for signs and symptoms (CSS +) based on these criteria, all of which had bacterial burden exceeding 10^7 CFU/g (range 1.1×10^7 CFU/g to 5.10×10^8 CFU/g). Accordingly, assessment of CSS based on IWII criteria produced a sensitivity of 6.8%, meaning that most wounds harbouring high loads were missed (Figure 2). Accuracy of CSS was similarly low (29.3%). In contrast, specificity of CSS was 100%, likely because of the low number of wounds deemed CSS+ and therefore low number of false positives detected. Table 3 lists the IWII criteria included in the CSS assessment and the corresponding frequency of detecting each CSS in wounds with $>10^4$ CFU/g. CSS were rare (<15%) across all surgical sites with $>10^4$ CFU/g. Even the criteria included in the CDC definition of a superficial incisional SSI (presence of purulent draining from the superficial incision, localised pain or tenderness, swelling, erythema, or heat[13]) were rarely observed. Erythema (13.6% of all surgical wounds) was the most common CSS detected, followed by pain (11.4%) and swelling (9.1%). Delayed wound healing beyond expectation (covert delayed healing (36.4%) and overt delayed healing (38.6%)) was the most common sign or symptom detected.

3.4 | Improved detection of bacterial loads with fluorescence imaging

After completing the clinical assessment of CSS, clinicians then captured standard and fluorescence images of the surgical wounds to determine whether elevated levels of bacteria were present. Wounds were considered positive for fluorescence (FL+) if red or cyan fluorescence signals were detected by clinicians on fluorescence images. Point-of-care fluorescence imaging raised sensitivity to detect wounds with elevated bacterial loads from 6.8% with CSS, to 38.6%, an improvement of 5.7-fold (Figure 2A; $P = .0005$). Similar improvements were observed for accuracy, which increased from 29.3% with

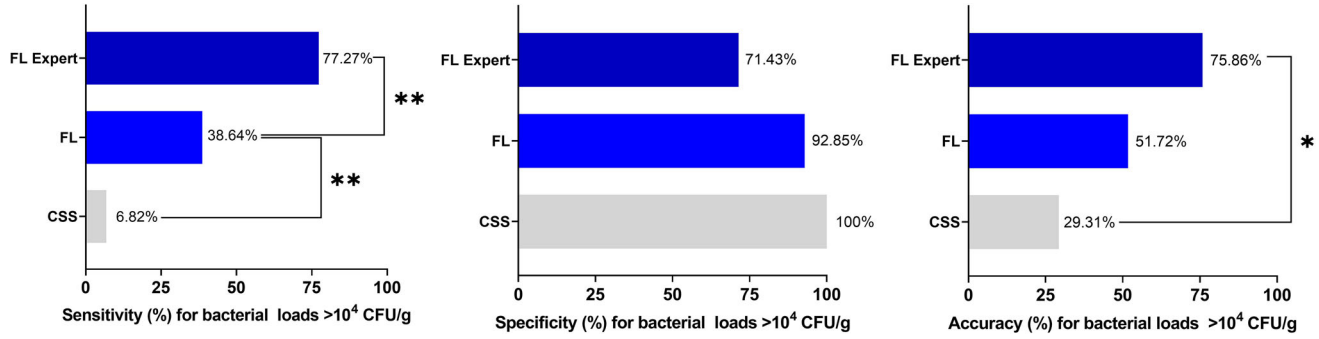

FIGURE 2 A, Sensitivity, B, specificity, and C, accuracy of clinical signs and symptoms of infection (CSS) and fluorescence imaging (FL) alone. Comparisons were also made based on imaging interpretation performed by expert users of fluorescence imaging (FL expert) included in the reader study. *$P < .05$ and **$P \leq .0005$ derived from McNemar's one-sided test

TABLE 3 Frequency of clinical signs and symptoms of infection detected among wounds with $>10^4$ CFU/g

