Bacterial Infections Profile and Patterns for Diabetic Foot Ulcers in Nongovernmental Hospitals of Jordan

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Authors' contributions

This work was carried out in collaboration among all authors. Author HAAH planned the basic framework for manuscript designed the study. Author AS conducted the analysis and improved the final version of manuscript Author HMD managed the literature searches. Authors HAAH and AAAS performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript Authors AJAQ and ED collected the data and prepared the initial manuscript. All authors read and approved the final manuscript

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ABSTRACT

Background: Many types of infection can cause diabetic foot ulcers Infections involving the bacteria; E. coli, *Acinetobacter* spp (MDR) and *K. pneumoniae*, pseudomonas aeruginosa, so the assessment of Bacterial profile and patterns is needed to understand the source and management of these injuries.
Objective: To determine Bacterial infections profile and patterns for diabetic foot ulcers in nongovernmental.
Method: During a period of eleven months, 148 patients with diabetic mellitus foot syndrome (DMFS). Patients were involved, out of 130 which foot ulceration infections. data analysis was done using SPSS version 20. p value was set at <0.05.

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Results: Out of 607 Patients with diabetic foot ulceration (DFU) were 130 out of 148 with diabetic mellitus foot syndrome (DMFS). Diabetic foot ulceration (DFU) therefore contributed 20.3% of DMFS among these subjects. Microbiological culture pattern was total of 17 different pathogenic microorganisms were isolated from the participants, one yeast and 16 types of bacteria, from the diabetic foot swabs for ulcers. *S. aureus* was the most frequent pathogen followed by E.coli then *Acinetobacter* spp (MDR) and *K. pneumonia*, then pseudomonas aeruginosa , then *p. mirabilis* then *Streptococcus agalactiae* (group b) then (*Enitrobacteria* spp and *pseudomonas s*pp and Candida spp and *P. vulgaris* and *K. oxytoca* ESBL) then *S. viridanse* and *Enterobacter* spp ESBL and *Staphylococcus* coag. negative). The Enterobacter spp ESBL was the less frequent pathogen.

Conclusion: Diabetic Foot Ulcerations (DFU), is forming about a quarter of the diabetic patient's tissue infections, the causative agents were bacterial and fungal(yeast). Most of the causative pathogens were; *Staphylococcus aureus, and Acinetobacter* spp (MDR). The risk of development of High resistant drug isolates of diabetic foot ulcers to be multidrug resistance were high by 53% of total isolated pathogens specially with *K. pneumonia (K. pneumoniae), Escherichia coli (E. coli)* and *Proteus mirabilis bacterial.*

Keywords: Diabetic ulcers; foot ulcer; antibiotic susceptibility; Staphylococcus aureus; diabetes; Jordan.

1. INTRODUCTION

The term diabetes mellitus describes a metabolic disorder with heterogeneous etiologies which is characterized by chronic hyperglycemia and disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both [1,2]. It is one of group of metabolic disorders characterized by a high blood sugar level over a prolonged period of time, with will determined symptoms often include frequent urination, increased thirst, and increased hunger. Diabetes; If left untreated, can cause short term or long-term complications. Acute complications can include diabetic ketoacidosis, hyperosmolar hyperglycemic state, or death. Serious long-term complications include cardiovascular disease, stroke, chronic kidney disease, foot ulcers, damage to the nerves and damage to the eyes [3]. WHO recommend the cut point for diagnosing diabetes is HbA1c of 6.5%, thus the value less than 6.5% does not exclude diabetes diagnosed using glucose tests. The consultants concluded that there is currently insufficient evidence to make any formal recommendation on the interpretation of HbA1c levels below 6.5% [4].

Foot infections in diabetic patients were the main cause of morbidity and amputations, and could be categorized clinically as limb threatening or non-limb threatening. The main causes of foot infections were; *Staphylococcus aureus* (often methicillin-resistant) and group B *streptococci* and gram-negative bacilli and anaerobes. "Multidrug-resistant pathogens are found in chronic infections, especially after exposure to health care and antibiotics. Effective treatment combines appropriate antimicrobial therapy with wound management and, if needed, surgical debridement. Osteomyelitis is common, often requiring surgical debridement for effective therapy [5]. Diabetic mellitus foot syndrome (DMFS) is one of the most destructive complications of diabetes mellitus (DM), and the common cause of prolonged hospitalization. The costs associated with diabetic foot ulcers (DFU) is very high for the patients and his family and/or governmental and health care systems [2].

