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Microbiological Detection of Some Pathogenic Bacteria in Diabetic Foot Ulcers of Type 1 and 2 Diabetic Mellitus Patients

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***Abstract*: Globally, diabetes mellitus (DM) is a serious health issue. Different aerobic bacteria, that can colonize DFUs including Gram positive cocci, such as *Staphylococcus aureus*, and *(beta-haemolytic) Streptococcus*. *Coagulation-negative Staphylococcus* (CNS), *Corynebacterium*, *Enterococcus*, and *Cutibacteria* are frequently found in ulcer cultures in clinical practice; Gram-negative bacteria account for about one third of DFIs. About 70 participants in all, including 29 individuals with type 1 diabetes and 41 patients with type 2 diabetes who have diabetic foot ulcers. The aims of study: is detecting of some aerobic of pathogenic bacteria in diabetic foot ulcers. Scarping of ulcers takes place after regular saline wound cleansing to eliminate surface pollutants, and then exudate is sampled. Pus, or discharges from the base of the ulcer, is then submitted to the laboratory as quickly as possible using an aseptic approach. Then, the bacteria were inoculated onto various culture media, such as MacConkey and Blood agar as enrichment media, and incubated aerobically at 37°C for 24 hours to isolate aerobic bacteria. The following day, the bacteria were checked for growth, and a pure colony was then prepared. According to morphological examination of bacteria in the culture, microscopic examination of slides, and particular cards of the automated VITEK2 system, isolated bacteria were identified. Results: based on the bacteriological profile of diabetic foot ulcers, *S. aureus* among the gram-positive isolates and *E. coli* among the gram-negative isolates were the predominant pathogens. In recommendations, further studies are needed for isolation and identification of another microorganism such as anaerobic bacteria, fungi and virus.**

Keywords— Diabetic foot ulcers, Diabetic mellitus, Pathogenic bacteria, VITEK2 system.

I. Introduction

Globally, diabetes mellitus (DM) is a serious health issue. High blood glucose levels brought on by inadequate insulin synthesis or action indicate this metabolic illness. An inflammatory reaction is brought on by the immunological response to elevated blood glucose levels as well as the presence of inflammatory mediators made by adipocytes and macrophages in adipose tissue. Low and persistent inflammation harms pancreatic beta cells, which reduces the amount of insulin produced and raises blood sugar levels. Diabetes hyperglycemia is hypothesized to lead to immune system malfunction, which makes it difficult for diabetic people to stop the spread of invasive infections. Patients with diabetes are known to be more vulnerable to infections as a result [1,2].

Aerobes and anaerobes frequently cause diabetic foot wounds to become infected; this additional ischemia, necrosis, and progressive gangrene eventually lead to amputation [3]. Patients with diabetic foot have reduced microvascular circulation, which restricts phagocyte availability and encourages the development of infection. A variety of microorganisms have been isolated from diabetic foot infections in recent research, which illustrates the persistent, open nature and anatomical location of these infections. DFIs can be mono- or polymicrobial, with polymicrobial l frequently present in persistent infections previously treated with antibiotics [4].

Human skin is home to a wide variety of pathogenic and non-pathogenic microorganisms. An infected DFU often contains three to five different types of bacteria, including the following: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Corynebacterium* species, gram-positive aerobes; gram-positive anaerobes; *Enterococcus* species; *Streptococcus* species; *Peptostreptococcus* species; gram-negative aerobes; *Proteus mirabilis*; *Escherichia coli*; *Bacteroides* species; and fungi (*Candida* species). Gram-negative pathogens, of which *Pseudomonas aeruginos* is the most prevalent, are more widespread in low-income countries. *Streptococcus* and gram-positive cocci, particularly *Staphylococci*, are frequently isolated [5,6].