Covert sign	Prevalence (%)	Overt sign	Prevalence (%)
Hypergranulation	4.5	Erythema[a]	13.6
Bleeding, friable granulation	4.5	Local warmth[a]	6.8
Epithelial bridging and pocketing in granulation	0	Swelling[a]	9.1
Wound breakdown and enlargement	9.1	Purulent discharge[a]	2.3
Delayed wound healing beyond expectation	36.4	Delayed wound healing	38.6
New or increasing pain	4.5	New or increasing pain[a]	11.4
Increasing malodour	9.1	Increasing malodour	9.1

[a]Symptoms included in the Centres for Disease Control definition of surgical site infection.[6]

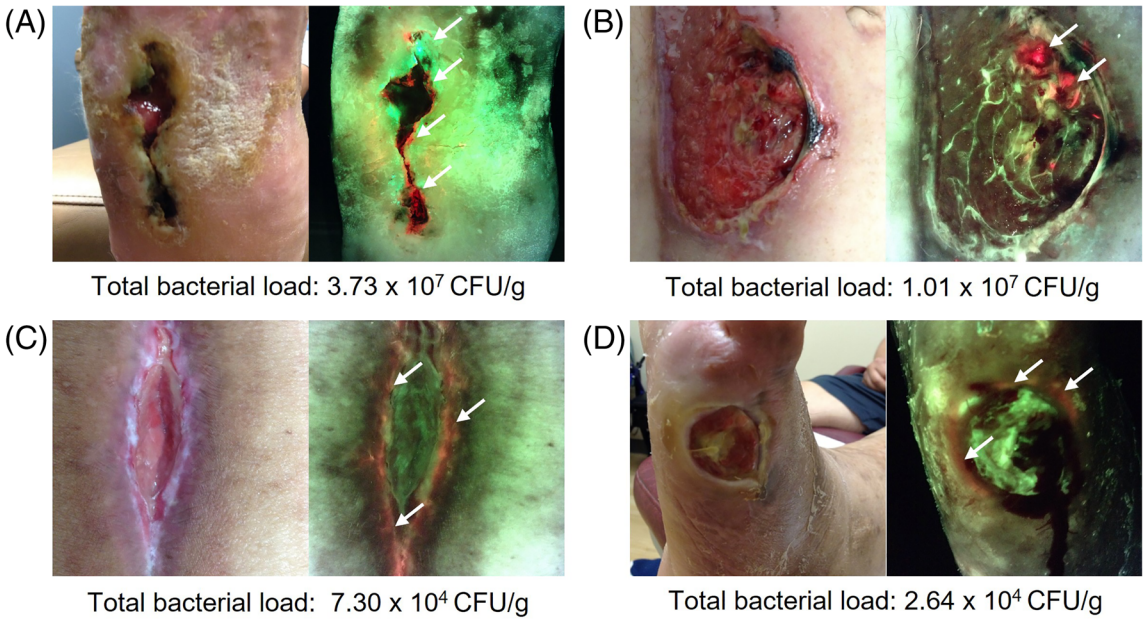

FIGURE 3 Example of standard and fluorescence images of surgical site wounds that were negative for clinical signs and symptoms of infection but positive for fluorescence from bacteria. Total bacterial load of each wound was determined by quantitative culture of wound biopsy. A, Wound on plantar foot; B, Torso wound; C, Lumbar surgical wound; D, Diabetic foot wound. Arrows indicate regions of red or cyan fluorescence indicative of elevated bacterial loads. Collagen, fibrin, and other matrix components in skin, slough, and other wound tissues fluorescence green

CSS to 51.7% fluorescence imaging (Figure 2C; $P < .05$). Specificity of fluorescence imaging (92.9%) and CSS (100%) were comparably high (Figure 2B). Example fluorescence images of surgical site wounds are depicted in Figure 3. Presence of red or cyan fluorescence indicative of bacterial loads $>10^4$ CFU/g was detected in 18 of 58 wounds. In each example shown in Figure 3, clinicians deemed the wound to be negative for clinical signs and symptoms of infection (CSS-). Analysis of wound biopsies later revealed the presence of clinically significant bacterial loads exceeding 10^4 CFU/g.

