Utility of a real-time fluorescence imaging device in guiding antibiotic treatment in superficial skin infections

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Abstract

The prescription of antibiotics empirically without confirmation of an infective etiology is on the rise. Administration of appropriate antibiotics can be guided by real-time fluorescence imaging using a point-of-care device. These composite images show the presence, type and the burden of infection. The time saved by this method over microbiological testing, especially in resource-poor settings, can lead to a paradigm shift in treatment by facilitating prompt and adequate antimicrobial therapy, surgical debridement as well as follow-up. Thumbnail sketches of a series of four cases highlighting different scenarios in which a fluorescent imaging device utilizing artificial intelligence and machine learning was found useful is presented in this report.

Key words: Antimicrobial stewardship, fluorescence imaging, Illuminate, infections, wound care

Introduction

Most of the skin and soft tissue infections resolve with treatment, however, some worsen forming chronic ulcers that impair not only the patients' quality of life but strain an already overburdened healthcare system. An escalating incidence of secondarily infected chronic wounds has led to increased hospitalization of patients as well as antibiotic usage.[1]

The incidence of acute and chronic wounds in India is 10.5 and 4.5 per 1000 population respectively.[2] Secondary bacterial infections commonly complicate the healing of diabetic ulcers leading to the loss of a lower limb every 30 seconds.[3] Initial clinical evaluation and microbiological correlation of an infected wound typically takes up to 10 days. An already tedious recovery may get prolonged by incorrect therapy consequent to a cursory examination, pending culture and sensitivity results, or inaccurate reports following improper swabbing. Moreover, despite proper swabbing/aseptic techniques, all organisms may not be isolated especially in comparison with that of deep tissue biopsies (current gold standard).[4,5]

We used a novel handheld imaging device, "Illuminate," developed by Adiuvo Diagnostics (Chennai, India), which aims to accelerate the treatment of skin infections by rectifying some of the above-mentioned lacunae. This device leverages autofluorescence, artificial intelligence and machine learning to accurately classify/locate the microorganisms and provide semiquantitative data regarding the infective burden of the wound. Pointed to the region of interest, the device captures 16 serial images at different excitation emissions and produces a clinical image overlaid with the superimposed infection, if any, marking the presence of gram-positive bacteria in red, gram-negative in green, and fungus in blue. Four cases where the device was found invaluable are summarized below.

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Case Reports
Case 1
A 73-year-old female had multiple and painful non-healing recalcitrant ulcers on both thighs [Figure 1a] for 9 months, despite multiple courses of tablet amoxicillin/clavulanic acid, tablet azithromycin, and injectable amikacin as per sensitivity reports of the culture isolates of *Staphylococcus epidermidis* and *Klebsiella*. The ulcers were well defined, punched out, 0.5 to 3 cm in diameter with erythematous margins and seropurulent discharge. A punch biopsy revealing neutrophilic infiltrate was described as 'non-specific'. Repeated use of Illuminate for many days consistently failed to capture the fluorescence indicative of any pathogenic organisms, although repeated pus swabs showed growth of coagulase-negative *Staphylococcus* [Figure 1b]. Deep excisional biopsy thence demonstrated a dense band of neutrophilic infiltrate and perivascular lymphocytes suggestive of pyoderma gangrenosum. The patient ultimately responded well to corticosteroids (systemic and intralesional) without any antibiotic coverage.

Case 2
A 58-year-old female, known diabetic for 10 years developed an ulcer over her left great toe [Figure 2a] along with difficulty in walking for 4 months. *Escherichia coli* were isolated but despite the complete course of cefoxitin as per sensitivity,

the ulcer did not heal. Cyan and green fluorescence in two different areas of the ulcer examined by Illuminate indicated the co-colonization of *Pseudomonas species* and *Escherichia coli* and the same was subsequently confirmed by image-guided swabbing [Figures 2b and c]. Prompt clinical response followed with the use of ciprofloxacin.

Case 3
A 45-year-old HIV-positive patient presented with a crusted ulcer on left shin for 15 days. [Figure 3a] Initial assessment with Illuminate suggested a high gram-positive bacterial load (subsequently confirmed to be methicillin-sensitive *Staphylococcus aureus*) and hence cotrimoxazole was given empirically. Seven days later, the sensitivity report confirmed it to be the appropriate choice, during which serial assessment demonstrated reduced fluorescence at every visit indicating a satisfactory response at the end of 10 days of treatment, obviating the need for repeated wound swabs [Figures 3b-d].

