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Original Article

Bacterial autofluorescence in infected perineal wounds: A prospective cohort study

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ABSTRACT

Diagnosis of perineal wound infection based solely on clinical signs and symptoms is subjective, and often incorrectly identifies wounds with clinically significant bacterial loads. New advances in wound care such as bacterial fluorescence imaging allow point-of -care assessment of bacterial burden. This single-center, prospective observational study included 80 women with perineal wound infection and aimed to determine the incidence of significant bacterial colonization identified with bacterial fluorescence imaging. Also, to evaluate the diagnostic accuracy of bacterial fluorescence imaging. 30 women (37.5%) had fluorescence in their wounds despite antibiotic therapy. The sensitivity of bacterial fluorescence imaging in the diagnosis of wounds with a clinically significant bacterial burden was 83% and specificity was 90%. The positive predictive value was 92% and negative predictive value was 80%. Overall, diagnostic accuracy was substantial. The results of this study demonstrate that bacterial fluorescence imaging can provide real-time information surrounding the bacterial burden of perineal wounds.
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1. Introduction

Perineal wound infection can affect up to a quarter of women following vaginal delivery [1]. This condition is a major source of anxiety for postpartum women, and is reported within the first month following perineal trauma [2,3]. However, inadequate priority is given to the postnatal management of this condition [4]. Moreover, women often have poor experiences with the clinical management of perineal wound complications due to a paucity of information about clinical management and recovery [5]. Therefore, perineal clinics have been established to improve the care of perineal trauma in the postpartum period, however there is disparity with regards to the availability of these services [6].

At present, perineal wound infection is diagnosed taking into account clinical signs and symptoms such as perineal pain, purulent discharge and wound dehiscence [7]. Wound swabs are often also taken to guide and direct management, despite results from microbiological analysis taking up to 5 days [8]. Although wound swabs are the most practical, least invasive and widely available method, microbiological results obtained using this technique are particularly dependent on sampling method, the area sampled, duration of sampling and wound preparation prior to sampling [9,10]. Other practical factors can increase the unreliability of wound swabs such as transport medium, storage and the time taken for transport to the laboratory for analysis. For example, it is recommended that samples should be transported within no later than 2 hours following collection [11].

Antimicrobial stewardship is a significant health priority, as inappropriate antibiotic prescription and as a result, the overuse and misuse of antibiotics, promote bacterial resistance [12]. It is therefore a compelling initiative to improve the diagnosis of perineal wound infection and prescribing practice. Advances in wound imaging, such as bacterial fluorescence imaging, have been created to address this and can be performed at the bed-side to allow real-time bacteria localization [13]. The use of this technology has mainly gained popularity for use in chronic wounds to investigate bacterial burden and to target treatments such as wound cleaning, debridement and dressing change [14]. The Fluorescence imaging Assessment and Guidance (FLAGG) study demonstrated that in chronic wounds such as diabetic foot ulcers, venous leg ulcers, pressure ulcers and surgical site wounds, that bacterial fluorescence imaging significantly improved diagnosis of bacterial burden and wound care [15]. However, to date no study has evaluated the use of bacterial autofluorescence imaging in the assessment of the bacterial burden of perineal wounds.

The aim of this study was to investigate the incidence of significant bacterial burden diagnosed using bacterial fluorescence in infected perineal wounds and to identify associated risk factors.

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Secondly, we aimed to evaluate the diagnostic accuracy of bacterial fluorescence imaging in the detection of significant bacterial loads within perineal wounds using wound swabs as a reference standard.

2. Materials and methods

This is a secondary analysis of the Prospective Observational Study Evaluating the Sonographic Appearance of the Anal Sphincter in Women With Perineal Wound Infection Following Vaginal Delivery (PERINEAL Study). The design and methodology have been registered previously (ClinicalTrials.gov NCT 04480684). Briefly, this was a prospective cohort study at Croydon University Hospital (CUH), which was conducted to assess the clinical progression of perineal wound infection and its effect on anal sphincter integrity using endoanal (EAUS) and transperineal ultrasound (TPUS). The primary aim of the PERINEAL study was to evaluate the effect of perineal wound infection on anal sphincter integrity in women with and without obstetric anal sphincter injuries (OASIs) using 3D EAUS and 4D TPUS [6]. Ethical approval was obtained from NHS Health Research Authority, London - Surrey Research Ethics Committee (20/LO/0304). Written consent was obtained for publication of the images included in this review as per the recruitment for the PERINEAL study.