3.5 | Significance of expert image interpretation

Clinicians participating in the FLAAG trial had minimal experience with fluorescence image interpretation prior to study commencement. This posed a challenge when interpreting the diagnostic accuracy results as lack of expertise in identifying red or cyan signals on fluorescence images may have confounded clinician's ability to identify wounds with elevated bacterial burden. To address this, a reader study was conducted in which clinicians with experience in fluorescence image interpretation ("FL experts") reviewed standard and fluorescence images from the 58 surgical site wounds. These expert readers reviewed each wound in duplicate to evaluate inter- and intra-reader reliability. Sensitivity, specificity, and accuracy of fluorescence imaging were compared between three expert readers to the non-expert clinicians from the FLAAG trial. Expert readers doubled sensitivity of fluorescence imaging to 77.3% compared with the non-expert results from the FLAAG trial (38.6%, $P < .0001$; Figure 2A); this corresponded to an 11.3-fold increase in sensitivity of fluorescence imaging compared with CSS ($P < .0001$). Specificity of expert FL readers (71.4%) was not statistically different from non-experts (92.9%) or CSS (100%). In contrast, accuracy of expert FL readers increased to 75.9% from 51.7% (FL non-experts) and was significantly higher than the accuracy of CSS alone (29.3%). To determine the consistency of image interpretation within and among the three expert readers, we next calculated the inter- and intrareader variability. Since the readers were asked to indicate the presence of either red or cyan fluorescence on images, a separate value was calculated for each fluorescence signal. There was moderate agreement between the three expert readers for the detection of red fluorescence on images (Fleiss $\kappa = 0.583$; 95% CI 0.480-0.687). A similar result was observed for cyan fluorescence (0.569, 95% CI 0.465-0.672). Fleiss Kappa was also used to calculate intrareader variability for the three readers. The average kappa for red fluorescence was near perfect at 0.873; in contrast, fair agreement (0.491) was observed for the presence of cyan in images. Of the 58 images, there were four images in which no consensus was reached for red fluorescence and 7 images were there was no consensus reached for cyan fluorescence. A tie-breaker reading was performed in these instances. Some of the image interpretation challenges the readers faced are shown in Figure 4.

