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SPECTROSCOPIC ANALYSIS OF DIABETIC FOOT ULCER PATIENTS

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ABSTRACT
An infected diabetic foot ulcer is the common cause of morbidity in diabetic patients leading to complications like gangrene and amputation. Illuminate, A Device that helps in the identification and type of bacteria (Gram-positive or negative) and In point of care settings by Multispectral autofluorescence imaging is considered simple, affordable, and non-time consuming and initiates appropriate antibiotics without any delay. The device excites the bacterial organisms with ultraviolet and blue light which in turn have characteristic emission of fluorescence from the organism that has been captured by the device, records the spectral signature, An Inbuilt image is processed and the artificial intelligence algorithm in the machine detects the native fluorescence emitted by the bacteria according to their Gram type.
This study was done in a tertiary care hospital With a total of 15 number of Patients, where we evaluated both spectroscopy and standard culture sensitivity test and found that the illuminate device was able to detect the microorganism with 92.9% Sensitivity and 50% Specificity to the culture sensitivity test and with the accuracy of 71.15%. This device has the potential to help clinicians to identify wound with significant bacteria which maximizes the treatment for the infected diabetic foot ulcer with early start of appropriate antibiotics.

INTRODUCTION
Antimicrobial resistance has the same negative effects on healthcare as global warming[1]. The most huge challenge to overcoming antimicrobial resistance is the creation of a point-of-care (POC) diagnostic test to identify the presence of a bacterial infection that is affordable, precise, quick, and simple to use. This test would allow clinicians to administer the appropriate antibiotics at the appropriate time. When bacteria are quickly found in wounds, blood, or bodily excretions, the specific pathogen is treated. Currently, a tissue swab from the ulcer is used to do a culture and sensitivity test in order to detect wound infection.
A microbiology laboratory is needed for this, and it takes roughly 48-72 hours to determine the type of bacteria. Although there are numerous phenotypic and molecular methods for detecting microbes, they are labor- intensive, reagent-intensive, and demand for specialized expertise, making them unsuitable for quick screening. Recent innovations have been found to be sensitive and successful for the quick screening of bacterial infections and classification [3], taking advantage of the fluorescence of bacterial cell molecules. [3].

AIM
To study the effectiveness of this handheld device (Illuminate) to determine the presence of infection, classify the gram type of infecting bacteria, and to compare the results with those obtained by the standard tissue culture method.

METHODOLOGY
Study design: a prospective Observational study

Sampling method: Simple random sampling**. Sampling size:** 15
Study tool: Illuminate imaging device, swab culture results

Inclusion criteria: Type2 diabetic patients with diabetic foot ulcer.

Data analysis: Data collected will be entered in MS Excel and will be analyzed using Statistical package for social sciences (SPSS) software**.**

ILLUMINATE DEVICE

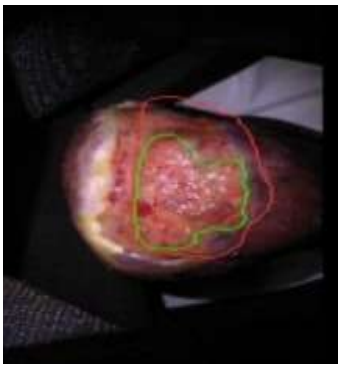
A start up primarily based in India has developed and patented (IN Patent–323,440) a unique handheld device, (Illuminate®), which makes use of the native fluorescence of bacteria for its detection [4] (Fig. 1).

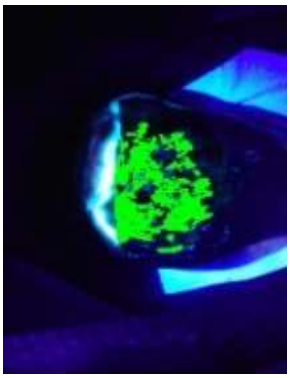
Each bacterium has characteristic specific fluorescence when excited with an extraordinary wavelength[5, 6]. The device was based on multispectral imaging combined with state of an art AI engine for spatial mapping and type of bacteria. The device captures the spectral signature markers of the microorganism reasons for infection, to locate and assess the bacterial gram type. The device leverages the Autofluorescence property exhibited through bacterium [such as Nicotinamide adenine dinucleotide hydrogen (NADH), flavins] And infectious markers [Pyoverdine, Porphyrin] present. In microorganisms and fungi [6] Use of such handheld devices Has been carried out very effectively in diagnosing skin and smooth tissue. Contamination—a bacterial and fungal infection.

All patients attending plastic surgery OPD , admitted in ward with diabetic foot ulcers included in the study. After clinical examination and thorough wound wash, wound swab for culture and sensitivity taken from the clinically suspecious area. After this, the ulcer was imaged by this device either in a dark room or under a black hood.The Illuminate device captures images of the ulcer, in a dark room at a distance of 7 to 10 cm away from the wound. The Gram type displayed immediately by the device was recorded in a prepared proforma. After 48 hrs, culture reports were compared with the device report (Gram type).

Figs. 2 show the images taken by the device and clearly indicating the site of infection, the Gram type of bacteria infecting the wound and size of the ulcer.The device finds the presence or absence of bacteria in the ulcer, maps the exact location of the infection, and classifies the Gram type of bacteria within 2 minutes.