Case 4
A 48-year-old male, known diabetic, presented with a malodorous, discharging ulcer over the right toe since 1 month. [Figure 4a] Earlier culture of pus swab taken from the center of the lesion yielded no growth. Clusters of methicillin-resistant *Staphylococcus aureus* were isolated from an image-guided swab taken from the periphery of the ulcer [Figure 4b] The wound was surgically debrided with repeated imaging till the device failed to show any fluorescence, signifying appropriate debridement depth [Figure 4c].

Discussion
Studies have shown that significant bacterial load in wounds is correctly identified only in 52.5% instances by clinical assessment as against 74.6% with fluorescent imaging-based techniques.[6] Antibiotic resistance, a growing global concern can be prevented, at least in part, by antimicrobial stewardship practices.[7,8] All of the key factors listed by the European Wound Management Association contributing to antimicrobial misuse in wound care, namely— diagnostic

Figure 1a: Clinical image of recalcitrant thigh ulcers

Figure 1b: Lack of fluorescence on imaging with "Illuminate"

uncertainty regarding presence of bacterial infection, clinician ignorance regarding class and duration of antibiotic to be used, and patient demands for prescription of inappropriate antibiotics can be overcome by a point-of-care device providing immediate guidance.[9]

Our case series underscores that real-time fluorescence imaging of microorganisms can greatly facilitate the appropriate use of antibiotics. The patient with pyoderma gangrenosum, having received unnecessary and prolonged antibiotic therapy due to a misleading clinical appearance, responded promptly to systemic steroids, due to the instant result provided by this device.

Swabbing from inappropriate sites demonstrated false-negative culture results in cases 2 and 4. Sapico *et al*. reported only a 63% concordance of isolates between skin biopsy samples taken from the periphery and in the center of 25 pressure sores.[10]

The resistant-to-local-cleaning periphery of wounds was suggested to be a better swabbing site for accurate detection of isolates in some cases. Also, a mixed infection may go undetected by a single swab taken even from the area of maximal clinical involvement. Image-guided sample collection overcomes this problem.
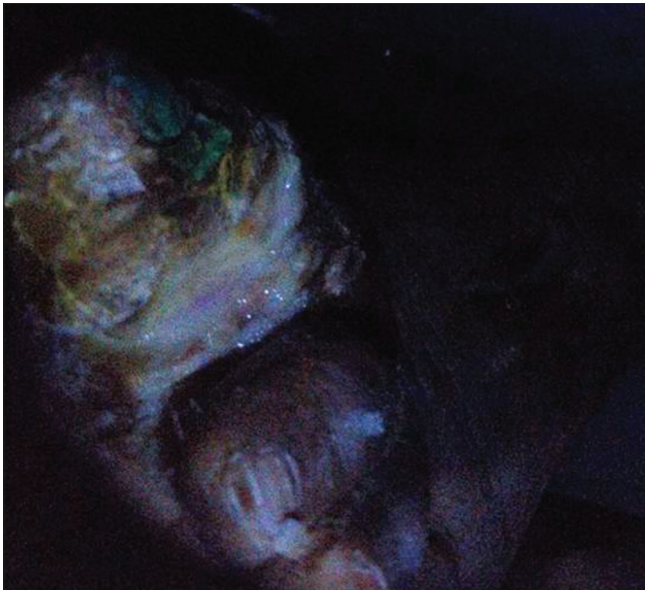

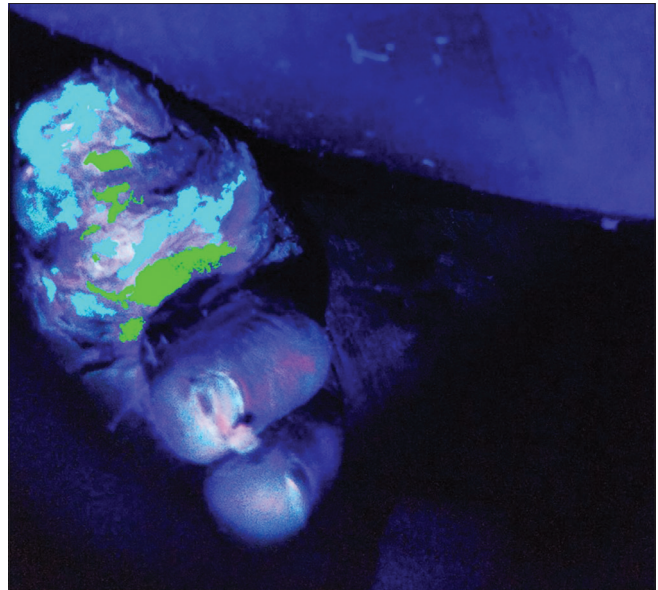**Figure 2a:** Ulcer on the great toe with slough

