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Estimation rate of S. aureus and MRSA carriage in diabetic type-2 and effect of Aspergillus Gliotoxin on bacterial carriage in type-2 diabetes

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ABSTRACT

Diabetes mellitus is a serious public health problem, *S. aureus* and *MRSA* are the most common bacteria isolated from ulceration of diabetic patients. The aim of the study was to estimation rate of *S. aureus* and *MRSA* carrier in diabetes type-2 and determine the antimicrobial effect of Gliotoxin on the previous bacterial carriage in type-2 diabetes. The study was conducted on 450 diabetics' patients, attended the outpatients clinic in Baquba Teaching Hospital, their ages ranged from 15-65years, with mean age of 36.15, and 150 healthy group, who were randomly selected, during the period from May 2016 to April 2017, patients were classified into two groups according to the type of diabetes, group1 included: 184 with type1-diabetes, and group2: included 266 with type2-diabetes, 97 patients with foot ulcers. Swabs were taken from anterior nares, toe and axillae for each diabetic patient type-2, identified based on standard bacteriological methods. Using the Kirby -Bauer method for detection the antibacterial effect of Gliotoxin. The results showed rates of the bacterial carriage in anterior nares of type-2diabetic patients without complications were (11.4%), (4.4%), respectively for *S. aureus* and *MRSA*, in type-2 diabetes with complications were (8.6%), (2.1%) respectively for *S. aureus* and *MRSA*. In the toe of type-2diabetic patients without complications were (6.7%), (2.5%) respectively for *S. aureus* and *MRSA*. In with complications were (9.2%), (5%) respectively. In the axillae of diabetic patients, type-2 without complications was (5.5%), (4.5%) respectively. *Aspergillus fumigatus* Gliotoxin was effective against bacterial carriage in diabetes type-2 with the foot ulcer, for *S. aureus* inhibition diameter was (20.50, 16.40, 12.20) mm for different concentrations of Gliotoxin, to *MRSA* was (8.25, 6.1, 4.20) mm. Increasing rate of *S. aureus* and *MRSA* carrier in diabetic patient's type-2 which lead to a significantly increased risk of bacterial infections. Gliotoxin was effective as an antibacterial agent against *S. aureus* and *MRSA* in type-2diabetis with the foot ulcer.

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INTRODUCTION

Diabetes mellitus is a serious public health problem, that is rapidly expanding worldwide (Shaw J *et al.,* 2009) Infections with diabetes are one of the leading causes of human morbidity and mortality. It represents a severe complication of diabetes and the most common cause of diabetes associated hospital stagnation (Lavery L *et al.,* 2007). Diabetes is a chronic infection occurs when pancreas yield in sufficiently amount of insulin and when the body cannot efficiently use the insulin (Prompers L *et al.,* 2008), result in several abnormalities of the host

Table 1: The rate of positive and negative bacterial growth from different regions in type -2 diabetes and healthy group

Study groups	Positive result N (%)	Negative result N (%)	Total N (%)
Type-1diabetes	136(22.5)	48(8)	184(30.6)
Type-2diabetes without complications	140(23.17)	29 (4.8)	169(28.1)
Type-2diabetes with complications	77(12.74)	20(3.6)	97 (16.2)
Healthy group	61(10.09)	93(15.39)	154 (25.4)
Total	414(69)	190(31)	604(100)

Table 2: The rate of positive bacterial growth in different regions in type - 2 diabetes and healthy group

Type-2diabetes 266						Healthygroup150		
Without complications 140			With complications 77			61		
Anterior nares	Toe	Axillae	Anterior nares	Toe	Axillae	Anterior nares	Toe	Axillae
62	36	42	34	36	7	27	16	18

Table 3: The rate of S. aureus, MRSA carriage in anterior nares of diabetics patients type-2 and healthy group

Bacteria Total no.185(100)	Type-2 diabetes without complication 62(33.5)	Type-2 diabetes with complication 34(18.5)	Healthy group 27(14.5)	P value
Staphylococcus aureus	21(11.4)	16(8.6)	9(4.8)	0.05
Methicillin Resistance S. aureus	8(4.4)	4(2.1)	1(0.5)	0.06
Other types of bacteria	29(15.6)	12(6.4)	16(8.6)	0.01
P value	0.01	0.05	0.05	