2.1. Clinical parameters

Women were diagnosed with perineal wound infection if clinical signs and symptoms such as perineal pain, purulent discharge and wound dehiscence were present [7]. Broad spectrum antibiotics were prescribed for 7 days prior to recruitment in keeping with local guidelines. In cases of superficial wound infection, the following antibiotics were given: oral Co-amoxiclav 625mg, in case of mild penicillin allergy; oral Cefalexin 500mg and Metronidazole 400mg and in cases of severe penicillin allergy; Clindamycin PO 300mg and Ciprofloxacin PO 500mg. In the presence of deep wound infection, intravenous antibiotics were commenced (Cefuroxime 1.5 g and Metronidazole 500 mg or in cases of penicillin allergy: Clindamycin 900mg and Ciprofloxacin 400mg). Deep wound infection was defined as a wound infection that involved the deep soft tissues of the dehisced incision (muscle/fascia) and/or was associated with systemic symptoms such as fever (>38 ^{0C}) [16]. Wound swabs were taken prior to recruitment to guide clinical management. If a wound swab had not been taken prior to recruitment or wound swab results were not available, 1 was taken at the first appointment to detect the causative organisms and guide management. Appropriate antibiotics were then given to cover the detected organism if required.

2.2. Microbial analysis

All PERINEAL participants underwent bacterial fluorescence imaging of their wound with the hand-held MolecuLight device (MolecuLight, Toronto, Canada). The MolecuLight i:X imaging device detects bacteria (at loads of $>10^4$ CFU/g) using a clinically safe violet light at a wavelength of 405-nm and uses optical filtration to only permit signals from wavelengths of interest and to prevent reflection of the violet light which can affect the image generated [13]. Due to the iron chelating compounds present in bacteria, when at high loads ($>10^4$ CFU/g), the fluorophores they emit can be detected [13]. Therefore, the MolecuLight i:X imaging device has been shown to be highly predictive in diagnosing wounds with significant bacterial loads ($>10^4$ CFU/g) [13,17] (Fig. 1). Sampling of the wound to detect bacterial load using wound swabs for microbiological analysis was targeted to areas of fluorescence. To ensure bacterial fluorescence corresponded with the bacteria within the wound at that time point. Any wound with exposed suture material had this removed and sent for microbiological analysis. Microbiological culture was performed off-site at the St George's University Hospital laboratory. St George's University Hospital laboratory follows the UK National Standard Microbiology Investigations (SMI) Standard Operating Procedures (SOP). For the processing of swab samples in this report, the laboratory uses mainly the principles as outlined in the SMI B 28 (investigation of genital tract and associated specimens) SOP [18]. Any pus [19] or tissues [20] samples such as suture material were processed following the relevant SMIs. All clinically significant isolates are identified using Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF). Results from swabs with more than 2 mixed *Enterobacteriaceae* are reported as mixed coliforms. Sensitivity testing on significant isolates is undertaken using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [21]. Semi-quantitative analysis was used with results being reported as none (0), light (1+) moderate (2+ to 3+) or heavy (4+) growth. Counts of moderate to heavy indicated significant bacterial loads, which are equivalent to $>10^4$ CFU/g on quantitative analysis [8]. As this was an observational study, to avoid deviation from normal practice, unless the wound exhibited clinical signs of ongoing infection despite antibiotics, in the presence of bacterial fluorescence, no additional antibiotics were given.