Some studies have shown that the presence of anaerobic organisms is related to deeper DFIs. Diabetes patients are more prone to foot infections due to neuropathy, vascular dysfunction, and lowered neutrophil activity. In between 30 and 50 percent of diabetic patients, peripheral neuropathy plays a significant role in developing a foot infection [7,8].

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Anaerobes are present in a smaller minority of DFIs, while gram-negative bacteria account for about one third of DFIs. Impairments in innate and adaptive immune responses within the hyperglycemic milieu have been associated with increased frequency and severity of bacterial infections in diabetes. Bacterial infections and diabetes have a reciprocal link in that diabetes make people more vulnerable to bacterial infections and their consequences [9, 10]. The aims of study is detecting of some aerobic of pathogenic bacteria in diabetic foot ulcers [DFUs] and confirmative that by VITEK2 compact system.

II. MATERIALS AND METHODS

A.Ethical approval

Before collecting any samples, all of the study participants' verbal consent was obtained after they had been fully told of the study's objectives. The Karbala Medical College's Scientific Council gave its approval to this study.

B.Experimental design

A case control study including 70 people was carried out during a six-month period between August 2022 and January 2023. Of them, 29 patients with type 1diabetes and 41 patients with type 2 diabetes had diabetic foot ulcers. After the area of the diabetic foot ulcers had been cleaned with normal saline to remove any surface contaminants, the ulcers were scarped before being sampled for exudate; pus or discharges were then taken under aseptic conditions and sent to the laboratory as soon as possible. Then, the bacteria were inoculated onto various culture media, such as MacConkey and Blood agar as enrichment media, and incubated aerobically at 37°C for 24 hours to isolate aerobic bacteria. The following day, the bacteria were checked for growth, and a pure colony was then prepared. According to morphological, microscopic, and particular cards of the automated VITEK2 system, isolated bacteria were identified.

III. RESULTS

A. Isolation of Pathogenic Bacteria of diabetic foot ulcer (DFU):

Out of 70 samples were taken from individuals with diabetic foot ulcers; 67 (95.71%) of those samples tested positive for microbial growth, and 3 (4.29%) of the samples had no growth, as shown in Table 1.

Table (1): Characteristics of aerobic bacterial culture.

Gram staining property	Type of Bacteria	Frequency	Percentage
Gram-Negative Bacteria	*E. coli*	15	36.58 %
	K. pneumoniae	11	26.83 %
	P.mirabilis	7	17.07 %
	P.aeruginosa	4	9.76 %
	A.baumannii	3	7.32 %
	Morganella morganii	1	2.44 %
Total		**41**	**61.19 %**
Gram-Positive Bacteria	*S. aureus*	20	76.93%
	streptococcus group B	4	15.38%
	Enterococcus spp.	2	7.69%
Total		**26**	**38.81%**
Number of isolated Bacteria	Mono-infection	**29**	43.28%
	Mono microbial infection with gram-positive bacteria	10	34.48%
	Mono microbial infection with gram-negative bacteria	19	65.52%
	Poly-microbial infection	**38**	56.72%

Of 67 positive cultures, 29 patients had monomicrobial infections and 38 had polymicrobial infections. *E. coli*, which exhibits a high percentage of 15(36.58%), followed by *K. pneumoniae* 11(26.83%), revealed a high rate of 41(61.19%) in a culturing inquiry based on morphological and VITEK compact system results. Then came P. mirabilis 7 (17.07%), *P. aeruginosa* (4 (9.76%), *A. baumannii* (3 (7.32%), and *Morganella morganii* 1 (2.44%), in that order. At the same time, *S. aureus* was the most isolated bacterium in this study with a percentage 20(76.93%), followed by Streptococcus group B 4(15.38%) and *Enterococcus* 2(7.69%), Gram-positive bacteria recorded 26(38.81%), as shown in (table 1 and figure 1).

