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Non Anthrax Bacillus Species Association In Non Healing Diabetic Foot Ulcers And Their Drug Susceptibility

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Abstract

INTRODUCTION

Non-anthracis Bacillus species are ubiquitous gram-positive spore-forming organisms that were once believed to be non pathogenic but are now recognized as causing a variety of infections.A Diabetic foot Ulcer is an open wound in the skin that does not heal within days or weeks and people with diabetes are at even more risk of developing diabetic foot ulcers and pain.Diabetic Foot ulcers are open sores or lesions that will not heal or that return over a long period of time.

AIM

This study is done to find out the association of Non anthrax Bacillus species in the non healing diabetic foot ulcer

OBJECTIVE

To know the significance of the presence of Non anthrax Bacillus species in diabetic ulcers.To know about the specific treatment outcome and the drug of choice

MATERIALS AND METHODS

Study is done in patients visiting the diabetic clinic OP department. Samples were collected from patients who are clinically proven diabetic and having non healing foot ulcers and elected for medical management. Swabs samples were collected and transported to the department of microbiology. Samples were stored at 4 deg.C till it was processed. Samples were then inoculated on Blood agar, Nutrient agar and MacConkey agar and incubated at 37 deg.C for 24 hours. The growth on Macconkey agar was identified by gram's staining for the presence of gram positive bacilli with spore and for chain formation. Those colonies are biochemically identified as Bacillus species and tested for their drug susceptibility. The most common species identified is Bacillus cereus. The results were tabulated and analysed.

Results

In this study a total number of 82 samples were collected from foot ulcers in diabetic patients. The samples were subjected for microbiological examination. In this 13 bacterial isolates were identified and the proportion of Bacillus sps association is determined. Bacillus sps were isolated from 8 patients which was the fourth highest in number of the bacterial sps isolated. Bacillus sp is not a common finding in a healthy patient.

Conclusion

Isolation of bacillus species in the diabetic patients should be given due importance, because it is found as a single isolate in many diabetic patients with non healing foot ulcers.

Keywords: Non anthrax Bacillus species, Non healing diabetic foot ulcers,drug susceptibility

Introduction

Anthrax is a serious infectious disease caused by gram-positive, rod-shaped bacteria known as Bacillus anthracis. Anthrax can be found naturally in soil and commonly affects domestic and wild animals around the world.Non-anthracis Bacillus species are ubiquitous gram-positive spore-forming organisms that were once

believed to be non pathogenic but are now recognized as causing a variety of infections(Bergman, 2011). A Diabetic Ulcer is an open wound in the skin that does not heal within days or weeks and people with diabetes are at even more risk of developing diabetic foot ulcers and pain. Diabetic Foot ulcers are open sores or lesions that will not heal or that return over a long period of time. These sores result from the breakdown of the skin and tissues of the feet and ankles and can get infected(Sibbald, Gary Sibbald and Ayello, 2018). Symptoms of foot ulcers can include swelling, burning, and pain.Diabetic foot infections are classified as mild, moderate, or severe. Gram-positive bacteria, such as Staphylococcus aureus and beta-hemolytic streptococci, are the most common pathogens in previously untreated mild and moderate infection(Stefanopoulos *et al.*, 2021).

Diabetic Foot ulcers lead to gangrene. Without prompt and proper treatment, a foot ulcer may require hospital treatment. Or, it may lead to deep infection or gangrene and amputation.People with diabetes have an increased risk of developing gangrene(Lin, Liu and Sun, 2020). This is because the high blood sugar levels associated with the condition can damage your nerves, particularly those in your feet, which can make it easy to injure yourself without realising.A diabetic foot will be amputated,when the Tissue damage or death (gangrene) may occur, and any existing infection may spread to your bone. If the infection cannot be stopped or the damage is irreparable, amputation may be necessary. The most common amputations in people with diabetes are the toes, feet, and lower legs(Bekele and Chelkeba, 2020).Non-healing diabetic foot ulcer will occur When they are not treated ulcers can lead to severe complications such as gangrene and amputation.Diabetic foot ulcers will not heal because of Poor blood circulation is a form of vascular disease in which blood doesn't flow to your feet efficiently. Poor circulation can also make it more difficult for ulcers to heal. High glucose levels can slow the healing process of an infected foot ulcer, so blood sugar management is critical(Harris and Fang, 2021).Diabetic foot ulcers are a common and much feared complication of diabetes, with recent studies suggesting that the lifetime risk of developing a foot ulcer in diabetic patients may be as high as 25% . Foot ulceration requires long and intensive treatment, has important effects on quality of life of both patients and care-givers and is associated with major healthcare costs(Ndong *et al.*, 2021).

