University of Thi-Qar Journal of Science (UTJsci)
E-ISSN: 2709-0256, P-ISSN: 1991-8690, Vol. 11, No. 1, Jun. 2024

Screening and Isolation of Protease Producing Bacteria from Diabetic Foot Ulcers

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Received: 2024-03-01, Revised: 2024-03-13, Accepted: 2024-03-15, Published: 2024-06-19

Abstract*—This study was conducted to detect bacterial causative pathogens in patients with diabetes foot ulcer infections and determine the protease enzyme. Samples were collected from 75 swabs were collected from patients suffering from diabetic foot ulcers referred to The Diabetic Center /Thi-Qar province. Data collected from patients included: age, gender and Period of infection. The results revealed that positive bacterial growth appeared in 54 (72 %) of diabetic foot ulcer specimen, and the most common isolates were *Enterobacter cloaca* 15(27.77%), followed *by proteus mirabilis* 12 (22.22%), *Staphylococcus aureus* 10 (18.51%), *Escherichia coli* 8 (14.81%), for each one *Klebsiella pneumonia, Pseudomonas aeruginosa* and *Streptococcus pyogens* 3(0.84%). The results of the Protease enzyme test were performed for all 54 isolates to determine the variable ability of these bacterial species isolated from didactic foot ulcers to produce this enzyme. The highest percentage production protease enzyme was 100 % for *Pseudomonas aeruginosa, Staphylococcus aureus* and 76.92% for *proteus mirabilis*, while the isolates showed a low production of 20.0 % for *Enterobacter cloaca.

***Keywords*— Diabetes Foot Ulcer Infections, Protease Enzyme.**

I. INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia due to impaired insulin secretion or defective action. Chronic hyperglycemia is linked to long-term microvascular complications in the eyes, kidneys, and nerves and an increased risk of cardiovascular disease [1-2]

Diabetic foot ulcers (DFUs) are ulcers of the foot and destruction of deep tissues in diabetic patients due to vascular and neuropathic lesions of the lower extremities [3]. The progression of these wounds from superficial to debilitating infections be facilitated by impaired defense mechanisms of the body and delayed treatment. The subcutaneous tissue infections can spread to the deeper structures, which will ultimately progress into complications, including gangrenous changes and also amputations. Poor control of DM puts a patient at higher risk of skin infections as being in a state of hyperglycemia impairs the efficiency of the body's immune or defense mechanism. Diabetic foot infections (DFIs) are complex, rampant and costly ramifications of DM. These infections account for the majority of DM-related hospitalizations other than non-traumatic amputations. Few have studied the relationship between a number of organisms identified and the types of infections that occur from it. They have suggested that gram-positive bacteria predominate in acute diabetic foot infections and that gram-negative bacteria and anaerobes may be involved in chronic infections. Bacterial involvement in diabetic foot ulcers may be polymicrobial. Enzymes known as proteases are responsible for the breakage of peptide bonds. They are found in all living things–and perform a wide range of physiological tasks, from broad protein breakdown to more specialized and controlled activities, including hormone activation, blood coagulation, and the passage of secretory proteins across membranes. Proteases can be classified as endopeptidases, which break internal peptide bonds, or exopeptidases, whose activities are controlled by the amino- or carboxyl terminal of proteins. Four classes of endopeptidases, commonly known as proteinases, exist Aspartic, serine, cysteine, or metallo- (often zinc) proteases [4].

Proteases are the class of enzymes which occupy key position key their applications in both physiological and commercial fields. Proteases are also known as peptidyl – peptide hydrolases and are industrially useful enzymes that catalyze the hydrolysis of peptide bonds from protein molecules. Proteases constitute 50-65% of the global industrial enzyme market, most of which are alkaline protease [5-6]. The most essential proteins are those made by bacteria because their characteristics can easily be altered through genetic modification to fit a variety of uses. Microorganisms are a great source of enzymes because of their wide range of biochemical activities and genetic modification susceptibility [7]. Because microbial proteolytic enzymes are used in many different sectors, research on them is developing quickly. Since microbial

https://doi.org/10.32792/utq/utjsci/v11i1.1183

proteolytic enzymes have nearly all the properties needed for biotechnological applications, they are chosen over plant and animal proteolytic enzymes [8].

The present study aimed to determine the ability of some bacterial species to produce proteolytic enzymes To achieve this aim, the following objectives were conducted:

1. To isolate and identify Gram-Positive and Gram-Negative bacteria in patients with diabetes.
2. Determination the ability of the isolated bacteria to produce protease enzyme.