Figure 2b: Composite algorithmic image after processing demonstrating co-colonization
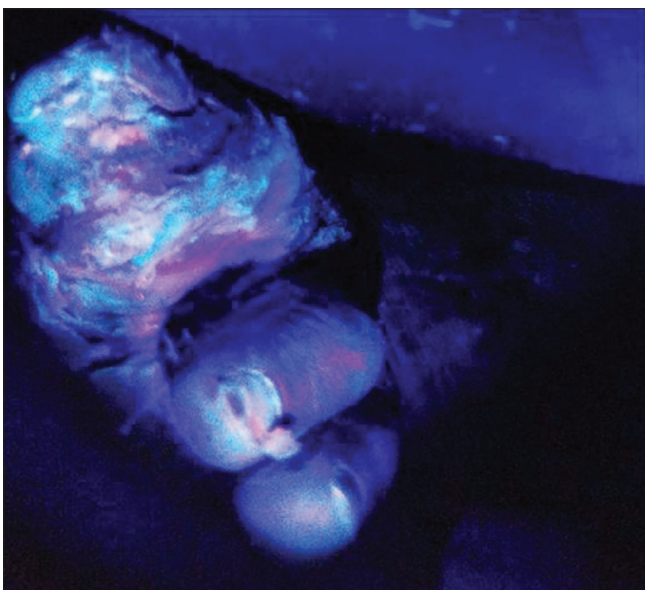

Figure 2c: Fluorescence highlighting areas of bacterial colonization with gram-positive species (red) and *Pseudomonas*
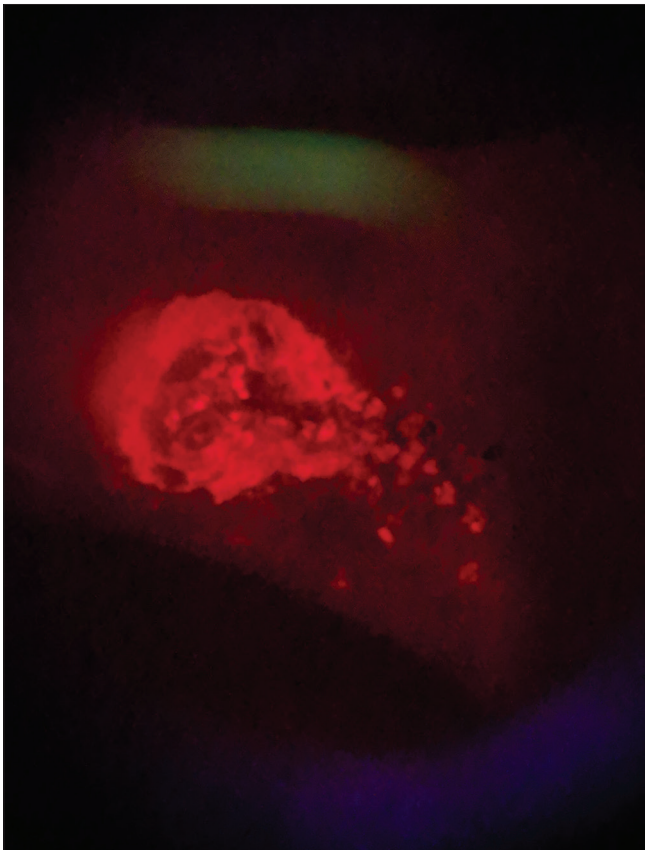

