"To Study the Microbiological Profile and MDR Strains of Chronic Non Healing Diabetic Ulcers with Special Reference to Bio film Formation"

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Abstract:

Background: Diabetes mellitus is a major health problem that affects approximately 171 million people globally. One of its most severe complications is the development of diabetic foot ulcers (DFU). Multidrug Resistance (MDR) is basically caused by the Bio film which developers a barrier for the immune system and the antimicrobial agents.

Aim and Objective: To Study the Microbiological Profile and MDR Strains of Chronic Non Healing Diabetic Ulcers with Special Reference to Bio film Formation.

Material and Methods: This is a cross-sectional study which was carried out in the Department of Microbiology, RMCH &RC, Mandhana, and Kanpur for a period of 1 year January 2020 to December 2020. Clinical samples from Pus and tissue bit samples were taken from 200 diabetic patients with non healing ulcers as per standard microbiological procedures. Antimicrobial susceptibility test was performed as per Clinical and Laboratory Standards Institute (CLSI) guidelines. Detection of Bio film formation by the tissue culture plate method was done.

Results: Out of 200 diabetic patients, the ratio of Males were more than the Females with 122 (61%) and 78 (39%). Patients with type 2 diabetes related ulcer were more than Type 1. The most common sites of ulcers were the plantar surface of foot 101 (50.5%), toes 49 (24.5%), and dorsal surface of foot 50 (25%). Among the gram negative aerobes 120 (60%) are predominant than gram positive aerobes 47 (23.5%). The rest of the growth showed 22 (11%) Fungal and 11 (5.5%) anaerobic growth.

The predominantly isolated pathogens were Pseudomonas aeruginosa and Staphylococcus aureus among aerobic bacteria, Peptostreptococcus among the anaerobes and Candida alb cans was most predominantly isolated among fungus.

Staphylococcus aureus was the strong biofilm producer, followed by Pseudomonas aeruginosa, baumannii. Gram-negative bacteria showed high sensitivity to piperacillin-tazobactam, meropenem, Coniston, gram-positive cocci to vancomycin and linezolid .

Conclusion: Bio films and polymicrobial infection have a crucial role in DFIs and contribute to delay healing. These wounds are characterized by a complex micro biome and a polymicrobial organization, especially within the bio film. The development of processes and methodologies to study bio films is needed. This represents the next step to validating new anti bio film molecules with a promising therapeutic potential.

Keywords: MDR, DFU, CLSI

Introduction

Bacteria within bio films are sheltered from various stresses, including immune responses and antimicrobial agents. The bio film-forming ability of bacteria has been associated with increased antibiotic resistance and chronic recurrent infections especially in diabetics [1].The global prevalence of DFU is 6.3%. India has the largest diabetic population and is expected to increase by 2025 to 57 million [2].

Studies have suggested that wounds with a high microbial load of greater than 105 Colony Forming Unit (CFU) per gram of tissue are considered critical for Diagnosing infection and are associated with an increased incidence of wound sepsis [3-5]. Microorganisms stick to each other and become embedded in a self-secreted Extracellular Polymeric Substance (EPS) form a structured community over the surfaces [6]

The ability of a microorganism to form bio film is an important virulence factor as it establishes a protective environment for the organisms to survive and evade antibiotics. These bio films are the main cause of many chronic infections such as diabetic foot ulcers, and they pave the way for the re-emergence of multidrug-resistant strains and result in treatment failure [2]. Bio films are difficult to eradicate using conventional antibiotics; hence the identification of bio film producers among clinical isolates may lead to better management of wound infections in diabetics who, in spite of repeated antibiotic treatment, fail to respond to treatment because bio films are not being tested for routinely.

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Material and Methods

This is a cross-sectional study which was carried out in the Department of Microbiology, RMCH &RC, Mandhana, and Kanpur for a period of 1 year January 2020 to December 2020. Clinical samples from Pus and tissue bit samples were taken from 200 diabetic patients with non healing ulcers as per standard microbiological procedures. Antimicrobial susceptibility test was performed as per Clinical and Laboratory Standards Institute (CLSI) guidelines. Detection of Bio film formation by the tissue culture plate method was done.