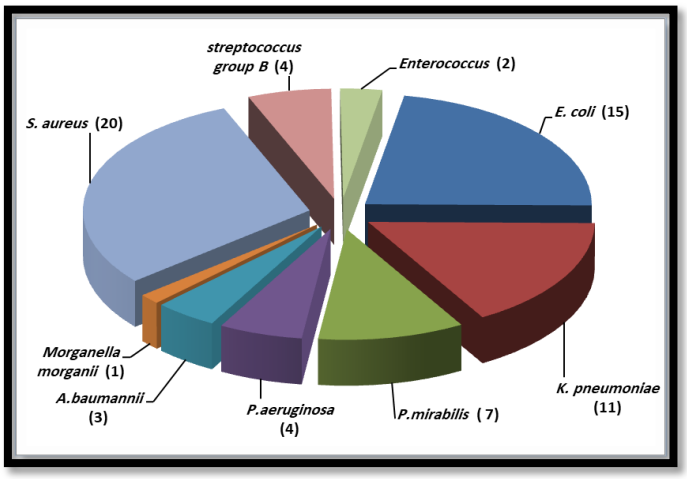

Figure (1): Distribution of Bacterial Species isolates.

B. Distribution of Isolated Bacteria according to gender and participant groups:

Bacterial types isolated according to test diabetic population groups are shown in table (3.20). The most frequent pathogen isolated from all diabetic groups was *S. aureus* (20 isolates) with male predominance in type 1 DM, followed by *Escherichia coli* (15 isolates) with female predominance in type 2 DM. Also, *Klebsiella pneumonia* (11 isolates) with male predominance of type 1 DM percentage (54.55%). *Proteus mirabilis* was the fourth microorganism isolated (7 isolates) in this study with (85.71%) percentage in type 1 DM. *Pseudomonas aeruginosa* uncommon diabetic pathogen were isolated from diabetics group (4 isolates) with approximately prevalence in type1DM, as well as *streptococcus group B* uncommon diabetic pathogen were isolated from diabetics group (4 isolates) with equal prevalence in T1DM and T2DM. Also, least isolated diabetic pathogens were; *Enterobacter* spp. (2 isolate) isolated from both T1DM and T2DM and *Morganella morganii* (1 isolates) in T2DM only. *Acinetobacter baumanni* (3 isolates) in T1DM females.

Table (2): The results of DFU culturing in investigated groups.

Bacterial Species		T1DM male	T1DM female	T2DM male	T2DM female	Total
S. aureus	NO.	8	3	5	4	20
	% within Bacterial type	40.0 %	15.0 %	25.0 %	20.0 %	100.0%
E. coli	NO.	0	5	3	7	15
	% within Bacterial type	0.0%	33.33 %	20.0 %	46.67 %	100.0%
K. pneumoniae	NO.	6	0	2	3	11
	% within Bacterial type	54.55 %	0.0%	18.18 %	27.27 %	100.0%
P.mirabilis	NO.	2	4	0	1	7
	% within Bacterial type	28.57 %	57.14 %	0.0%	14.29 %	100.0%
P.aeruginosa	NO.	0	3	0	1	4
	% within Bacterial type	0.0%	75.0 %	0.0%	25.0 %	100.0%
streptococcus group B	NO.	1	1	2	0	4
	% within Bacterial type	25.0 %	25.0 %	50.0 %	0.0%	100.0%
A.baumannii	NO.	0	2	1	0	3
	% within Bacterial type	0.0%	66.67 %	33.33%	0.0%	100.0%
Enterococcus	NO.	0	1	1	0	2
	% within Bacterial type	0.0%	50.0 %	50.0 %	0.0%	100.0%
Morganella morganii	NO.	0	0	0	1	1
	% within Bacterial type	0.0%	0.0%	0.0%	100.0%	100.0%
No growth	NO.	1	0	0	2	3
	% within Bacterial type	33.33 %	0.0%	0.0%	66.67%	100.0%
Total	NO.	18	19	14	19	70
	% within Bacterial type	25.72 %	27.14 %	20.0 %	27.14 %	100.0%

The Table 2 shows the positive and negative diabetic cultures in investigated groups, with no growth also reported accounting for 4.29% of the overall population under research and the majority having T2DM.