Figure 3a: Encrusted ulcer on the left shin

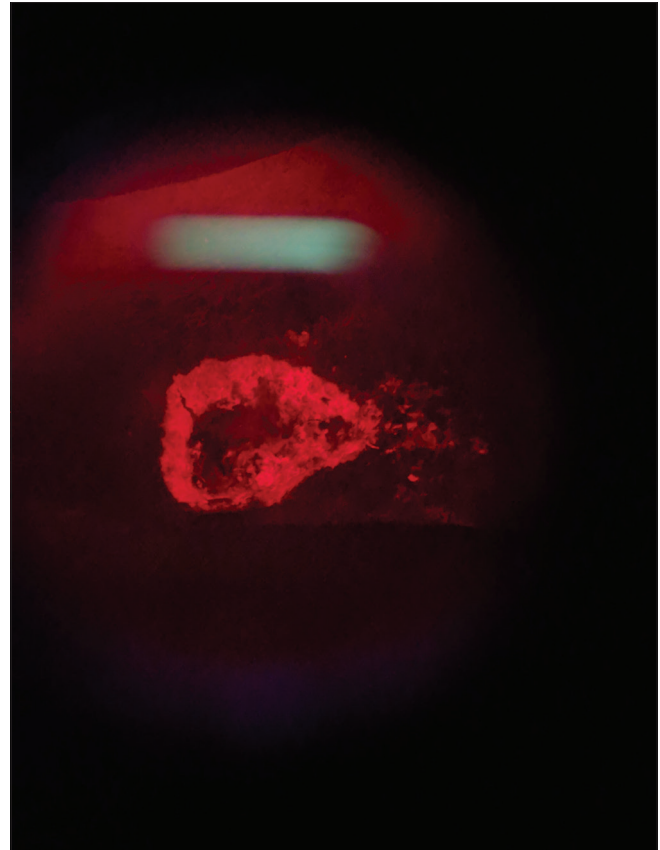**Figure 3b:** Fluorescence at initial assessment

Figure 3c: Day 5 of follow-up

The reduced bioburden following appropriate antibiotics/wound debridement seen on serial imaging in cases 3 and 4 not only led to rapid healing but also supplanted the subjective clinical and/or time-consuming microbiological assessment.

Illuminate leverages the autofluorescence property exhibited by pathogens [such as nicotinamide adenine dinucleotide hydrogen (NADH), flavins] and infectious markers (pyoverdine, porphyrin) present in bacteria and fungus. NADH has a strong emission peak at 470 nm producing blue fluorescence, while flavin and porphyrin peak at around 525 nm and 620 nm corresponding to green and red fluorescence, respectively. Gram-negative organisms have a higher intensity of NADH and flavin than gram-positive organisms thus making it possible to differentiate between gram types. Pseudomonas possesses pyoverdine, a unique marker having blue-green color enabling its detection.

An image processing and machine learning algorithm processes the spectral images to detect and classify pathogens based on the intensity of autofluorescence variation amongst these biomarkers. It can detect bacterial loads as less as 10^3 CFU/gm (colony-forming unit per gram) allowing for early intervention (Indian patent No. 323440, 2019). Contaminants, if present, are usually at a lower density. Contamination may also occur at later stages of sample collection, processing, or in the laboratory.[11]

The skin itself has a natural fluorescence contributed by collagen/elastin; bacteria interact with the tissue via heme pathway and produce porphyrin displayed as red fluorescence while fungi have a characteristic blue fluorescence due to chitin and dityrosine linkages. *Pseudomonas aeruginosa* produces a greenish-cyan fluorescence signal due to the presence of pyoverdine. There are spectral and textural changes between the green illumination of specific organisms (especially *Pseudomonas* giving a cyan color) and surrounding dermis, which enable their identification. The device overlays this composite autofluorescence image on regions of infections. Gram-positive bacteria are displayed in red and gram-negative in green by comparing the difference in autofluorescence intensity.

However, limitations of the technology exist in detecting deep-seated bacterial infections and differentiating pathogenic from commensal bacteria. While an approximation of significant bacterial load is possible; the determination of accurate levels thereof still requires tissue culture. The device also cannot act as a surrogate for culture and sensitivity, which is often required to choose appropriate antibiotics in the era of drug resistance.

