

Original article

# Microbiological Profile and Antimicrobial Susceptibility Patterns in Patients with Tinea Pedis at Diabetic Center In EL-Beyda City

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## ARTICLE INFO

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Received: 04-12-2023

Accepted: 09-01-2024

Published: 13-01-2024

**Keywords.** Diabetes Mellitus, Tinea Pedis, Bacterial Super Infection, Antibiotic Sensitivity, DFU.

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## ABSTRACT

The purpose of this study was to determine the microbiological profile and antimicrobial susceptibility patterns in patients with Tinea Pedis at the Diabetic Center in EL-Beyda City (Libya). Samples were collected from the diabetic patients with intertrigo of foot attending the out-patients' department in the Diabetic Center from December 2020 to February 2021 by using sterile swabs. Samples were inoculated into blood and MacConkey agars for bacterial isolation. The microbiologic isolation and antimicrobial susceptibility tests were performed according to standard microbiological methods. Antibiotic susceptibility patterns of all the isolates to different antibiotics was determined by antimicrobial susceptibility testing. Overall, 50 patients (50% male and 50% female) had a mean age of  $58.4 \pm 9.4$  years and a mean duration of diabetes of  $14.6 \pm 6.6$  years. Most isolates (76.8 %) were Gram-negative bacilli (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella Spp* and *Proteus Spp*), followed by Gram-positive cocci (23.2%) (*Streptococci Spp* and *Staphylococcus aureus*). Most bacteria were sensitive to Ciprofloxacin (84.0%) and resistant to Erythromycin (42.0%). The majority of isolated bacteria from patients were found to be multi-drug resistant. The results demonstrate the importance of time in the identification of the intertrigo sample collection for the identification of the pathogens and their antibiotic susceptibility pattern before initiating antimicrobial treatment.

**Cite this article.** Altabouli S, Qowaider S, Hamad M, Ali M. Microbiological Profile and Antimicrobial Susceptibility Patterns in Patients with Tinea Pedis at Diabetic Center In EL-Beyda City. 2024;7(1):50-55. <https://doi.org/10.54361/ajmas.2471009>

## INTRODUCTION

Diabetes affects persons of all ages and in all socioeconomic segments of the population. World health organization predicts that by the year of 2010 the world's diabetic population will double to over 200 million people [1,2]. Tinea Pedis is a dermatophyte infection of the feet especially between the toes (interdigital or web space) and the soles of the feet. This disease is estimated to affect 10% of the world's population [3]. Patients with diabetes appear to be prone to interdigital Tinea Pedis infections at an earlier age than those without diabetes. Previous study found that, the fungal infection is frequent in diabetic patients attending to diabetic center [4]. Diabetic patients are very often prone to fungal infections, because of higher blood glucose levels which help for the growth of fungi. Tinea Pedis

often occurs in the 3<sup>rd</sup> and 4<sup>th</sup> interdigital spaces, may extended to the dorsal and plantar aspects [5]. Tinea Pedis especially the interdigital type is often involving of bacterial super infection which causes the treatment to be difficult [6,7]. Although antifungal medications are effective, dermatomycosis remains undertreated [8]. In addition to cause permanent damage to the affected area, dermatomycosis can progress to severe disease in elderly and immunocompromised patients if left untreated [9-11]. Furthermore, untreated Tinea Pedis or improper treatment may lead to secondary infection by bacteria [12]. This infection may be caused by pathogenic bacteria originating from the external environment as well as by bacteria forming physiological micro flora of the skin (e.g. *staphylococcus epidermidis*, *staphylococcus aureus*, and *propionibacterium acnes*) [13,14]. Neglected acute and chronic Tinea Pedis can become a problem for persons with diabetes. Because of the lower extremities compromise seen in high-risk diabetes, Tinea Pedis presents a significant concern because interdigital web-space Tinea Pedis may lead to cracks and fissures. These can become secondarily infected, resulting in serious deep space infection, then ulcerating and if there is neuropathy or ischemia may lead to amputation which very burden to the patients and to health care system [15]. This study was aimed to determine most common bacterial super infection in Tinea Pedis and antimicrobial susceptibility patterns in patients with this infection at diabetic center in EL-Beyda City.