C. The age groups Distribution based on results of bacterial growth:

The present study findings of bacterial growth showed that all age groups have bacterial growth with age level of (≥ 60) years were the most associated with infection as recorded in table (3.21) both in T1DM and T2DM, while, some persons in age groups (30 - 39 Years) showed negative bacterial growth results in T1DM. Besides, the (<20+50-59Years) age groups revealed negative growth results in T2DM patients. However, the data of bacterial growth analyzed compared to the age groups revealed there were a significant variation between all the age intervals of study patients as in Table (3).

Table (3): Distribution of age groups based on results of bacterial growth.

Variable	Categories	Bacterial Growth				P-Value
		T1DM		T2DM		
		Positive	Negative	Positive	Negative	
Age	< 20	3(10.71%)	0 (0.0%)	5 (12.82%)	1(50.0%)	0.01 Sig.
	20 - 29 Years	7(25.0%)	0 (0.0%)	8 (20.51%)	0 (0.0%)	
	30 - 39 Years	2(7.15%)	1 (100%)	2 (5.13%)	0 (0.0%)	
	40 - 49 Years	3(10.71%)	0 (0.0%)	4 (10.26%)	0 (0.0%)	
	50-59 Years	5(17.86%)	0 (0.0%)	11(28.21%)	1(50.0%)	
	≥ 60	8(28.57%)	0 (0.0%)	9 (23.07%)	0 (0.0%)	
Total		28 (100.0%)	1(100.0%)	39 (100.0%)	2(100.0%)	

Chi-square test, significant difference at $P \leq 0.01$.

IV. DISCUSSION

Some samples from the infected area demonstrated positive culture growth, while others revealed negative culture growth. If the wounds were not infected at the time of the study or the antibiotics were effective, the negative growth could be due to the other infectious agents, such as anaerobic bacteria, fungi, and viruses (11), or it could be due to other factors [12]. Initial characterization of bacterial isolates derived from clinical specimens was done using cultural morphology. Culture results revealed pink-colored colonies on MacConkey agar with bile salt precipitating around the colonies; these are E. coli isolates. These findings are diagnostic for *E. coli* [13, 14]. Vitek2

was used for additional confirmation. In study by [15] reported similar outcomes.

Pink lactose fermenter mucoid colonies on MacConkey agar were used to identify *K. pneumoniae [16]*. Vitek2 is used for additional confirmation, which yields comparable outcomes. Similar results were also noted by [17].

On mannitol, *S.aureus* fermenters appeared as yellow colonies, but *S. aureus* was not the only *Staphylococcus species* that was mannitol-positive. On the mannitol salt agar, add yellow colonies surrounded by yellow regions as well [18]. Raheema made similar discoveries (19) and blood hemolysis in blood agar [13]. Gram negative bacteria were identified in 64.5% of the DFU patients in the study by [20], while gram positive bacteria were isolated in 35.5% of the patients. The current investigation has noticed a prevalence of gram negative bacteria over gram negative bacteria, which is consistent with [21, 22].

Variations in environmental factors, such as sanitary practices, such as the use of water for perianal wash (ablution) after defecation, which frequently causes contamination of hands with fecal flora that is rich in Gram-negative bacteria, have been suggested as a possible explanation for the difference in the nature of microbes infecting the diabetic foot infection [23]. Similar to [24,25], the most common gram negative and positive bacteria isolated in our study were *E. coli* and *S. aureus*, followed by *K. pneumoniae and proteus.*

S. aureus was the most common gram-positive bacteria and *E. coli* was the most common gram-negative bacterium responsible for DFUs, according to a similar finding by [22,26]. Additionally, similar outcomes in India and Iraq, respectively, were reported by [27, 28]. On the other hand, a study from Pakistan found that the most common bacteria were *Staphylococcus aureus* (25%), *Pseudomonas aeruginosa* (18.18%), and *Escherichia coli* (16.16%) [29]. The diversity in sample collection techniques, geographic locations, treatment modalities, and illness severity may all have an impact on the bacterial profiles recovered from individuals with DFUs [30].