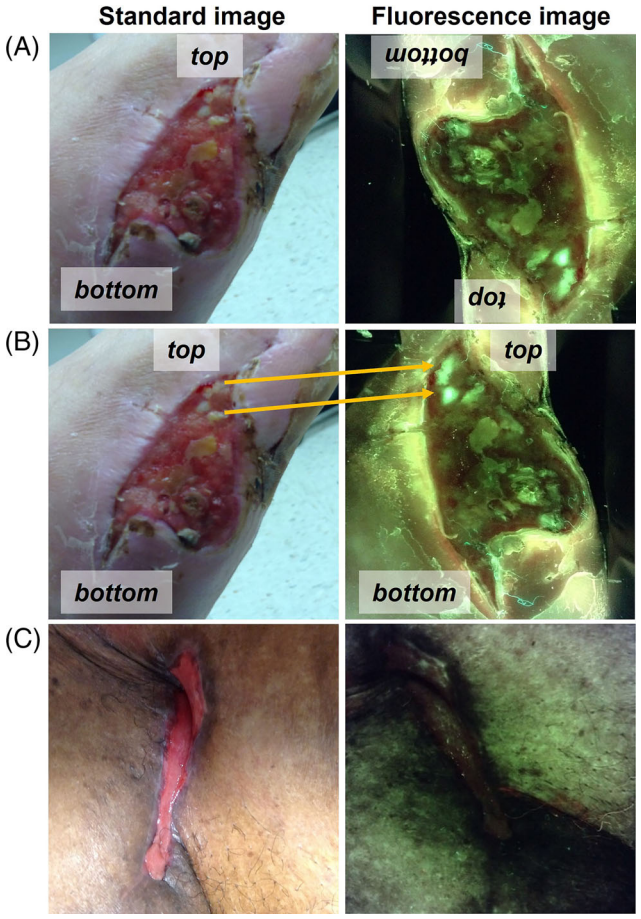

FIGURE 4 Significance of advanced image interpretation. A, The original fluorescence (FL) image for this surgical wound was taken upside down compared with the standard (ST) image. This led clinicians, including the expert readers, to misinterpret the fluorescence from slough as a positive signal from Pseudomonas bacteria. B, When the FL image is oriented to match the ST image, the bright green signal observed clearly aligns precisely with an anatomical structure (slough or a tendon) and is not because of *Pseudomonas*. C, Example of a false positive image identified by expert readers as having red or cyan fluorescence when neither colour signal was present. False positive calls because of incorrect image alignment or misinterpretation of fluorescence during interpretation decreased FL and FL-expert specificity

4 | DISCUSSION

In this post hoc analysis of 58 surgical wounds, 76% had bacterial loads of clinical concern that went largely unnoticed because of the poor sensitivity and accuracy of

CSS. Early detection of high bacterial burden in surgical wounds is critical to prevent SSIs. Point-of-care fluorescence imaging significantly enhanced detection of surgical wounds with high bacterial burden by 5.7-fold compared with CSS. Advanced training on image interpretation further increased sensitivity of fluorescence imaging up to 11.3-fold compared with CSS alone. These findings are part of an important initiative by the International Surgical Wound Complications Advisory Panel (ISWCAP) to study SSI on a global scale and highlight the need for more objective diagnostic techniques to support the early and accurate detection of clinically concerning bacterial burden in surgical wounds. To the best of our knowledge, this is the first study reporting the use of an advanced diagnostic device for the visualisation and diagnosis of bacterial burden in surgical wounds.

Despite a wealth of data linking bioburden and biofilm to surgical wound complications and healing impairment, the importance of wound bioburden is often overlooked when considering surgical wound management. As part of the intra and postoperative patient monitoring, clinicians are taught to proactively manage any changes in blood pressure, temperature, and oxygen to reduce risk of post-surgical complications. In contrast, surgical wound bioburden is often managed only after signs of infection manifest. In this study, most surgical wounds evaluated (>75%) had bacteria at loads that are known to increase risk of infection, healing impairment, and other postoperative complications.[17-19] Yet, CSS were detected in only three wounds. Because of unreliable diagnostic methods, the frequency of high bacterial loads and their ability to be entirely asymptomatic may have been previously underappreciated, resulting in a dearth of information in guidelines advising on how to appropriately manage high bacterial loads in surgical wounds. With the advent of imaging technology, bacterial burden can now be readily detected at the point-of-care, enabling clinicians to detect and manage high loads prior to manifestation of infection. Management of bacterial burden prior to the manifestation of infection should always begin with wound hygiene strategies (eg, cleansing, debridement) and only escalate to antibiotics when essential. This proactive approach has been highly successful at decreasing antibiotic usage in diabetic foot ulcer wounds.[32] Note that the visualisation of bacterial burden can be combined with ISWCAP tools (surgical wound dehiscence grading system[10]) to clarify what we are seeing. This enables a new approach to management of surgical wounds—one that turns attention away from solely focusing on infection management and towards proactive detection and informed, hygiene-based bacterial removal.

Clinical "expert users" highly familiar with image interpretation resulted in the highest sensitivity to detect elevated bacterial burden, but also tended to over-read select fluorescence images. All diagnostic imaging modalities have an associated learning curve (eg, MRI, ultrasound)[40,41]; colour-based images present an additional interpretation challenge, primarily because of the multidimensional nature of colour images. In this study, experience with image interpretation (>200 clinical encounters imaged and interpreted) resulted in a 2-fold increase in sensitivity over novice clinicians participating in the clinical trial. However, this expertise also resulted in a slight, although not statistically significant, decline in specificity among expert readers because of a tendency of experts to "over" interpret fluorescence images. Over interpretation is a concern across all imaging modalities because of the production of false positives. However, the implications for "over" interpretation (false positives) in the context of wound management must be considered relative to the false negatives that imaging and expert interpretation avoids. For every false positive created by imaging, 10 false negatives were avoided. The first line strategy when a positive signal is detected on images should always be hygiene. Often, vigorous scrubbing can remove the signal, and this should always be attempted before antibiotics are considered.[42] Additional actions to address problematic bacterial burden in wounds may include microbiological testing, and potential use of antimicrobial dressings. These added efforts based on imaging information—additional hygiene, a potential increase in microbiological testing, and use of antimicrobial dressings all outweigh the risks of underdetecting bacterial burden in wounds—namely the development of SSI, and other wound complications including sepsis and amputation.