The foot infection is infra-malleolar infection in a person with diabetes mellitus, the infection could include; paronychia, cellulitis, myositis, abscesses, necrotizing fasciitis, septic arthritis, tendonitis and osteomyelitis. Actually the infection began when the protective layer of skin is breached and bacteria start colonization, in the wound may progress to become actively infected, and, by contiguous extension, the deeper tissues could be involved, this sequel of events can be rapid, especially in an ischemic limb. In presence of week immunity especially with patients that involve polymorphonuclear leukocytes, may affect some diabetic patients, and these likely increase the risk and severity of foot infections [3]. Diabetic foot infections (DFI) considered as one of the most common diabetes related cause of hospitalization, Foot ulceration is one of the most serious complications of diabetes, it affects approximately 15-20% of people, and frequently become infected with very serious sequelae lead to amputation making diabetes is the most common cause of lower

extremity amputations.The rapid diagnosing of the infections are required urgently , which increase the morbidity (and/or mortality) which means that they represent significant clinical events, requiring immediate attention in relation to local and systemic complications thus requiring well-coordinated management, unfortunately diabetic foot infections (DFI) frequently fail to display overt signs and symptoms of infection including purulence, erythema, pain, tenderness, warmth and induration, it's difficult to be detected to make timely interventions to limit the highly undesirable consequences [4,2].

Diabetic foot infections are mild to moderate in severity,while the severe, infection would lead to serious sequelae are often accompanied by osteomyelitis, and they could leads to cause diabetes-related hospitalization and lower extremity amputation in the United States, in the other hand, the acute infections in patients who have not recently received antibiotic therapy were found be predominantly caused by aerobic gram-positive cocci, as an infection caused by one microbe, while the chronic wounds tend to develop more-complex flora [5]. Several classification systems have been proposed and utilized for the assessment of diabetic foot ulceration (DFU) and DFI. Actually, there is no one universally accepted classification system. Most employedsystema matrix of grades based upon depth and size of wound. The foot ulcers infections also were classified as a mild, moderate, or sever infections; The mild infections: caused by *Staphylococcus spp* and Streptococcus *spp*. Which could be purulence and cellulites to superficial soft tissue, while, the moderate infections cause deep tissue abscess in muscle, joint, tendon and bone and/or gangrene. The sever infections also called as limb- threatening is the most difficult diabetic foot infection to cure. Chronic osteomyelitis, usually, caused by polymicrobial; *Staphylococcus* spp, *Streptococcus* spp, *E. coli, K. pneumoniae, Proteus spp, Acinetobacter spp*, *Pseudomonas aureginosa* and anaerobes (Bacteroides *spp*) [2].

Staphylococcus aureus and the Beta-hemolytic *streptococci* (groups A, C, and G, but especially group B) were the predominant microorganism that colonize and acutely infect breaks in the skin aerobic gram-positive cocci., on the other hand, the chronic wounds develop a more complex colonizing flora, including *enterococci*, various Enterobacteriaceae, obligate anaerobes, *Pseudomonas aeruginosa,* and, sometimes, other no fermentative gram-negative rods in the Hospitalized patients , surgical procedures, and, especially, prolonged or broad spectrum antibiotic therapy may predispose patients to colonization and/or infection with antibiotic-resistant organisms (e.g., MRSA or vancomycin-resistant *enterococci* VRE) [6].

The high resistance and Multidrug resistant bacteria for drugs were common in patients with diabetic foot ulcers. Multi drug resistance MDR bacteria were resistant to two or more different families/types of antibiotics so they require treatment with extended spectrum antibiotics for long durations. Infection with MDR and high resistance drug resistant bacteria can cause prolonged stays in hospital, leading to higher treatment costs and increase the risk of nosocomial infections chances which is leading to a risk of increased morbidity and mortality [2].

The aim of this study was to further enhance understanding the the source and management of diabetic foot ulcers management of infections in nongovernmental hospitals of Jordan

2. MATERIALS AND METHODS

2.1 Study Area

This was for more than months period from 1-1-2018 to 11-11-2018 for both of in and outpatients in one of the biggest nongovernmental hospitals in Jordan. In and outpatients were involved from surgery and admitted wards. All the patients fulfilled the criteria above were consecutively enrolled in the study.

2.2 Study Design and Study Population

A hospital base study was performed to determine the multidrug resistant bacterial profile and patterns for pus isolates and recurrent wound infections in nongovernmental hospitals of Jordan and the percent of infections among the causative agents, within the perid from 1-1-2018 to 11-11-2018 for both of in and outpatients in one of the biggest nongovernmental hospitals in Jordan.