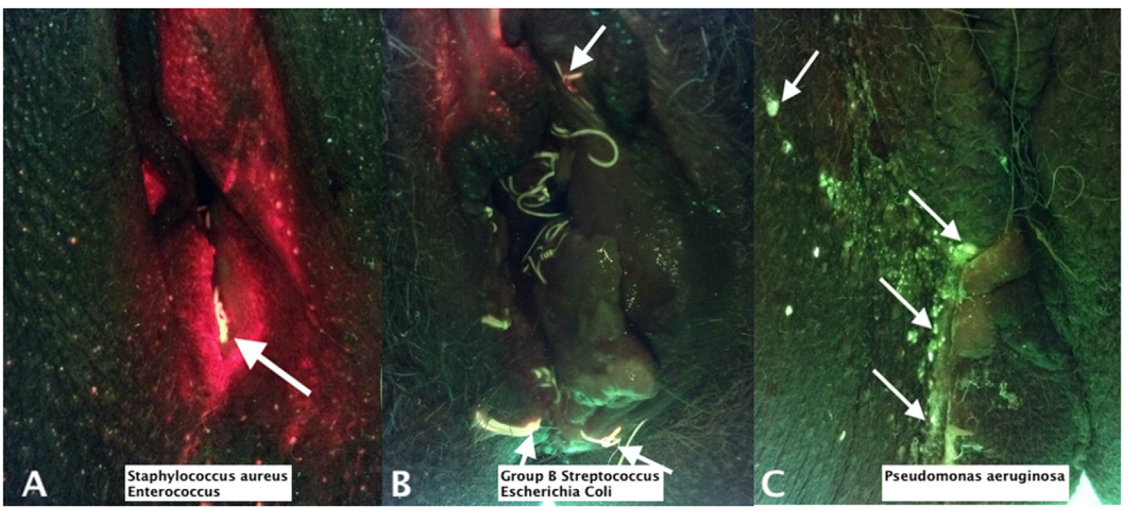

Fig. 1. Bacterial fluorescence images of infected perineal wounds taken using the MolecuLight i:X imaging device. Wounds showing red (A, B) or cyan (C) fluorescence (arrows) with microbiological culture results below.

2.3. Study outcomes

The primary study outcome of this analysis was the incidence of bacterial fluorescence in infected perineal wounds and factors associated with the presence bacterial fluorescence. The secondary outcome was to assess the accuracy measures of bacterial fluorescence imaging relative to moderate (2+ to 3+) or heavy (4+) bacterial loads on microbiological analysis. The STROBE guidelines were used to ensure the reporting of this observational study [22].

2.4. Statistical analysis

Data was analyzed using SPSS version 26.0.0.0. Demographic and obstetric variables that were considered in this analysis included co-morbidities (for example diabetes, gestational diabetes and thyroid disease), smoking status, grade of perineal tear (diagnosed using the Sultan Classification [23]), perineal repair technique (continuous subcuticular or interrupted suture), wound swab status and antibiotic use. Continuous clinical variables included body mass index (BMI) and pain severity using a verbal analogue scale (VAS). We also examined the time between symptom onset, antibiotic receipt and perineal clinic review and its effect on the presence of bacterial autofluorescence. The Shapiro-Wilk test was used to check normality of continuous variables including, BMI and pain severity. Nominal data is expressed as number and percentage. Continuous variables were compared using Student's *t* test, or the Mann- Whitney U test where appropriate. The Fisher's exact test or Chi-Squared test was used for categorical variables where appropriate. The multivariate analysis used a logistic regression model which included significant factors identified on univariate analysis. A corresponding *P* value of < 0.05 was considered statistically significant. Sensitivity, specificity and positive and negative predictive values of bacterial autofluorescence were calculated in comparison to the reference test (microbiological analysis). Receiver operating characteristic (ROC) curves were constructed and the area under the curve (AUCs) were calculated with 95% confidence intervals (95%CIs).

3. Results

3.1. Study population

Eighty women diagnosed with perineal wound infection were recruited between August 2020 to August 2021. Of the 80 women with perineal wound infection recruited, 75 (93.7%) had a wound swab for microbiological analysis taken prior to referral to the perineal clinic; 54 (72%) of the wound swabs were positive. Seventy-six (95.0%) women were given broad spectrum antibiotics on average 6 days prior to referral to the perineal clinic. Four of the 80 women recruited (5.0%) were diagnosed with a deep wound infection. One of these women (1.3%) was pyrexial and required intravenous antibiotics. The time to wound infection resolution and complete wound healing ranged between 1-16 weeks (median 2 [IQR 1–4]).