II. MATERIALS AND METHODS

A. Collection of Specimens

Specimens were collected from 75 patients of both genders with different ages, who suffered from diabetic foot ulcer referred to The Diabetic Center /Thi-Qar province. During the period from August to September 2023. Data collected from patients included: age, gender and period of infection, the patients' verbal consent was obtained before taking the ; the patient's verbal. During the sampling, precautions were taken to ensure the safety of the participants. A total of 75 Swabs were collected from patients using disposable transport media.

Isolation and Identification of Bacteria swabs which were from diabetic foot ulcer patients were cultured on blood agar, MacConkey agar and mannitol salt agar and incubated at 37 ° C for 24 hours. Bacterial species was isolated according to morphological and biochemical tests,, including the IMViC test catalase, coagulase and API 20 E Kit (biomerix).

B. Skim Milk Agar

Suspended 51.5 grams of skim milk agar in 1 liter of distilled water And heated to boil to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates. Proteolytic bacteria hydrolyze casein to form soluble nitrogenous compounds which are indicated as a clear zone surrounding the colonies.

C. Statistical Analysis

Data were analyzed using Excel-software for Windows 2010. Descriptive statistics were applied to analyze all data and to calculate Chi. square to assess the significant differences (p-value<0.05) between different parameters used in this study.

III. RESULTS AND DISCUSSION

The results of swab cultures showed that only 54(73.33%) gave positive growth on blood agar. The The diagnosis of the 54 isolated bacteria showed that 15 (27.77%) were *E.cloacae* , 12 (22.22%) were *p. mirabilis* , 10 (18.51%) were *S. aureus*, 8 (14.81%) were *E.coli*, 3 (0.84%) for each *K. pneumonia*.

A. Identification of Isolated Bacteria According to Age Group

The results showed that the higher percentages of infections were in patients aged 56-70 years 26 (48.14%). Other age groups showed infection percentage below 40 years 3(5.55%), 40-55 years 23(42.59%) and above 70 years 2(3.70%).

The result also indicated that a statistically significant difference between patients infected with isolated bacteria according to age groups and type of infectious bacteria at P. value <0.05.

B. Identification of Isolated Bacteria According to Gender

The results of the current study recorded that the most bacteria isolated was *E.cloacae* (27.77%) followed by *p.mirabilis* (22.22%), while the lowest isolated bacteria was *S.pyogens*, *P.aeruginosa* and *K. pnemonea* (0.84%). Moreover, the results indicated the females were higher rate of infection with isolated bacteria (62.96%), while the males recorded infection percentage (37.03%).

The result indicates that there is a non-statistically significant difference between patients (males and females) infected with isolated bacteria at (P < 0.05)

C. Identification of Isolated Bacteria According to Period of Infection

The results showed that below one month was the predominant 22(40.74%), followed by 1-3 months 16(29.62%), 4-7 month and above 7 months 9(16.66%), 7(12.96) . Also all the causative bacterial species were isolated in high percentages from below one month and Above 7 month. Additionally, the result indicate that a statistically significant difference between patients infected with isolated bacteria according to Period of infection at P. value <0.05.

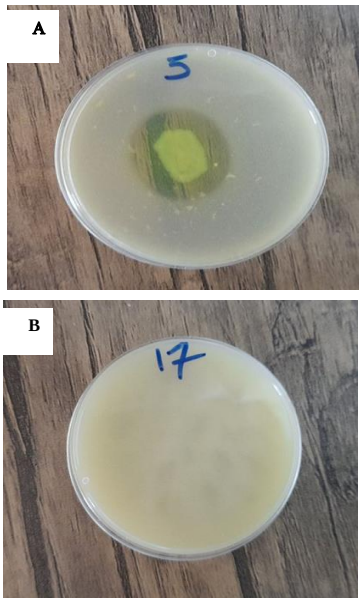

Figure 1: Protease enzyme test positive test (A) and negative test (B).

Table 1: Identification of bacteria according to age group

Age Group / Bacteria	below 40 years		40-55 years		56-70 years		above 70 years		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
Enterobacter	1	6.66	5	33.33	9	60.0	0	0.0	15	27.77
Proteus	0	0.0	5	41.66	6	50.0	1	8.33	12	22.22
S. aureus	0	0.0	7	70.0	3	30.0	0	0.0	10	18.51
E. coli	0	0.0	3	37.5	4	50.0	1	12.5	8	14.81
Pseudomonas	0	0.0	3	100.0	0	0.0	0	0.0	3	0.84
Klebsiella	2	66.66	0	0.0	1	33.33	0	0.0	3	0.84
Streptococcus	0	0.0	0	0.0	3	100.0	0	0.0	3	0.84
Total	3	5.55	23	42.59	26	48.14	2	3.70	54	100
$CalX^2 = 37.221$					DF = 18		P. value = < 0.005			