1)example of gram-positive bacteria- infected ulcer(showing red area)





2) example of gram-negative bacteria-infected ulcer(showing green area)

Fig 2 Pictures captured in an illuminate device

RESULTS

Out of 15 patients 10 were male and 5 were female. Diabetic foot ulcer gram-type results obtained by the illuminate device are compared with the swab culture study from the microbiology department. The device displayed a red and green color covered over the surface of the ulcer indicating Gram-positive and Gram-negative bacteria respectively.

From our study, Results of the Illuminate device showed 46.7% of gram- positive bacteria, 40% of gram-negative bacteria in the spectral study and culture method showed about 46.7% of gram-positive and gram Negative bacteria in the culture study. No growth seen in 13.3% of the spectral study and 6.7% in culture study.

As for as the type of organisms concerned, our study shows about 33.3% of staphylococcus aureus-infected diabetic foot ulcers followed by 26.7% of E.coli infected diabetic ulcers. The sensitivity of the illuminate device for detecting the growth in infected ulcers is 92.9%.Our study showed a sensitivity of 85.71%, and a specificity of 83.33%for identifying the gram type of bacteria in infected diabetic foot ulcer with the spectroscopy device.

The Illuminate spectroscopy device has shown a positive predictive value of 85.71%for identifying the gram type of the Bacterium with an accuracy of 84.62%.Cohen's Kappa (K) to set on the agreement between the culture method and the illuminate device is K= 0.659 showing substantial agreement between the spectroscopy study and culture study. The illuminate device shows mostly the correct gram type of organism in the infected diabetic foot ulcer.Cramer's V is used to interpret the values of the association between the spectral and culture study for diagnosing the bacteria which is 0.688. It shows that the illuminate device has a very strong interpretation of association with the standard culture method for diagnosing the microorganism.

Table 1, 2: Test results of culture vs Spectroscopy

Screening test culture/spectroscopy		**Growth according to culture report**		
		Growth seen	**No growth seen**	**Total**
Growth according to spectroscopy	Growth seen	12(TP)	1(FP)	13
	No growth	1(FN)	1(TN)	2
	Total	13	1	15

Sensitivity of spectroscopy	92.3%
Specificity of spectroscopy	50%
Positive predictive value	92.3%
Negative predictive value	50%
Accuracy	**71.15%**

Table 3: Comparison of detection of Gram-negative, Gram-positive, and no growth by spectroscopy and culture method.

Culture/spectroscopy		**Growth according to culture**		
	Gram- positive	**Gram-negative**	**No growth**	
Growth according to spectroscopy	Gram- positive	6	1	0
	Gram- negative	1	5	0
	No growth	0	1	1

Table 4: Sensitivity specificity for spectroscopy method FOR gram type

Sensitivity of Spectroscopy	85.71%
Specificity Spectroscopy/Culture	83.33%
Positive predictive value	85.71%
Negative predictive value	83.33%
Accuracy	84.22%

Table 5: Results of bacteria obtained through the culture method

Culture: organisms	Frequency	Percent
E.coli	4	26.7%
Enterococcus	2	13.3%
Klebsiella	2	13.3%
No growth	1	6.7%
Pseudomonas aeruginosa	1	6.7%
S.aureus	5	33.3%
Total	15	100.0

DISCUSSION

The mechanism underlying diabetic foot ulcers is critical for effective treatment and the prevention of associated complications. However, the application of spectroscopy in studying diabetic foot ulcers remains nascent. Therefore, precise methodologies and objectives are essential for clinicians to utilize this technology effectively and to administer appropriate antibiotics. Our study revealed a sensitivity of 85.71% in detecting both gram-negative and gram-positive bacteria, in contrast to the 91.3% sensitivity reported by Viswanathan et al(3)., which suggests a comparable capability in pathogen detection.

Furthermore, our study demonstrated an accuracy of 71.15% in identifying bacterial growth, while Parkhe's research indicated a higher accuracy of 92.3%(6), also aligning closely with our findings. We noted a specificity of 83.3% for detecting gram-negative organisms, compared to the 93.2% specificity reported by Vijay(6), who focused exclusively on varicose ulcers, potentially enhancing specificity due to soft tissue involvement. In our study, the presence of exposed bones and tendons likely contributed to instances of false gram-negative fluorescent readings.

The most prevalent organism identified in our study was Escherichia coli, accounting for 26.7% of infections. This contrasts with findings by Bansal(7), who reported E. coli infections at 18%, reinforcing its status as a significant pathogen in diabetic foot ulcers(9).

Our findings suggest that the illumination device can promptly determine the gram type of organisms involved in diabetic foot ulcers within clinical settings. This capability allows for its application in operating rooms to identify organisms in surgical wound areas, facilitating early antibiotic administration and potentially preventing complications(8). Additionally, the device can be employed to detect microorganisms on clinical instruments within hospital settings. It also measures the extent of ulcers, enabling tracking of wound healing progress during patient follow-up. The instant test results, along with data storage and transfer capabilities via USB or cloud connectivity to hospital records, highlight the device's practicality for widespread use.

CONCLUSION

Among the many recently discovered methods for detecting bacterial infections, those that rely on the bacteria's fluorescent properties can be easy, affordable, and quick. This recently created tool is a perfect illustration of the same which helps in early start of appropriate antibiotics.

Currently, this device just displays the Gram type of bacteria, not the species. The AI in the gadget will eventually be able to distinguish between the gram type of infectious bacteria on future studies. The instrument's accuracy, which is about 71.15%, is above average, but the accuracy can be improved with larger population study.

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