3.2. Primary outcome: - Bacterial fluorescence

Despite antibiotics, 30 (37.5%) of wounds had significant bacterial loads identified using bacterial fluorescence. Univariate analysis (Table 1) identified 3 factors associated with the presence of bacterial fluorescence in infected perineal wounds: Pain severity score ($P < 0.001$), perineal skin suturing technique ($P = 0.04$) and exposed suture material within the wound (loose sutures/exposed knots) ($P = 0.01$). There were no other factors significantly associated with the presence of bacterial

Table 1
Factors associated with the presence of bacterial fluorescence.

	Positive bacterial fluorescence (*n*=30) Mean(SD)/Median (IQR) /*n* (%)	Negative bacterial fluorescence (n=50) Mean(SD)/Median (IQR) /*n* (%)	*P*-value
BMI (kg/m^2)	24.5 (5.3)	24.9 (5.1)	0.71[c]
Pain (VRS)	8 (7–9)	6 (5–7)	*<0.001*[d]
Smoker (*n*=7)	4 (13.4)	3 (6.0)	0.42[a]
Non-smoker (*n*=73)	26 (86.7)	47 (94.0)	
Co-morbidities (*n*=18)	10 (33.3)	8 (16.0)	0.07[b]
No co-morbidities (*n*=62)	20 (66.7)	42 (84.0)	
OASI[e] (*n*=10)	3 (10.0)	7 (14.0)	0.74[a]
No OASI (*n*=70)	27 (90.0)	43 (86.0)	
Subcuticular skin repair (*n*=31)	9 (29.0)	22 (71.0)	*0.04*[a]
Interrupted skin repair (*n*=12)	8 (66.7)	4 (33.3)	
Positive wound swab (*n*=54)	20 (74.1)	34 (70.8)	1.00[b]
Negative wound swab (*n*=21)	7 (25.9)	14 (29.2)	
Antibiotics received (*n*=76)	29 (96.7)	47 (94.0)	0.56[a]
No antibiotics received (*n*=4)	1 (3.3)	3 (6.0)	
D between review and antibiotics	6 (3–7)	6 (3–8)	0.35[d]
D from symptom onset to perineal clinic review	19 (15–26)	17 (14–22)	0.31[d]
Exposed sutures (*n*=47)	24 (76.7)	23 (48.0)	*0.01*[b]
No exposed sutures (*n*=33)	7 (23.3)	26 (52.0)	

OASI = Obstetric anal sphincter injury; VRS = verbal rating scale.
[a] Fishers Exact.
[b] Chi-Squared.
[c] Independent *t* test.
[d] Mann-Whitney U.
[e] Five women had a missed OASI diagnosed on endoanal ultrasound scan.

Table 2
Factors associated with the presence of bacterial fluorescence: multivariate analysis.

	OR (95%CI)	*P*-value	adj OR (95%CI)[a]	*P*-value
Pain (VRS)	1.39 (1.09–1.77)	<0.001	1.34 (1.05–1.71)	*0.01*
Subcuticular skin repair[a] (*n*=31)	0.20 (0.50–0.90)	0.03	-	-
Interrupted skin repair[a] (*n*=12)				
Exposed sutures (*n*=47)	3.56 (1.29–9.79)	0.01	2.95 (1.03–8.45)	*0.03*
No exposed sutures (*n*=33)				

adjOR = adjusted odds ratio; OR = odds ratio; VRS = verbal rating scale.
[a] Suture technique was not added to the regression model as this was unknown in 37 (46.3%) patients.

fluorescence. Logistic regression analysis demonstrated the use of a subcuticular repair of the perineal skin reduced the odds of bacterial fluorescence by 80% (OR 0.20 [95%CI 0.50–0.90]). On multivariate analysis, pain severity score (OR 1.34 (95%CI 1.05–1.71)) and the presence of exposed suture material (OR 2.95 (95% CI 1.03–8.45)) remained independent risk factors (Table 2). Table 3 shows the bacteria colonized from exposed suture material with bacterial fluorescence (*n* = 23). Twenty-one (91.3%) wound bacterial colonies were polymicrobial and 2 (8.7%) were a single organism. 15 bacterial species were identified. The most common bacterial species identified were *Enterococcus Faecalis and Escherichia Coli*. All bacteria apart from *Pseudomonas aeuroginosa* (cyan fluorescence) exhibited a red fluorescence.