2.3 Sample Size Determination and Samplings Techniques

The total number of patients for the study was 607; 331 of them wound infected patients, which forms 54.4% of the total population the female number of patients was 124 females (63%) and

the male's numbers was 207(37%). The pus culture's patients were 128, which forms 21.1% of the total population, the female number of pus's patients was 40 females (31%) while the male's numbers were 88(69%). The overall percent's female over male 41/59. The aim and benefits of the experiment was clearly illustrated for the participants prior to data collection, the participation was on voluntary basis and they have informed them it is there right to withdraw from the study at any time during the course of data collection.

2.4 Sample Collection and Laboratory Quality Control and Methods

Data were from volunteers collected using structured questionnaire consisting of the patient's demographic information, two sets of deep wound samples were obtained by rolling two sterile swab sticks one after the other over the surface of the sampling site, Biopsies and aspirated material were preferred over swabs for deeper wound Site, after debridement of superficial exudates, one swab specimen was immediately transferred into a thioglycollate medium, and sent with the second specimen to the microbiology laboratory for analysis under the supervision of medical microbiologist. Appropriate conditions had created for aerobic and anaerobic bacteria and fungi to multiply and isolate a pure culture of microorganisms in culture medium to determine and identify the type of organism in wound, pus, soft tissue, diabetic foot, skin, ulcer, cyst, bile, abscess or any sterile swab, in addition to the antibiotic susceptibility testing. agar's expiration date and QC, cracked dishes, thin or unequal fill, hemolysis, evidence of freezing, desiccation, bubbles and contaminated agars had excluded, the performance of prepared media was tested by inoculation control stands; S. Aureus ATCC 25923 and E. coli ATCC-25922 to confirm the results.

2.5 Antimicrobial Agents and Antibiotics Susceptibility Testing

The isolated organisms were inoculated onto nutrient agar plates, and anti-microbial susceptibility testing was carried out using the modified Kirby-Bauer disc diffusion method, discs for available, anti-microbial agents were used. Attempts were made to incorporate discs' representative of different classes of anti-microbial. Disc of the following anti-microbial was used: Ceftriaxone (30 µg), Ceftizoxime (30 µg), Cefoxitin (30 µg), Gentamicin (10 µg), Amoxicillin/Clavulanate (30 µg), Cefuroxime (30 µg), Nitrofurantoin (100 µg), Ceftazidime (30 µg), Ciprofloxacin (10 µg), Ofloxacin (10 µg), Pefloxacine (30 µg), Clindamycin (2 µg), Ampicillin/Sulbactam (10/10 µg), Imipenem (10µg), Meropenem(10 µg), Ertapenem (10 µg), Clarithromycin (10 µg), Ampicillin (30 µg), Erythromycin (10 µg), Ampicillin/Cloxacillin (30µg), Cefixime (5 µg), Levofloxacin (10 µg), Norfloxacin (10 µg), And Metronidazole (5 µg).

2.6 Data Analysis and Interpretation

Data analysis was done using Statistical Package for the Social Sciences (SPSS) version 23 (IBM Corp, 2015). Qualitative data were described as proportions or percentages; cross-tabulation was used where necessary. Test of significance for differences for quantitative and categorical variables was tested with T-test and Chi-square analyses respectively. A p-value of < 0.05 was considered significant.

3. RESULTS

General characteristics of study population: Subjects with diabetic foot ulceration (DFU) were 130 (65 male and 83 female participants respectively) out of 148 with diabetic mellitus foot syndrome (DMFS). Diabetic foot ulceration (DFU) therefore contributed 20.3% of DMFS among these subjects. The age range of the participants was 18 to 80 years. The age group with the highest number of participants was 41 — 60 years (57.7%). The gender and percentage of each sex presented in Table1, Glycaemic control was generally poor: 10.5% and more see Table 1 and Table 2.

Pattern of bacteriological flora in the participants with diabetic foot ulcers:

Out of 607 total volunteers of diabetic mellitus foot syndrome (DMFS), 517(85.2%) for give growth and 90(14.8%) gives no growth of samples, 148(24.4%) were diabetic foot ulcer patients, 128(21.1%) were pus for culture, and 331(54.5%) were wound swab all patients were uncontrolled glycation, from the diabetic foot swabs for ulcers 18 samples ware not get growth for pathogenic bacteria and 19 non-pathogenic bacteria see Table 3 and Table 4.