Point-of-care devices may substantially aid the evaluation of wounds and help institute appropriate treatment promptly by instant confirmation of infective etiology thereby reducing
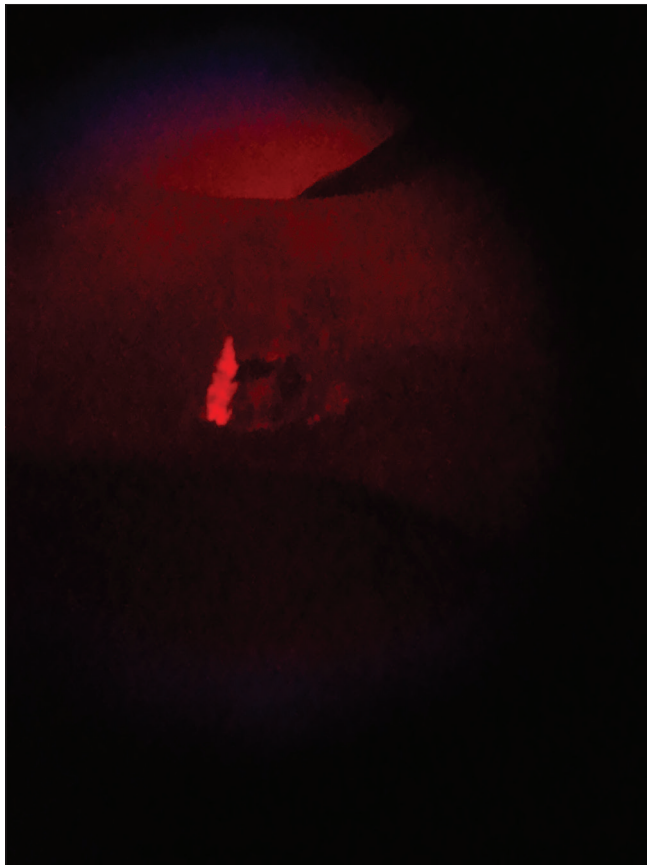
Figure 3d: Day 10 of follow-up

Figure 4a: Ulcer over the right great toe with serous discharge

Figure 4c: Reduction in fluorescence evident after the initial cleaning

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent.

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Conflicts of interest

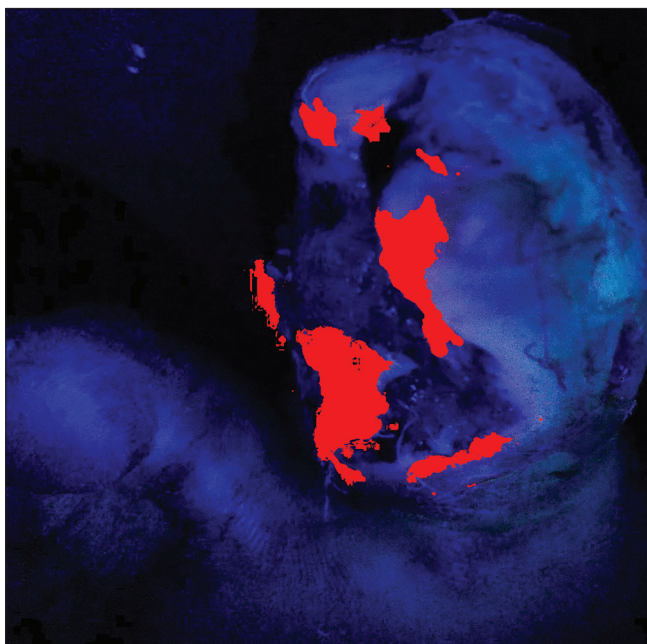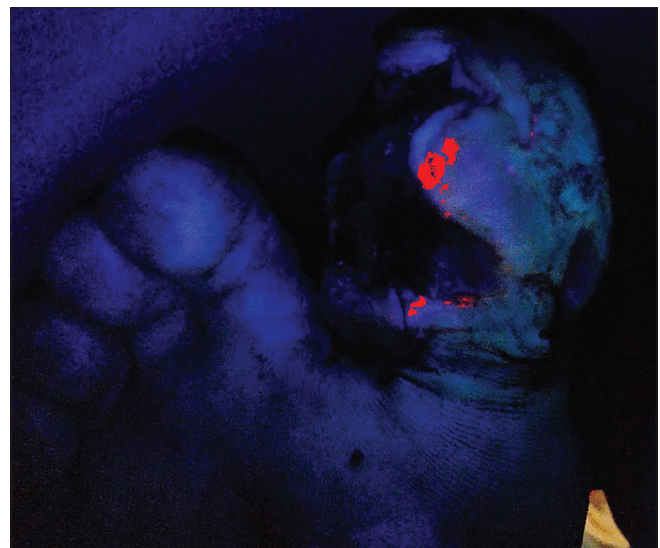There are no conflicts of interest.

Figure 4b: Highlighted areas showing clusters of bacterial colonization primarily over the periphery of the wound

the investigative/financial burden, especially in resource-poor settings where facilities for microbiological evaluation may not be available.

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