Table 3
Bacterial and fungal species identified from exposed sutures.

Organisms	n (%)[a]	Fluorescence color
Polymicrobial	*n*=21 (91.3)	
Gram positive cocci		
Enterococcus faecalis	6 (28.6)	Red
Group b ß-haemolytic streptococcus	2 (9.5)	Red
Staphylococcus aureus	1 (4.8)	Red
Streptococcus anginosus	3 (14.3)	Red
Streptococcus oralis	4 (19.0)	Red
Gram positive bacilli		
Corynebacterium amycolatum	4 (19.0)	Red
Gram negative anaerobic bacilli		
Bacteroides fragilis	1 (4.8)	Red
Bacteroides thetaiotaomicron	1 (4.8)	Red
Bacteroides vulgatus	1 (4.8)	Red
Prevotella bivia	1 (4.8)	Red
Gram positive anaerobic cocci		
Peptostreptococcus anaerobius	1 (4.8)	Red
Gram negative aerobic bacilli		
Enterobacter bugandensis	1 (4.8)	Red
Enterobacter cloacae	4 (19.0)	Red
Escherichia coli[b]	8 (38.1)	Red
Klebsiella aerogenes	1 (4.8)	Red
Klebsiella pneumoniae	1 (4.8)	Red
Morganella morganii	1 (4.8)	Red
Proteus mirabilis	1 (4.8)	Red
Pseudomonas aeruginosa	1 (4.8)	Cyan
Serratia marcescens	1 (4.8)	Red
Yeasts		
Candida albicans	2 (9.5)	Red
Candida lusitaniae	1 (4.8)	Red
Other		
Mixed anaerobes	5 (23.8)	Red
Mixed coliforms	1 (4.8)	Red
Single organism	*n*=21 (91.3)	
Gram negative aerobic bacilli		
Morganella morganii	1 (50.0)	Red
Enterobacter cloacae	1 (50.0)	Red

[a] 23 wounds had loose sutures, however, 21(91.3%) bacterial colonies from exposed suture material were polymicrobial and 2 (8.7%) were a single organism.
[b] One of the Escherichia Coli were found to be Extended-Spectrum B-Lactamase Producing.

3.3. Secondary outcomes

Diagnostic properties of bacterial fluorescence are presented in Table 4. Twenty eight women were excluded from analysis as they had a wound swab taken prior to prior to recruitment or had no loose suture material within their wound. The sensitivity of bacterial fluorescence imaging in the diagnosis of wounds with a clinically significant bacterial burden was 83% and specificity was 90%. The positive predictive value (PPV) of was 92% (95% CI 0.74–0.98) and the negative predictive value (NPV) was 80% (95% CI 0.58–0.92). The area under the ROC (AUC) for the diagnosis of significant bacterial loads in wounds using the MolecuLight was 0.87 (0.76–0.98).

3.4. Discussion

This novel study is the first to investigate the presence of bacterial fluorescence in infected perineal wounds. It demonstrated that approximately 40% of infected perineal wounds emitted bacterial fluorescence on initial review despite antibiotics. Factors associated with the presence of bacterial fluorescence include an interrupted suturing perineal repair technique at delivery, severe pain scores and exposed suture material within a wound. Furthermore, bacterial fluorescence imaging was found to have substantial accuracy in diagnosing perineal wounds with significant bacterial load.

Sutures have been described to be a nidus for bacterial biofilm formation [24]. In this study, we only included samples with moderate (2+ to 3+) or heavy (4+) bacterial loads (equivalent to $>10^4$ CFU/g on quantitative analysis). This bacterial load is associated with infection as it is the agreed level at which the host can become overwhelmed and generate an inflammatory response [25]. Moreover, Edmiston et al [26] showed that bacterial biofilms associated with infected suture material were $>10^4$ CFU/cm of suture segment [26]. We found that the odds of bacterial fluorescence significantly increased four-fold when exposed suture material (loose sutures or knots) was present in perineal wounds. To date, no study has used the Moleculight device to identify bacterial fluorescence in foreign bodies such as suture material. This could be used to facilitate early removal and improve wound healing. Moreover, we found that a subcuticular repair of the skin in comparison to interrupted suturing technique significantly reduced the risk of bacterial fluorescence by 80%. A continuous subcuticular technique in comparison to interrupted sutures for perineal repair has been shown to be associated with less short-term perineal pain (10 days post-partum) and a reduction in suture removal [27]. Pain is an important clinical sign associated with wound infection and has been shown to be significantly higher in infected acute wounds in comparison to non-infected wounds [28]. We measured pain using a validated verbal rating scale [29]. We found that each unit increase in verbal rating scale score was associated with a 40% increase in the odds of bacterial fluorescence presence. However, a number of women with perineal trauma following