The area of the ulcer site was cleansed and decontaminated with 10% povi done iodine and normal saline and samples such as tissue bits, pus, exudates were collected by rubbing the deepest accessible area ulcer covering an area of 1cm with two sterile swabs after adapting aseptic measures. One among the two swabs was used for culture and the other for gram staining. Tissue bits were collected aseptically in a sterile closed container containing normal saline without preservative (to keep the tissue moist) was transported within an hour, and homogenized in a tissue grinder or minced before mycological evaluation. The specimens were inoculated into Mac Conkey agar, Blood agar for bacteriological analysis and incubated at 36±1˚C for 24-48 hours. In addition, the prepared specimens were inoculated into two tubes of Sabouraud Dextrose Agar and incubated at 25˚C and 37˚C, respectively. Tissue bits were collected in Robertson cooked meat broth and incubated at 36±1˚C for 48 hours for anaerobic culture and processed using the Gas pack in a McIntosh fields jar. Bacterial and fungal pathogens were identified by direct microscopy, colony characterization and biochemical parameters. Bio film detection by tissue culture plate method [7] and the AST was performed as per CLSI guidelines.

Results

Here in our study out of 200 patients with non healing diabetic ulcers, 70 (35%) of cases belonged to the age group of 51-60 years followed by 55 (27.5%) in 41-50 years, 35 (17.5%) in 61-70 years, 20 (10%) in 71-80 years, 15 (7.5%) in 20-40 years and 5 (2.5%) in >80 years. Among 200 patients the ratio of Males was more than the Females with 122 (61%) and 78 (39%).

Table1: Distribution of Patients according to age.

S.NO.	Gender	No. (n=200)	Percentage
1.	Male	122	61%
2.	Female	78	39%

Patients with type 2 diabetes related ulcer were more than Type 1. There was 158 (79%) with Type 2 and with Type 1 diabetes related ulcer, was 42 (21%).

The most common sites of ulcers were the plantar surface of foot 101 (50.5%), toes 49 (24.5%), and dorsal surface of foot 50 (25%). Among the gram negative aerobes 120 (60%) are predominant than gram positive aerobes 47 (23.5%). The rest of the growth showed 22 (11%) Fungal and 11 (5.5%) anaerobic growth.

The predominantly isolated pathogens were Pseudomonas aeruginosa and Staphylococcus aureus among aerobic bacteria, Peptostreptococcus among the anaerobes and Candida alb cans was most predominantly isolated among fungus.

Other infections among aerobes include Escherichia coli, Klebsiella species, Acinetobacter baumannii , Proteus species ,Coagulates Negative Staphylococcus . (87%) of bacterial isolates and (13%) of fungal isolates were bio film producers. Staphylococcus aureus was the strong bio film producer, followed by Pseudomonas aeruginosa, baumannii . Gram-negative bacteria showed high sensitivity to piperacillin-tazobactam, meropenem, gram-positive cocci to vancomyc in and linezolid . 15 of the GNB isolates were MDR. And on testing were Celestin found 100%sensitive.

Table no. 2: Distribution of pathogens in diabetic ulcer

Group	Isolates	Number	%
GPC 47 (23.5%)	Staphylococcus aurous	30	15
	Enterococcus facials	10	5
	Staphylococcus epidermidis	7	3.5
GNB 120 (60%)	Pseudomonas aeruginosa	45	22.5
	Acinetobacter baumannii	18	9
	Klebsiella pneumonia	21	10.5
	Klebsiella oxytoca	5	2.5
	Escherichia coli	19	9.5
	Citrobacter freundii	6	3
	Enterobacter species	2	1
	Proteus vulgar is	4	2
	Proteus mirabilis	11	5.5
Fungal 22 (11%)	Candida alb cans	10	5
	Candida tropical is	3	1.5
	Candida parapsilosis	6	3
	Candida globate	1	0.5
	Aspergillus flavus	1	0.5
	Aspergillus niger	1	
Anaerobes (11isolates) 5.5%	Peptostreptococcus anaerobius	9(4.5%)	
	Bactericides	2(1)	

Discussion

Here in our study out of 200 patients with non healing diabetic ulcers, the maximum number of patients were

in the age group of 70 years followed by the age group of 51-60 years and least in the age of 20-40 years and >80 years. This corresponds to the study by PRama Prabha et al., where the maximum numbers of cases were in the age group of 70 years and minimum in the age above 80 years [8].

Among 200 patients the ratio of Males was more than the Females with 122 (61%) and 78 (39%), which correlate with the study of other author [8].

Patients with type 2 diabetes related ulcer were more than Type 1. The most common sites of ulcers were the plantar surface of foot 101 (50.5%), toes 49 (24.5%), and dorsal surface of foot 50 (25%). Among the gram negative aerobes 120 (60%) are predominant than gram positive aerobes 47 (23.5%). The rest of the growth showed 22 (11%) Fungal and 11 (5.5%) anaerobic growth. This was supported by other authors also [9] [10] .

The predominantly isolated pathogens were Pseudomonas aeruginosa and Staphylococcus aureus among aerobic bacteria, Peptostreptococcus among the anaerobes and Candida albicans was most predominantly isolated among fungus, and this supported our study [8]. Arun CS et al., (40%) [11].