## METHODS

### *Study setting*

This study was carried out during the period between December 2020 to February 2021, after obtaining informed consent form from the patients or relatives. The study protocol was reviewed and approved by scientific research and ethics committee at University of Tripoli (SREC-UOT) with Ref No: SREC/010/34/2023. Additionally, permission to perform the study was obtained from the center manager.

### *Samples*

Fifty consecutive patients (25 males and 25 females) with more than 5 years diagnosed of diabetes mellitus and having Tinea Pedis resistance to pure antifungal therapy were enrolled in this study. All the patients underwent a detailed demographic profile including: name, age, diabetes duration and dermatological examination.

The samples were obtained from patients who aged above 20 years, diabetic more than 5 years with Tinea Pedis based on clinical examination with positive KOH examination before start antifungal treatment and willing to participate in research by signing informed consent. Exclusion criteria are being treated with oral and topical antibiotics in the past week.

### *Sampling technique*

First, the area is cleaned with normal saline, then awaited dry. For lesions in the form of vesicles or bulla, done by opening the roof of the lesion using a sterile scalpel and then scrape the base of the lesion by sterile cotton swab, then inserted into dry transport system container and labeled with patients' identity. The tubes immediately transported to the microbiology laboratory. The specimens were removed from the dry transport swab, applied to the blood agar and Mac Conkey agar media, incubated at 37<sup>0</sup> C for 24 hours. The mold species were identified on the basis of their microscopic and macroscopic appearance. Biochemical tests were done to identify and confirm all isolates. Aantibiotics susceptibility patterns of all the isolates to different antibiotics was determined by the disc diffusion methods, as recommended by the Clinical and Laboratory Standard Institute CLSI, using commercial antimicrobial discs (Mast. Co., UK). Antimicrobial sensitivity testing of all isolates was performed on diagnostic sensitivity test plates according to the Kirby-Bauer method [16].

### *Statistical analysis*

Recorded data were analyzed using the statistical package for social sciences, version 23.0 (SPSS Inc., Chicago, Illinois, USA). Quantitative data were expressed as mean± standard deviation (SD). Qualitative data were expressed as frequency and percentage.

The comparison between groups with qualitative data was done by using Chi-square test and Fisher's exact test instead of Chi-square test only when the expected count in any cell less than 5. The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was considered P- value ≤ 0.05 was considered significant, P- value > 0.05 was considered insignificant.

## RESULTS

This is a cross sectional study to determine bacterial super infection in Tinea Pedis at the Diabetes Center in AL-Jabal AL-Akhdar at EL-Beyda City (Libya). Fifty Libyan patients, 50% (n=25), 50% (n=25) were female and male respectively. Gram negative and positive isolates were recorded in this study; the most isolates (76.8 %) were Gram negative bacilli (*Escherichia coli* (22 patients), *Pseudomonas aeruginosa* (13 patients), *Klebsiella Spp* (4 patients), and *Proteus Spp* (3 patients), followed by Gram positive cocci (23.2%) (*Streptococci Spp* (7 patients), and *Staphylococcus aureus* (6 patients), with a significant difference between gram positive and gram negative, as shown in table 1.

**Table 1. Gram negative and positive isolates from male and female subjects.**

Gram Stain	Sex				Total		Chi-square test	
	Female (n=25)		Male (n=25)		No.	%	x <sup>2</sup>	P Value
	No.	%	No.	%				
Gram negative Bacilli	19	38	24	48	43	76.8	4.153	0.042*
Gram positive cocci	7	14	6	12	13	23.2		

The most common bacterial culture results in this study were gram-negative bacterial infections with no significant difference between genders (96 % and 76% male and female, respectively). However, gram-positive bacterial infections were found with a significant difference between genders (24 % and 28% male and female, respectively). *Candida* found only 1 patient (Table 2).

**Table 2. Characteristics of different isolations among 50 subjects**

Pathogen	Isolates		Female (n=25)		Male (n=25)		x <sup>2</sup>	P Value
	No.	%	No.	%	No.	%		
<b>Gram negative Bacilli (n=43)</b>								
<i>Escherichia coli</i>	22	51.1	13	26	9	18	1.273	0.259
<i>Pseudomonas aeruginosa</i>	14	32.6	7	14	7	14	0.00	1.00
<i>Klebsiella Spp</i>	4	9.3	1	2	3	6	1.065	0.302
<i>Proteus Spp</i>	3	7	3	6	0	0	3.128	0.077
<b>Gram positive cocci (n=13)</b>								
<i>Streptococci Spp</i>	7	53.8	2	4	5	10	4.052	0.044*
<i>Staphylococcus aureus</i>	6	46.2	0	0	6	12	6.682	0.010*
<i>Candida</i>	1	100	0	0	1	2	1	0.317