Table 4: The rate of S. aureus, MRSA and carriage in Toe of diabetics patients type-2 and healthy group

Bacteria Total no. 120(100)	Type-2 diabetes without complication 36(30)	Type-2 diabetes with complication 36(30)	Healthy group 16(13.4)	P value
Staphylococcus aureus	8(6.7)	11(9.2)	4(3.3)	0.11
Methicillin Resistance S. aureus	3(2.5)	6(5)	1(0.8)	0.13
Other types of bacteria	14 (11.6)	5(4.2)	6 (5)	0.06
P Value	0.06	0.05	0.09	

defense system might result in a higher risk of infections, from these abnormalities immunological impairments such as phagocytosis, impaired migration, intracellular killing and chemotaxis in leukocytes (Lipsk BA *et al.,* 2012). *S. aureus* and *P. aeruginosa* are the most common bacteria isolated from ulceration of diabetic patients (Thomas GW *et al.,* 2009). The presence of *S. aureus* carriage increased the risk of subsequent hospitalization with an *S. aureus* infection by over five-fold (Jeffcott WG *et al.,* 2008). Chronic leg ulcers affect (1–2%) of the general population and are related to increased morbidity and health costs (Munckhof WJ *et al.,* 2009). *S. aureus* was the most frequent pathogen (25.6%) in diabetic patients, and a high proportion of *S. aureus* isolates were *MRSA*(63.4%)(Shao-Hua W *et al.,* 2010). Almost two-thirds of *S. aureus* isolates were *MRSA* in diabetic patients with foot ulcers (Hartmann H *et al.,* 2004; Tentolouris N *et al.,* 2006). The pathology resulting from *S. aureus* and *MRSA* infections is of great importance due to the throughout nature, growing resistance to antimicrobial agents, increasing prevalence and ability to delay healing (Naimi TS *et al.,* 2003; Keen EF *et al.,* 2010). Multiple studies have also detected the presence of bacteria and the polymicrobial nature of chronic, non-healing wounds, and the frequency of *S. aureus* and *MRSA* infections has to be high (Frank D *et al.,* 2009; Gontcharova V *et al.,* 2010). *S. aureus* can produce biofilms and to express antimicrobial resistance and variety of virulence factors

Table 5: The rate of *S. aureus, MRSA* and carriage in Axillae of diabetics patients type-2 and healthy group

Bacteria Total no.109	Type-2 diabetes without complication 42(38.5)	Type-2 diabetes with complication 7(6.4)	Healthy group 18(16.6)	P Value
Staphylococcus aureus	(5.5) 6	2(1.8)	3 (2.7)	0.21
Methicillin resistance S. aureus	5(4.5)	1(0.9)	0.9) (1	0.17
Other types of bacteria	18(16.5)	3(2.8)	9(8.5)	0.05
P value	0.05	1(0.9)	0.07	

Table 6: The rate of the bacterial carriage in the healthy control group (not diabetic)

Bacteria Total no.154(100)	Anterior nares 63(40.9)	Toe 37(24)	Axillae 54(35.1)	P value
Staphylococcus aureus	9(5.8)	4(2.6)	3(1.9)	0.06
Methicillin resistance S. aureus	1(0.6)	1(0.6)	1(0.6)	0
Other types of bacteria	16(10.4)	6(3.9)	9(5.8)	0.05
No growth	36(23.4)	21(13.6)	36(23.4)	0.063
P value	0.03	0.04	0.03	

Table 7: Inhibition zone (mm) of Gliotoxin on *S.aureus* and MRSA carrier in type-2 diabetes with foot ulcers

Bacteria	2mg/ml	4mg/ml	6mg/ml	8mg/ml
Staphylococcus aureus	20.50	16.40	12.20	16.o
Methicillin resistance S.aureus	8.25	6.1	4.20	8.2

Table 8: Values of MIC and MBC of *Aspergillus fumigatus* Gliotoxin on bacteria isolated from type-2 diabetic patients with foot ulcers

Bacteria	MIC	MBC
Staphylococcus aureus	2	4
Methicillin resistance S.aureus	4	6

such as surface proteins, endotoxins, and exoenzymes which enhances its virulence especially *MRSA* (Frazil M *et al.,* 2009).