Table 4
Diagnostic performance of bacterial fluorescence in comparison to bacteria at moderate to heavy loads.

N=52[a]	≥2+ bacterial load *n*=30		<2+ bacterial loads *n*=22
Positive bacterial fluorescence *n*=27	25 (83.3)		2 (9.1)
Negative bacterial fluorescence *n*=25	5 (16.7)		20 (90.9)
Sensitivity (95%CI)		0.83 (0.65–0.94)	
Specificity (95%CI)		0.90 (0.69–0.98)	
Positive predictive value (95%CI)		0.92 (0.74–0.98)	
Negative predictive value (95%CI)		0.80 (0.58–0.92)	
AUC (95%CI)		0.87 (0.76–0.98)	

AUC = area under the curve.
[a] Fifty-two (65.0%) of patients were used for analysis as they had exposed sutures removed or a wound swab taken (as one was not taken in maternity triage) on review in the dedicated perineal triage

childbirth will experience pain: 53% will report mild pain, 33% moderate and 3.7% severe [30]. In addition, approximately a quarter of women will experience perineal pain up to 8 weeks following vaginal delivery [31]. It is important to note that in our study, the majority of women reported perineal pain. However, as pain was significantly higher in wounds with significant bacterial loads, this highlights that pain severity is an important factor to consider in the diagnosis of perineal wound infection.

The diagnostic accuracy of bacterial fluorescence imaging in comparison to microbiological analysis has been evaluated previously mostly in chronic lower leg wounds. Reference standards used included wound swabs, and tissue biopsy [13,15,17,32]. Tissue biopsy is the gold standard for microbiological analysis of wounds, however it is invasive and can potentially disrupt wound healing [8,33]. Wound swabs are therefore more commonly used in the outpatient setting. We found that the sensitivity of bacterial fluorescence imaging in the diagnosis of acute perineal wounds with a clinically significant bacterial burden was 83% and specificity was 90%. Hurley et al [32] showed that bacterial fluorescence imaging had a sensitivity of 100% and specificity of 78% in detecting chronic lower leg wounds colonized with bacteria. Moreover, the PPV and NPV was 95.4% and 100% respectively. However, the authors diagnosed a wound as infected with bacteria at any load [32]. Bacterial involvement in a wound can be defined as: contamination, colonization or wound infection, depending on the load of bacteria present within wound [8,34]. In our study we only included wounds with significant bacterial loads (moderate-to-heavy) following antibiotic therapy. This may be a plausible explanation for why our specificity was higher (90.0% vs 78%) and NPV was lower (80.0% vs 100%), as the Hurley et al [32] included wounds that we deemed to have a clinically insignificant bacterial load, as the bacterial load was light (1+). It is important to note that the presence of bacterial fluorescence using the Moleculight device corresponds with the quantity of bacteria (moderate (2+ to 3+) or heavy (4+)/[$>10^4$ CFU/g) and only corresponds with the species of bacteria in pseudomonas infections (cyan fluorescence).