Drug penetrating necrotising available treatments include intravenous vancomycin and intramuscular teicoplanin. Surgical debridement is needed to drain pus and abscess cavities and to remove all necrotic and infected tissue including devitalised and infected bone resulting from osteomyelitis. Deep tissue swabs should be sent to the laboratory.(Ahmed, Getti and Boateng, 2021)

Diabetic patient wound secretions are collected,When the wound is relatively dry and collect the specimen with two cotton-tipped swabs moistened with sterile non-bacteriostatic saline. Gently roll the swab over the surface of the wound approximately five times, focusing on an area where there is evidence of pus or inflamed tissue.From the diabetic patients samples have been collected from the swab and wound asparates collection(Travis *et al.*, 2020). A skin or wound culture is a test to find germs (such as bacteria or a fungus) that can cause an infection. A sample of skin, tissue, or fluid is added to a substance that promotes the growth of germs. If no germs grow, the culture is negative. If germs that can cause an infection grow,

the culture is positive(Nelson *et al.*, 2018).Aspiration of the wound is when the wound is open, they can press the tip of a cotton swab into the wound and gently rotate it to collect a sample. If the wound is closed, they can withdraw fluid or pus from the wound with a syringe and a small needle. This is called needle aspiration (Abdulbasith *et al.*, 2020).

Foot ulceration is the most common, affecting approximately 15% of diabetic patients during their lifetime. Despite this, foot ulcers remain an important clinical problem, often resulting in costly, prolonged treatment.A non-healing ulcer is also a strong risk factor for major amputation(Brindley and Cofield, 1985).

Many factors can interfere with wound healing, including the patient's general health status (i.e., nutritional condition indicated by albumin levels) or drugs such as steroids that can interfere with normal healing. Dressing for diabetic foot ulcers are Alginate and foam dressings provide high absorbency for moderate to heavy exudate. For a diabetic foot ulcer with dying tissue, hydrogels or dressings with collagen and silver are most effective(Ahmed, Getti and Boateng, 2021). Most important is matching the absorptive ability of the wound dressing to the amount of wound drainage.The most common drug susceptibility of diabetic foot ulcers are dicloxacillin, amoxicillin-clavulanate, or clindamycin are effective choices.(Dixon and Edmonds, 2021)

Our team has extensive knowledge and research experience that has translate into high quality publications(Priyadharsini *et al.*, 2018; Vijayashree Priyadharsini, Smiline Girija and Paramasivam, 2018; Ramalingam, Selvi and Jayaseelan, 2019; Vijayashree Priyadharsini, 2019; Girija, Shankar and Larsson, 2020; Jayaseelan and Arumugam, 2020; Kumar, Girija and Priyadharsini, 2020; Mathivadani, Smiline and Priyadharsini, 2020; Paramasivam and Vijayashree Priyadharsini, 2020; Paramasivam, Priyadharsini and Raghunandhakumar, 2020; Paramasivam, Vijayashree Priyadharsini and Raghunandhakumar, 2020; Ushanthika *et al.*, 2021),(Reddy *et al.*, 2020; Teja and Ramesh, 2020; Barma *et al.*, 2021; Samuel, 2021; Samuel *et al.*, 2021). (Jayaseelan and Paramasivam, 2020) (Iswarya Jaisankar *et al.*, 2020) (Girija, 2021)