Colonization of the anterior nares is a significant risk factor for infection and cross-sectional surveys of healthy adult populations have reported nasal carriage rates that are typically (20-55%) (Belkum A. Vanbrugh H., 1997). Diabetes has been associated with increased *S. aureus* nasal carriage in some studies (Ahluwalia A *et al.,* 2000; Amer A *et al.,* 2006). But not others (Yoho LY *et al.,* 2014; Duran N *et al.,* 2006; Julie H *et al.,* 2015). The increased carriage in patients with diabetes may reflect an association between diabetes and risk factors found in the general population such as bacterial carriers among diabetic patients (Van BA *et al.,* 2009). Mycotoxins are active secondary metabolites produced by some filamentous fungi or moulds under suitable temperature and humidity conditions causing severe risks for human and animal health. Aspergillus fumigatus is known to produce various mycotoxins including Gliotoxin, that is an alkaloid with low molecular size, and possess many immunosuppressive activities (Erman D *et al.,* 1987). Anti microbicidal activity cytokine release by leukocytes and T-lymphocyte-mediated cytotoxicity it is genotoxic and also causes apoptosis in macrophages. That is biologically active secondary metabolites causing severe risks for human and animal health (Pardo J *et al.,* 2006).

PATIENTS & METHODS

The study was conducted on 450 diabetic patients ascertained from a variety of sources, attended the outpatients clinic in Baquba Teaching Hospital, their ages ranged from 15-65years, with mean age of 36.15 (±9years), and 150 healthy non diabetic as a control group who were randomly selected, during the period from May 2016 to April 2017 in Baquba city in Iraq, patients were classified into two groups according to type of diabetes, group1 included:184 with type 1-diabetes, and group 2: included 266 with type2-diabetes, 97patients with foot ulcer. Swabs were taken from anterior nares, toe and axillae for each diabetic patient type-2, the specimens were inoculated on Blood agar and Mannitol salt agar plates by streaking methods for isolation of aerobic bacteria, incubated aerobically at 37C for 48 hour, the isolates were identified based on standard bacteriological methods (Collee

JG *et al.,* 2006). Isolates of *S. aureus* were inoculated on Muller–Hinton agar to determine Methicillin resistance *S. aureus* by using Cefoxitin 30µg and Oxacillin 1µg, *MRSA* was considered positive when were ≥13mm as susceptible, regarding using Oxacillin, and considered positive when were ≥20mm as susceptible when using Cefoxitin(Andrews JM,2008). Using Kirby-Bauer method for detection antibacterial effect of *Aspergillus fumigatus* Gliotoxin (GT) that performed and extraction with slight modifications with 50 ml of chloroform and extracts by thin layer chromatography technique (TLC) according to (Kosalec I *et al.,* 2005; Belkacemi L *et al.,* 1999).

Statistical analysis: Single Sample Z Score: This tool calculates the z score of the mean of a single sample. It can be used to make a judgment about whether the sample differs significantly on some axis from the population from which it was originally drawn.