Other infections among aerobes include Escherichia coli, Klebsiella species, Acinetobacter baumannii, Proteus species ,Coagulase Negative Staphylococcus . (87%) of bacterial isolates and (13%) of fungal isolates were bio film producers. Our study was in agreement with the study conducted by Gadepalli R et al., Kumar A et al., in India [12][13].

Staphylococcus aureus was the strong bio film producer, followed by Pseudomonas aeruginosa, baumannii. Gram-negative bacteria showed high sensitivity to piperacillin-tazobactam, meropenem, gram-positive cocci to vancomycin and linezolid . This was in agreement with the findings of Nithyalakshmi J and Banu A et al., [14] [15]. In our study 15 of isolates were MDR. And on testing were Celestin sensitive. [8]

Conclusion

In our study there is a increase in the Antimicrobial resistance and the emergence of MDR organisms are a potential threat in the community. Difficulty in eradicating a chronic diabetic foot infection associated with bio film formation has been reported, and bio film-producing bacteria have been shown to resist higher antibiotic and disinfectant concentrations than non-bio film producing bacteria. Therefore, additional screening of multidrug-resistant organisms as well as non-resistant organisms like MSSA often associated with bio films should be considered.

This reflects the need for global strategies to control the emergence and spread of MDR pathogens. So the Early identification and correction of modifiable risk factors such as anaemia may slow the progression and improve the patient survival in Diabetic foot syndrome.

References

[1]. Swarna SR, Radha M, Gomathi S. et al. A study of Biofilm on Diabetic Foot Ulcer. International Journal of Research in Pharmaceutical and Biomedical Sciences. 2012; 3(4):1809–14.

[2]. Shankar EM, Mohan V, Premalatha G, Srinivasan RS, Usha AR. Bacterial etiology of diabetic foot infections in South India. Eur J Intern Med. 2005; 16(8):567-70.

[3]. Gardner SE, Frantz RA. Wound bio burden and infection-related complications in diabetic foot ulcers. Biol Res Nurs. 2008; 10(1):44-53.

[4]. Global Advanced Wound Care Market- Analysis and Forecast, 2018-2024:

[5]. Clinton a, Carter T. Chronic wound bio films: Pathogenesis and potential therapies. Lab Med. 2015; 46(4):277-84.

[6]. Anaissie EJ, McGinnis MR, Pfaller MA. Clinical Mycology E-Book. Elsevier Health Sciences; 2009 Jan 26.

[7]. Clinical and Laboratory Standards Institute. Performance standards for Antimicrobial Susceptibility testing; Twenty First Informational Supplement. CLSI document M100-S21. Wayne, PA: 2012.

[8]. Prama prabha, CP Ramani, R kesavan. Study on Microbiome of Chronic Non Healing Diabetic Ulcers with Special Reference to Biofilm and Multidrug Resistant Strains. Journal of Clinical and Diagnostic Research. 2021; Vol-15(10): DC01-DC06.

[9]. Ekpebegh CO, Iwuala SO, Fasanmade OA, Ogbera AO, Igumbor E, Ohwovoriole AE. Diabetes foot ulceration in a Nigerian hospital: In-hospital mortality in relation to the presenting demographic, clinical and laboratory features. Int Wound J. 2009; 6(5):381-85.

[10]. Chuan F, Zhang M, Yao Y, Tian W, He X, Zhou B. Anaemia in patients with diabetic foot ulcer: Prevalence, clinical characteristics, and outcome. Int J Low Extrem Wounds. 2016; 15(3):220-26.

[11]. Arun CS, Raju P, Lakshmanan V, Kumar A, Bal A, Kumar H. Emergence of fluconazole-resistant candida infections in diabetic foot ulcers: Implications for public health. Indian J Community Med. 2019; 44(Suppl 1):S74-76.

[12]. Gadepalli R, Dhawan B, Sreenivas V, Kapil A, Ammini AC, Chaudhry R. A clinico-microbiological study of diabetic foot ulcers in an Indian tertiary care hospital. Diabetes Care. 2006; 29(8):1727-32.

[13]. Kumar A, Agrawal AK, Kumar M, Sharma AK, Kumari P. Aerobic bacterial profile of diabetic foot and its anti bio gram in RIMS, Ranchi- A tertiary care hospital. Proteus. 2017; 2:01-05.

[14]. Rani V, Nithyalakshmi J. A comparative study of diabetic and non-diabetic wound infections with special reference to MRSA and ESBL. Curr Microbiol App Sci. 2014; 3(12):546-54.

[15]. Banu A, Noorul Hassan MM, Rajkumar J, Srinivasa S. Spectrum of bacteria associated with diabetic foot ulcer and bio film formation: A prospective study. Austral as Med J. 2015; 8(9):280-85.