MATERIALS AND METHODS

Study is done in patients visiting the diabetic clinic OP department. The study was done in a period of 3 months. Eighty two patients were included in the study. Samples were collected from patients who are clinically proven diabetic and having non healing foot ulcers and elected for medical management. Samples were collected in patients who were not under any antibiotic therapy. Swabs and aspirates were collected after proper surface cleaning of the wound. Exudates from the deeper lesions were collected to avoid skin contaminations. samples were collected, labelled and transported to the microbiology lab immediately without any delay. Samples were stored at 4 deg.C till it was processed. Samples were then inoculated on Blood agar, Nutrient agar and MacConkey agar and incubated at 37 deg.C for 24 hours. All the organisms isolated were identified with standard microbiological protocol. The growth on Macconkey agar was identified by gram's staining for the presence of gram positive bacilli with spore and for chain formation. Those colonies are biochemically identified as Bacillus species and tested for their drug susceptibility. The results were tabulated and analysed.

RESULTS

S.NO	ORGANISMS	NUMBER OF ISOLATION
1	Enterococcus	19
2	Staph. epidermidis	15
3.	Staph. aureus	9
4.	Bacillus cereus	8
5.	Beta streptococcus	5
6.	Proteus sps	4
7.	Esch. coli	4
8.	Acinetobacter baumannii	3
9	Staph. citreus	2

10.	Micrococcus	2
11.	Gram negative non fermenter	1
12.	Candida albicans	1
13.	Klebsiella sps	1

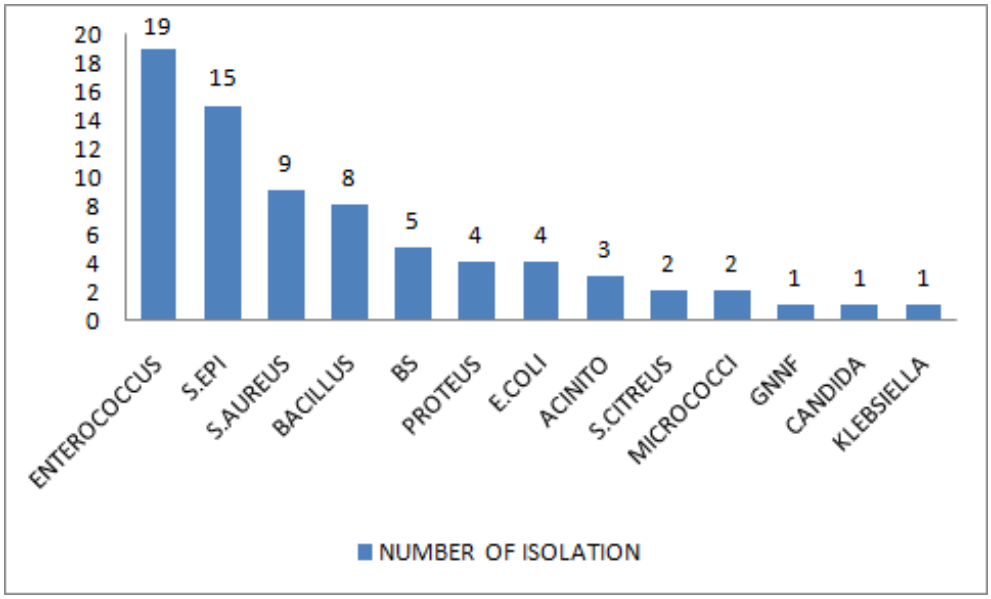

FIGURE 1: The above graph depicts the number of isolation of different sps from the wound samples

This study was conducted in diabetic patients who visited the OP clinic for regular checkup. Among the 82 samples collected from the patients, 13 different bacterial species were isolated.The figure 1 shows bacterial species isolated are Enterococcus 23%, Staphylococcus epidermidis18 %, Staphylococcus aureus 10%, Bacillus 9%, Beta streptococcus 5%, Proteus 4%, Escherichia coli 4%, Acinetobacter baumannii 3%, Staphylococcus citreus 2%, Micrococci 2%, Gram negative non-fermentative bacteria 1%, Candida albicans 1% and klebsiella sps 1%.