RESULTS AND DISCUSSION

The study was conducted on 450 diabetic patients ascertained from a variety of sources, attended the outpatients clinic in Baquba Teaching Hospital, their ages ranged from 15-65years, with mean age of 36.15 (± 9years), and 150 healthy non diabetic as control group who were randomly selected, during the period from May 2016 to April 2017 in Baquba city in Iraq. Patients were classified into two groups according to the type of diabetes, group1 included: 184 with type 1-diabetes, and group2: included 266 with type2-diabetes, 97 patients with foot ulcers. Swabs were taken from anterior nares, toe and axillae for each patient with type-2diabetes for bacterial detection carriers. The patient was considered to be an *S. aureus* and *MRSA* carrier when the positive bacterial swab was cultured from the anterior nares, axillae and toe on at least two separate occasions. The heavy carriage was defined as a positive direct culture. A negative culture was defined as when either directly or enrichment culture could not isolate the microorganism. The results as shown in table-1, Positive bacterial growth in type-2 diabetes without complications were140 (23.17%), in type-2 diabetes with complications were77 (12.74%), and61 (10.09%) in the healthy group. Table-2 explains the distribution of positive bacterial growth from different regions in type-2 diabetes and healthy group's. Rates of bacterial carriage in anterior nares of type-2diabetes without complications were 21(11.4%) and 4(2.1%) respectively for *S. aureus*, *MRSA* and29 (15.6%) for other types of bacteria, rates of bacterial carriage in type-2 diabetes with complications were16 (8.6%), 4(2.1%), 12 (6.4%) respectively for *S. aureus*, *MRSA*, and other type of bacteria. The rate of positive growth of *S. aureus* carriage in healthy group were 9 (4.8%) and 1(0.5%), 16(8.6%) for *MRSA*, another type of bacteria as explain in Table -3 and Figure -1. Rates of bacterial carriage in toe of diabetic patients type-2diabetes without complications were 8(6.7%), 3(2.5%), 14(11.6%) respectively for *S. aureus*, *MRSA*, and other types of bacteria, Rates of bacterial carriage in type-2 diabetes with complications were 11(9.2%), 6(5%), 5(4.2%) respectively for *S. aureus*, *MRSA* and another type of bacteria. The rate of positive growth of *S. aureus* carriage in healthy group were4 (3.3%), and 1(0.8%), 6(5%)for each *MRSA* and another type of bacteria as explain in Table-4 and Figure-1. Rates of bacterial carriage in axillae of diabetic patients type-2diabetes without complications were 6(5.5%), 5(4.5%), 18(16.5%) respectively for *S. aureus*, *MRSA* and other types of bacteria, Rate of bacterial carriage in type-2 diabetes with complications were2 (1.8%), 1(0.9%), 3(2.8%) respectively for *S. aureus, MRSA* and other type of bacteria. In the healthy group were 3(2.7%), 1(0.9%), 9(8.5%) respectively for *S.aureus MRSA*, and another type of bacteria as explain in Table -5 and Figure-1.Rates of the bacterial carriage in healthy control group explain in Table-6. The study showed the *Aspergillus fumigatus* Gliotoxin was effective against *S. aureus* and *MRSA* carrier in diabetics patients type-2 with foot ulcer as in Table-7, for *S. aureus* inhibition diameter was (20.50, 16.40, 12.20) mm for different concentrations of Gliotoxin, to *MRSA* was (8.25, 6.1, 4.20) mm. Its antibacterial effect was directly proportional with its concentration According to the values of MIC & MBC as in Table-8, the results revealed that the Gliotoxin of *Aspergillus fumigatus* was more effective as an antibacterial agent against *S. aureus* and *MRSA*. Diabetes and its complications were chronic and non-healing due to several factors such as bacteria were the predominant pathogens in the diabetic infections especially ulcers, our study revealed the high prevalence rate of bacterial carriage was observed especially in type-2 diabetes with a foot ulcer. *S. aureus* was the most common bacteria of community and hospital-acquired infections that can cause morbidity and mortality, *S. aureus* and MRSA nasal carriage varied between diabetic patients but increased in type-2 diabetes. Increase risk of *S. aureus* and multidrug resistance bacteria especially MRSA carriage in patients with diabetes may association with many factors such as obesity, old age, inappropriate previous antibiotics treatment and prolonged hospital stay, and state for several months, patients with *MRSA* frequently colonisation at readmission (Shankar *et al.,* 2005). Almost 50 % of bacterial isolates from diabetic patients with foot ulcers were *S. aureus* (Tentolouris N *et al.,*2006) and two-thirds (63.4%) of *S. aureus* isolates were

MRSA, 25-28 *MRSA* nasal carriers had previous hospital admissions for medical problems such as chronic renal failure and diabetic ulcers. The result of this study revealed that Gliotoxin was relatively effective as an antibacterial agent, and *S. aureus* was more sensitive than MRSA (Suen Y *et al.,* 2001). Gliotoxin was observed that it has a very good antibacterial property against bacteria *E. coli*, *Proteus sp., Pseudomonas sp., Micrococcus sp.*in many studies (Neeraj M and Behal K, 2010; Jayaveera KN *et al.,* 2010). Their mechanism of toxicity can be due to the inhibition of protein synthesis, inhibition of DNA synthesis or inhibition of the mitochondrial electron transport system.

CONCLUSION

Increasing rate of *S. aureus* and *MRSA* carrier in diabetic's patient type-2 which lead to a significantly increased risk of bacterial infections. Gliotoxin was effective as an antibacterial agent against *S. aureus* and MRSA in type-2diabetis with a foot ulcer.

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