A total of 17 pathogen organisms were isolated from the participants one yeast and 16 types of bacteria, from the diabetic foot swabs for ulcers.

S. aureus was the most frequent pathogen followed by E. coli then *Acinetobacter* spp (MDR) and *K. pneumoniae*, then pseudomonas aeruginosa, then *P. mirabilis* then *Streptococcus agalactiae* (group b) then (*Enterobacter* spp and *Pseudomonas* spp and Candida spp and *P. vulgaris* and *k.oxytoca* ESBL) then *S.viridanse* and *Enterobacter* spp ESBL and *Saphylococcus* coag. negative). The *Enterobacter* spp ESBL was the less frequent pathogen , Candida spp had counted to be one of the causes of infection in two cases. 19 patients had pathogenic microbes see Table 5.

The High resistant drug isolates of diabetic foot ulcers were; S.aureus MRSA, acinetobacter spp. (MDR), k.pneumoniae ESBL, k.p. carbapenemase XDR, p.mirabilis ESBL, k.oxytoca ESBL, p.vulgaris ESBL, enterobacter spp ESBL. The most frequent one was s.aureus MRSA the followed by acinetobacter spp. (MDR), then followed by p.mirabilis ESBL, then followed by k.pneumoniae ESBLthen K.oxytoca ESBL, then k.p.carbapenemase XDR Table. 6.

S. aureus MRSA was the most frequent pathogen among multidrug resistant isolates (MDR) for diabetic foot ulcers, then followed by Acinetobacter spp. (MDR) then by k. p. carbapenemase XDR and Enterobacter spp ESBL Table. 7.

4. DISCUSSION

Diabetic Foot Ulcerations is an actual burden of foot lesions globally, this type of disease is forming about a quarter of the diabetic patient's tissue infections [7,8]. Most of the causative pathogens are frequent infectious cases for the foot ulcer over the world; *S. aureus, Acinetobacter spp (MDR), E. coli, K. pneumoniae, Pseudomonas aeruginosa, P. mirabilis, Streptococcus group D (entrococcus), Morganella morganii, Streptococcus agalactiae (group B), K. oxytoca esbl , P. vulgaris, Candida spp, Pseudomonas spp, Enterobacter spp, Enterobacter spp esbl, Staphylococcus coag. Negative and S. viridanse, while the S.aureus, E.coli, K.pneumoniae and Acinetobacter* spp(MDR) were the most frequent causes Table. 5.

These demonstrations are clarifying and show the actual facts and the actual burden of infections and the progression of the infections to develop the causative bacteria to be highly resistant bacteria and the future horror of multidrug resistance (MDR), and the challenge among Jordanian healthcare providers and the patients suffering from complications of diabetes mellitus since 24.4% of total tissue infections were caused by foot ulcer infections, which within the global ratios [9] Table. 2.

The highly signifecancy by p<0.001of MDR results bell the rings to focus more in handling and take care for the progression of managing and treatment of DFI in Jordan (Table 7), the risk of development of high resistant drug isolates of diabetic foot ulcers was high by 53% of total infections, and the progression of new types of bacteria specially; *e.coli ESBL and p.mirabilis ESBL* is serious, and should be taken in the new management policies Table. 5, 1,2, 4, 5, 8, 9.

The high cost and lack of well-trained multi-disciplinary medical personnel, facilities and standardized management protocols are possible contributory factors. Physicians also have an important role in the prevention, early diagnosis and management of diabetic foot complications [10]. Though the patients reported the ulcers as resulting from spontaneous blisters or physician reporting of ulcers as a wound, small percentage of overlapping between diabetic foot ulcers and diabetic wounds, so remains of a possibility that some of the ulcers may have resulted from unnoticed micro-trauma. inappropriate footwear might lead to spontaneous blisters; this was found to be the second commonest predisposing event for diabetic foot ulceration (DFU), also fitting of foot wears in patients with peripheral neuropathy may results in foot ulcerations in patients with insensate feet, use of disordered machines or tools in addition to abnormal weight-bearing in the areas of the foot in patients with peripheral neuropathy could make the foot susceptible to ulceration while wearing shoes.

The self-inflicted burns due to thermal injury resulting from application of hot compresses to numb feet precipitated two cases of diabetic foot ulceration (DFU) might cause ulcerations and wound or burns and should be taken care in foot ulceration studies [2].