Regarding of mono-microbial infections versus poly-microbial infections, the current study showed that 56.72% of DFU patients had poly-microbial infections (the isolation of two or more bacteria), In contrast studies [24, 31] revealed that 83% and 75% of patients, respectively, had poly-microbial infections. In contrast, a study by [32], which showed that 48.57% of samples revealed a single organism, a study by [32], which found that 28.57% of samples revealed two organisms, and a study by [33] which discovered that 62.2% of wound cultures had monomicrobial growth and 27.1% had polymicrobial development. These outcomes may be explained based on the length of DFIs, the severity of the ulcer, and empirical antimicrobial medicines. Additionally, monomicrobial etiologies are common in the early stages of diabetic foot infections, but as time goes on, polymicrobial infections become more prominent [34].

Compared to poly-bacterial infections, mono-bacterial infections are simpler to treat [35]. Because these bacteria produce virulence factors that increase inflammation, form biofilms that prevent antibiotics from working, and work in concert to create a chronic wound infection, poly-bacterial infections are challenging to treat [36].

According to the severity of the infection and foot involvement, the microbiological yield of diabetic foot wounds varies. Gram-positive aerobic cocci are typically the secondary cause of the superficial diabetic foot infections. However, deep, persistent, or ulcers that have been treated with antibiotics in the past are more likely to be polymicrobial. Along with the typical diabetic foot pathogens, such wounds may also contain *Enterococci, Enterobacteriaceae, Pseudomonas aeruginosa, and anaerobes* [37]. However, the distribution of Isolated Bacteria by participant group and gender revealed the following:

Gram-positive isolates (38.81%) outnumbered gram-negative isolates (61.19%) by a margin of 41/67. This result is consistent with a previous investigation conducted at the same study site, where 88.55% (54/61) of gram-negative bacteria were identified vs 7% (11.47%) of gram-positive bacteria [15]. Similarly, a study from Egypt found 56% gram-negative and 27.7% gram positives, while a study from northeast India found 79% gram-positive and 21% gram negatives [38].

S. aureus was the most common isolate in the current investigation (76.33%), in contrast to a recent study in Ethiopia that found *Klebsiella* species to be the most common bacteria (23.9%), followed by *Proteus* species (18.47%) (17/92) [39]. *P. mirabilis (16.8%) is the most prevalent isolate in Egypt [40], Pseudomonas species* (15.6%) in Saudi Arabia (n = 134) (27), and *Pseudomonas* species (18.8%) in South America [41].

Similarly in agreement with studies in Kenya 17.5% (14/80 [42], and in Iran 28% (n=92) [43]. A recent study also reported that the growth rate was 81.7% (98/120), and no growth of 22% (18.34%), respectively [38] in accordance with our study with negative growth results was 3(4.29%) and positive growth results 67 (95.71%).

This discrepancy could be a result of the various research' varying sample sizes as well as other distinctive features of each study site. This demonstrates that the main bacteria causing DFU infections may change depending on the environment.

In the study sites, DFU infection is brought on by both gram-positive and gram-negative aerobic pathogenic bacteria. According to the current study, this infection can progress to osteomyelitis and even amputation of the limbs.

V. CONCLUSION

Based on the bacteriological profile of diabetic foot ulcers, ***S. aureus*** among the gram-positive isolates and ***E. coli*** among the gram-negative isolates were the predominant pathogens**.** In recommendations, further studies are needed for isolation and identification of another microorganism such as anaerobic bacteria, fungi and virus.

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