Based on evidence from this study and others,[24-26,28] the authors recommend adoption of fluorescence imaging for detection of bacterial burden in surgical wounds. For groups adopting this imaging technology to evaluate surgical site wounds, the following recommendations are suggested to achieve the highest possible accuracy:

1. *Ensure sufficient darkness*. Images captured in insufficient darkness were more likely to miss bacterial loads. If the room cannot be made dark, use a darkening drape that is commercially available.

2. *Remove blood or debris from the region prior to capturing the image*. These could interfere with fluorescence signals and challenge interpretation.[43]

3. *Ensure proper image orientation*. Post-capture, review the FL images and compare to standard image field of view to ensure the orientation of the FL is aligned with the standard image so that anatomical landmarks in standard images can guide accurate image interpretation.

4. *Ensure correct image interpretation*. Readers are referred to various training resources including publications by Oropallo et al[38] and Rennie et al,[43] as well as free online training (learning.moleculight.com). Elect for on-site training support and give yourself time to familiarise

yourself with the various colours of the images, realising that there is a learning curve.

4.1 | Strengths and limitations

There are a number of strengths of this study, including minimal exclusion criterion, patient recruitment from multiple sites, a large number of participating clinicians, and inclusion of wound specialists with diverse clinical accreditations (eg, surgeons, podiatrists, nurse practitioners). The use of gold standard quantitative biopsies and microbiology to confirm true bacterial load, with appropriate blinding, was also a strength. However, there are some limitations to the study including the single timepoint data and lack of data collection on non-bacterial factors (eg, blood pressure, temperature, oxygen) contributing to surgical wound complications, as this study was focused specifically on bacterial burden. Additionally, only surgical wounds referred to wound care clinic were included in this study.

5 | CONCLUSION

Early identification of high bacterial burden is critical for the prevention of SSIs. Here we show that pathogenic bacterial burden is present in most (>75%) surgical wounds but remains largely undetected based on standard of care assessment of CSS, resulting in delayed infection management. Fluorescence imaging of bacterial burden is positioned to change contemporary paradigms of post-surgical wound management. Based on the results of this study, as well as other studies reporting the impact of this technology on wound management and antibiotic prescribing reduction,[24,25,28,32,42] we and a larger Delphi consensus expert panel[38] recommend the use of this imaging technology when performing surgical site wound assessment and management. Information from this study on the extent of the bacterial burden problem in surgical sites, and its asymptomatic tendency, can be used to inform clinical practice for early intervention in the prevention of postoperative wound complications such as SSI.

5.1 | Future approaches to surgical wound management

In this study, fluorescence imaging enabled immediate and accurate identification of surgical wounds with high bacterial burden among those wounds that failed to heal on a normal trajectory, requiring additional care at a wound care centre. However, management of surgical wounds begins much before this point; prior to surgery, numerous preventative measures are taken to avoid infection (eg, patient optimisation, incisional site preparation, adherence to SSI prevention guidelines, and use of prevention bundles); similarly, after the surgical procedure, wounds are cleansed using an aseptic technique, dressed, and monitored for signs of infection to determine the need for antimicrobials or antibiotics. Integrating fluorescence imaging at these pivotal points may improve detection and removal of bacteria around the surgical site to prevent development of SSIs. Additional studies are warranted to evaluate and define the clinical indications and timing at which fluorescence imaging may be used to aid in prevention of SSIs. These studies are currently underway.

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CONFLICT OF INTEREST

Dr Charles A. Andersen and Dr Thomas Serena have received funding from MolecuLight for speaking engagements.

DATA AVAILABILITY STATEMENT

Data available upon request.

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