Thus, there is indeed a need to ensure that better focused education and determination the best way to handle and take care about ulcer foot cases on appropriate foot wears, foot care and other harmful practices be intensified among these patients.

Table 1. Characteristics of study population

Type of culture	Male	Female	Total
Diabetic foot swab	65	83	148
Percentage	44%	56%	100%

Table 2. Study population

Types of test	Number of tests	Percentage of total subjects
Diabetic foot swab	148	24.4%
Pus for culture		21.1%
Wound swab		54.5%
Total		100.0%

Table 3. Number of growth and non-growth sample results of study population

Types of test	Number of tests	Percentage of total subjects
number of growth samples	517	85.2%
number of non-growth samples	90	14.8%
total	607	100.0%

Table 4. Diabetic foot swabs

Types of test	Number of tests	Percentage of total subjects
number of growth samples	130	87.8%
number of non-growth samples	18	12.2%
total	148	100.0%

Table 5. Diabetic foot swab infections

Type of pathogen	Number of infections	Percentage of Diabetic foot swab infections
S.aureus	31	23.8%
Acinetobacter spp(MDR)	10	7.7%
E.coli	18	13.8%
K. pneumoniae	10	7.7%
Pseudomonas aeruginosa	8	6.2%
P. mirabilis	7	5.4%
Streptococcus group D (enterococcus)	6	4.6%
Morganella morganii	5	3.8%
Streptococcus agalactiae (group B)	3	2.3%
K. oxytoca ESBL	2	1.5%
P. vulgaris	2	1.5%
Candida spp	2	1.5%
Pseudomonas spp	2	1.5%
Enterobacter spp	2	1.5%
Enterobacter spp ESBL	1	0.8%
Staphylococcus coag. Negative	1	0.8%
S. viridanse	1	0.8%
no pathogenic bacteria	19	14.6%
Total number	130	100.0%

Table 6. High resistant drug isolates of diabetic foot ulcers

Type of pathogen	Number of infections	Percentage of Diabetic foot swab infections
S. aureus MRSA	21	16.2%
Acinetobacter spp. (MDR)	10	7.7%
K. pneumoniae ESBL	4	3.1%
K .p. carbapenemase XDR	1	0.8%
P .mirabilis ESBL	5	3.8%
K. oxytoca ESBL	2	1.5%
P. vulgaris ESBL	1	0.8%
Enterobacter spp ESBL	1	0.8%
Total number	53	40.8%

Table 7. Multidrug resistant isolates of diabetic foot ulcers

Type of pathogen	Number of infections	Percentage of Diabetic foot swab infections
S. aureus MRSA	21	16.2%
Acinetobacter spp. (MDR)	10	7.7%
K. p. carbapenemase XDR	1	0.8%
Enterobacter spp ESBL	1	0.8%
Total number	33	25.4%

4.1 Bacteriological Pattern of Diabetic Foot Ulcers

In the present study, a total of 17 different microorganisms were isolated from the participants, with mixed gram-positive and gram-negative species and yeast- Candida albicans; an average of 1:4 gram-positive aerobic bacteria, 4:1 gram-negative aerobic bacterium, and yeast 1:16 an overall average of 0.13% (6.7) organisms per case. This is similar to the findings as a result of the larger sample size in the US study [10], Table 5.

The Predominancey of gram-negative aerobes have been reported also field workers and previous researchers [11]. These differences could be partly due to changes in the causative organisms occurring over time and the capability of microbes to get more resistance for antibiotics, also might be affected by geographical variations, or the types and severity of infection. Differences in results might be due to the use of a "relatively small number of specimens", and limited specimen collection techniques (which would fail to exclude superficial or colonizing organisms), the poor handling techniques and poor preservation methods might affect the cultivation of anaerobic organism [8,11,12].

5. CONCLUSION

Diabetic Foot Ulcerations (DFU), is forming about a quarter of the diabetic patient's tissue infections, the causative agents were bacterial and fungal(yeast). Most of the causative pathogens were; *Staphylococcus aureus, and Acinetobacter* spp (MDR), The risk of development of High resistant drug isolates of diabetic foot ulcers to be multidrug resistance were high by 53% of total isolated pathogens specially with *Klebsiella pneumoniae (K. pneumoniae), Escherichia coli (E. coli) and Proteus mirabilis bacterial*

CONSENT AND ETHICAL APPROVAL

Ethical approval was obtained from the Health Research Ethics Committee of the MOH, Jordan.As per international standard university standard, patients' written consent has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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