CHOICE OF ANTIBIOTIC

Among the drugs tested, the susceptibility to cefepime tazobactam is significant. Among the 8 isolates, cefepime tazobactam is sensitive in six isolates and 2 isolates shown resistance. In some isolates where multidrug resistance was seen, cefepime tazobactam was the only choice.

DISCUSSION

This study was conducted in diabetic patients who visited the OP clinic for regular checkup. Among the 82 samples collected from the patients, 13 different bacterial species were isolated.Bacillus species were isolated from 8 patients which is the fourth highest in number of the bacteria isolated. Bacillus species is not a common finding in a healthy patient.

As compared to this study,(Dezfulian *et al.*, 2012) this report describes a community–acquired and multi-drug-resistant S. aureus isolated from a diabetic patient . Among antibiotics used in this study, co-resistance to oxacillin and vancomycin is a critical issue because vancomycin is the first-line antimicrobial agent for the treatment of infection with MRSA.In this previous study,(Sekhar *et al.*, 2014)Prevalence showed Gram-negative bacteria was slightly more than Gram-positive bacteria in diabetic foot ulcers. This study

recommends doxycycline should be the treatment of choice for Gram-positive isolates and , cefoperazone, and meropenem should be considered for most of the Gram.negatives aerobes.

As compared to this study,(Aragón-Sánchez *et al.*, 2010)S. epidermidis was isolated from a bone biopsy and histopathological studies confirmed the diagnosis. S. epidermidis should be considered as a real pathogen, not only as a contaminant, in diabetic patients with foot osteomyelitis when the bacterium has been isolated from the bone.As we compared to this study,(MacDonald *et al.*, 2019)Diabetic foot ulcers contribute to 80% of non traumatic lower-extremity amputations and are associated with 5-year mortality rates of 43–55%. Most patients undertake initial pursuit of infected wound debridement followed by a six week course of intravenous (IV) antibiotic therapy.However other previous study,(Ramirez-Acuña *et al.*, 2019)Diabetic foot ulcers (DFU) pose a big burden on a patient's quality of life. Traditional DFU treatments should not be ceased, and their management according to guidelines needs to be retained. The use of PDGF shows an increase in the rate of wound healing.In this previous study,(Michelotti and Jonathan Bodansky, 2015)Cutaneous B. cereus skin infection is frequently related to open wounds, haematological malignancies and drug-induced neutropenia. There is a wide spectrum of its effects on the skin ranging from superficial necrosis to necrotising fasciitis and myonecrosis.

In our present study,we have collected samples of diabetic patient foot ulcers.Different OrganismsEnterococcus,bacillus,acineto,GNNF,micrococcus,candida,E.coli,S.aureus,S.epidermi

dis,S.citreus,Beta streptococcus, klebsiella,proteus.In our study,Bacillus sps were isolated from 8 patients which was the fourth highest in number of the bacteria isolated. Bacillus sp is not a common finding in a healthy patient.Among all,Isolation of bacillus species in the diabetic patients should be given due importance, because it is found as a single isolate in many diabetic patients with non healing foot ulcers.

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CONFLICT OF INTEREST

The author declares that there were no conflicts of interests in the present study.

CONTRIBUTORS

Harini Palani, principle investigator, Muralidharan N.P study designing, guidance and analysis of the results and interpretation.

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CONCLUSION

In this current study it is found that the bacillus species was the fourth highest in number of the bacteria isolated and are more common in diabetic patients with non-healing foot ulcers. Since it is an opportunistic infection, the predisposing factors should be given due importance. Isolation of bacillus species in the

diabetic patients should be given due importance, because it is found as a single isolate in many diabetic patients with non healing foot ulcers.

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