Strengths of this study include its prospective design and originality, as it is the first study to evaluate the use of bacterial fluorescence imaging in infected perineal wounds following childbirth. Although, this study was not powered for secondary outcomes, as we demonstrated a statistically significant association between factors including suturing technique, pain severity, and presence of exposed sutures, this suggests we had sufficient power to detect the size of association observed in the data. Furthermore, additional limitations of this study should also be considered. Firstly, in our study we used semi-quantitative cultures from wound swabs and suture material as the reference standard, however, these may be unreliable in comparison to the "gold standard" quantitative methods. Serena et al in their prospective study of 350 chronic wounds found that in each semi-quantitative category there was a wide range of bacterial loads within each group with significant overlap. In addition, that 94% of wounds categorized as light growth (1+) had quantitative bacterial loads $>10^4$ CFU/g [35]. However, in the FLAGG study, bacterial fluorescence imaging significantly increased the detection of bacteria loads of $>10^4$ CFU/g four-fold [15]. Meaning that in our study, those wounds with positive bacterial fluorescence were likely to have significant loads of bacteria. Therefore, bacterial fluorescence imaging could be used as an adjunct to semi-quantitative swab cultures to diagnose clinically significant bacteria within perineal wounds. Particularly as semi-quantitative swab cultures are widely used across sites instead of quantitative methods due to expense [15].

At our unit, microbiological samples are analyzed off -site, meaning specimens may not have been transported within the advised 2 hours following collection [11]. Also, microbiological results obtained with wound swabs are particularly dependent on sampling techniques (Z-stroke or Levine technique) [9]. The Levine technique has been shown to detect significantly more bacteria in both acute and chronic wounds in comparison to the Z technique [36]. In our study we did not control for the sampling technique when taking swabs from wounds which had not been sampled previously in maternity triage. Therefore, our findings should be interpreted with caution. In addition, 95% of patients in the study were treated with antibiotics prior to perineal clinic review, therefore, before bacterial fluorescence imaging and targeted sampling. All of these factors may have resulted in false-negative cultures. However, despite this, approximately 40% of wounds had positive cultures and 84% of these also had bacterial fluorescence within the wound. Furthermore, although factors associated with the presence of bacterial fluorescence were identified, due to the nature of observational studies, causality cannot be established. We acknowledge that as wound samples are superficial, they may not represent the true pathogens of infection. Tissue biopsy is the gold standard for microbiological analysis of wounds, however it is invasive and can potentially disrupt wound healing [8,33]. However, in perineal wound infection following childbirth, although there is no established reference standard, it is recommended that wound swabs are taken for culture and sensitivity [37]. Tissue biopsy is not the standard practice for the assessment of perineal wound infection so was not performed in this study.

On microbiological analysis, perineal wound infections are often found to be polymicrobial due to the complex microflora of the surrounding anatomy [37]. This means that even in the absence of infection, microbial swabs are likely to isolate a range of bacteria. Therefore, diagnosis and treatment of perineal wound infection is often based on clinical signs and microbiological swabs are used to guide antibiotic choice [38]. However, diagnosis based solely on clinical signs and symptom is subjective, and often incorrectly identifies wounds with moderate-to-heavy bacterial loads [39]. This was demonstrated in the FLAGG study [15] which found that 82% of wounds with clinical significant bacterial loads were missed when assessment of clinical signs and symptoms was used without bacterial fluorescence imaging. Yet, moderate-heavy loads of bacteria within wounds are associated with wound complications such as wound infection and delayed wound healing [40,41]. As wound

swabs take 2−5 days to grow on culture media, women may complete a course of antibiotics for a perineal wound that is not infected, before the wound swab results are received and reviewed by healthcare professionals [8]. Therefore, with assessment of the clinical signs of infection, the Moleculight device could be used initially as a diagnostic screening tool, whilst awaiting wound swab results. Further research is required to evaluate if bacterial fluorescence imaging can be used on first review of suspected perineal wound infection to restrict antibiotic provision by early diagnosis of wound infection.

4. Conclusions

This is the first study to use bacterial fluorescence imaging in the assessment of infected perineal wounds. This can provide real-time information surrounding the bacterial burden of perineal wounds, which can aid diagnosis and management at the bed-side. This study has also highlighted that the use of external sutures in the perineum should be avoided as it can become a nidus for infection and therefore subcuticular sutures should be used where possible.

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Declaration of competing interest

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

Authors' contributions

Nicola Adanna Okeahialam: Conceptualization, Investigation Formal analysis, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization. Ranee Thakar: Conceptualization, Methodology, Resources, Project administration, Writing - Review & Editing, Supervision. Abdul Sultan: Conceptualization, Methodology, Resources, Project administration, Writing - Review & Editing, Supervision.

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