Table 2: Identification of bacteria according to gender

Gender / Bacteria	Male		Female		Total	
	No.	%	No.	%	No.	%
Enterobacter	5	33.33	10	66.66	15	27.77
Proteus	7	58.33	5	41.66	12	22.22
S. aureus	4	40.0	6	60.0	10	18.51
E. coli	2	25.0	6	75.0	8	14.81
Pseudomonas	2	66.66	1	33.33	3	0.84
Klebsiella	0	0.0	3	100.0	3	0.84
Streptococcus	0	0.0	3	100.0	3	0.84
Total	20	37.03	34	62.96	54	100
$CalX^2 = 7.616$			DF = 6		P. value = 0.268	

Table 3 : Identification of isolated bacteria according to Period of infection

Table 1-3: Identification of isolated bacteria according to Period of infection

Period of infection / Bacteria	Below one month		1-3 Month		4-7Month		Above 7Month		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
Enterobacter	6	40.0	8	53.33	0	0.0	1	6.66	15	27.77
Proteus	6	50.0	1	8.33	1	8.33	4	33.0	12	22.22
S.aureus	3	30.0	1	10.0	4	40.0	2	20.0	10	18.51
E. coli	2	25.0	3	37.5	3	37.5	0	0.0	8	14.81
Pseudomonas	2	66.66	1	33.33	0	0.0	0	0.0	3	0.84
Klebsiella	2	66.66	0	0.0	1	33.33	0	0.0	3	0.84
Streptococcus	1	33.33	2	66.66	0	0.0	0	0.0	3	0.84
Total	22	40.74	16	29.62	9	16.66	7	12.96	54	100.0
$CalX^2 = 27.231$	DF = 18		P. value = 0.075							

Protease Enzyme A Protease enzyme test was performed for all of 54 isolates to determine the ability of these bacterial species isolated from didactic foot ulcer to produce this enzyme. The high percentage of production protease enzyme was for *P. aeruginosa*, *p.mirabilis*, and S. aureus. while the isolates showed low produced was for each of *S.pyogens*, *E.cloaca* and E.coli. As shown in Fig (1-1) and Table (1-4) a proportions of isolates capable producing this enzyme.

The aerobic bacterial isolates from the study's specimens revealed several bacterial species isolated from diabetic foot ulcers. The current study's findings demonstrate that every species of bacteria was recognized using a variety of characteristics, including microscopic inspection, morphological characteristics on culture medium, identification based on biochemical tests, and API-20E for gram negative bacteria [9].

Gram-negative bacteria outnumbered Gram-positive bacteria in the current investigation. This outcome was consistent with a number of previous research conducted in Malaysia, Brazil, and Morocco [10-12].

Table 1-4 the proportions of bacteria capable of producing protease

Bacteria	No.	Protease	
		No.	%
S.aureus	10	10	100
Streptococcus spp	3	0	0.0
Proteus spp	13	10	76.92
Enterobacter spp	15	3	20.0
E.coli	8	0	0.0
Pseudomonas spp	3	3	100
Klebsiella	3	0	0.0

However, prior research has shown that the majority of organisms linked to diabetic foot infections are gram positive bacteria [13]. Gram negative bacteria appear to be replacing gram positive bacteria as the most prevalent cause of diabetic foot infections, according to a changing trend in the organisms causing these infections [14]. Variations in the sample size may explain the heterogeneity in the microbial spectrum reported in various research, the distribution of risk variables, and patient behaviors and lifestyles, including food and exercise.

The study reveals that patients aged 56-70 years have the highest rate of diabetic foot ulcer infections (48.14%), possibly due to biological changes like hormonal disorders and weakened immune systems, as well as the prevalence of these conditions among this age group [15-16].

According to the data, the men had an infection percentage of 37.03%, while the females had a higher incidence of infection (62.96%) with isolated bacteria. This suggests that compared to men, women are more prone to diabetic foot ulcer infections. This is because, according to their cultural customs, women consist the most of the labor force in families and are not regular hospital visitors. [17] As a result, the presentation of a post-traumatic infected foot ulcer is delayed.

The study found that S. aureus bacteria produced the protease enzyme at 100% of their ability, possibly due to genetic variation. The genes responsible for producing the enzyme are present in all S. aureus isolates, but their expression depends on regulatory factors in host cells. The enzyme occurs at specific times and not throughout the infection period [18-20], with *P. aeruginosa* producing 100% of the enzyme, P. mirabilis at 76.92%, and E. cloacae at 20.0%. The rest of the isolates could not produce the enzyme.

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

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