In terms of different sensitivity patterns to antibiotics, the most isolated bacteria were sensitive to Ciprofloxacin (Cip) 42 patients (84.0%), followed by Gentamicin (Gn) 4 patients (8.0%), and then Augmentin (AMC) in 2 patients (4.0%). Less sensitive were found with Augmentin (AMC) 16 patients (32.0%), followed by Erythromycin (E) 11 patients (22.0%), then Doxycycline (DO) 8 patients (16.0%), Nalidixic acid (NA) 6 patients (12.0%), and Gentamicin (Gn) 4 patients (8.0%). Most bacteria were sensitive to Ciprofloxacin (88.0%) and resistant to Erythromycin (42.0%), as shown in table 3.

The same table shows statistically significant differences between males and females in terms of being less sensitive to antibiotics. Which was higher with Augmentin (AMC) in the female group compared to the male group. While Nalidixic acid (NA) was high in male group compared to the female group, Similar significant differences were obtained according to resistance between male and female patients. Which was higher with Nalidixic acid (NA) and Nitrofurantoin (F) in the female group compared to the male group.

**Table 3. Different sensitivity patterns to antibiotics among 50 patients**

Antibiotics	All patients (n=50)		Female (n=25)		Male (n=25)		Chi-square test	
	No.	%	No.	%	No.	%	x2	P Value
<b>Sensitive (+++)</b>								
Ciprofloxacin 5 mg (Cip)	42	84	22	88	20	80	0.583	<b>0.445</b>
Gentamicin 10 mg (Gn)	4	8	2	8	2	8	0.00	<b>1.00</b>
Augmentin 10 mg (Aug)	2	4	1	4	1	4	0.00	<b>1.00</b>
Nitrofurantine 20 mg (F)	1	2	0	0	1	4	1	<b>0.317</b>
Nystatin 100 mg (NY)	1	2	0	0	1	4	1	<b>0.317</b>
<b>Sensitive (+)</b>								
Augmentin 10 mg (Aug)	16	32	13	52	3	12	9.007	<b>0.003*</b>
Erythromycin 15 mg (Ery)	11	22	6	24	5	20	0.114	<b>0.735</b>
Doxycycline 30 mg (Dox)	8	16	4	16	4	16	0	<b>1</b>
Nalidixic acid 30 mg (NA)	6	12	0	0	6	24	6.682	<b>0.010*</b>
Gentamicin 10 mg (Gn)	4	8	2	8	2	8	0.00	<b>1.00</b>
Ciprofloxacin 5 mg (Cip)	1	2	0	0	1	4	1	<b>0.317</b>
Cloxacillin 10 mg (CX)	1	2	0	0	1	4	1	<b>0.317</b>
Miconazole 10 mg (MCL)	1	2	0	0	1	4	1	<b>0.317</b>
Nitrofurantine 20 mg (F)	1	2	0	0	1	4	1	<b>0.317</b>
Tetracycline 30 mg (TE)	1	2	0	0	1	4	1	<b>0.317</b>
<b>Resistant</b>								
Erythromycine 15 mg (Ery)	21	42	9	36	12	48	0.724	<b>0.395</b>
Nalidixic acid 30 mg (NA)	11	22	9	36	2	8	5.597	<b>0.018*</b>
Nitrofurantine 20 mg (F)	5	10	5	20	0	0	5.444	<b>0.020*</b>
Vancomycin 5 mg (VA)	4	8	0	0	4	16	1	<b>0.317</b>
Augmantine 10 mg (Aug)	3	6	1	4	2	8	0.348	<b>0.556</b>
Doxacycline 30 mg (Dox)	3	6	0	0	3	12	3.128	<b>0.077</b>
Cephalexin 10 mg (CL)	1	2	0	0	1	4	1	<b>0.317</b>
Clotrimazole 50 mg ( clo)	1	2	0	0	1	4	1	<b>0.317</b>
Gentamicine 10 mg (Gn)	1	2	0	0	1	4	1	<b>0.317</b>

## DISCUSSION

Diabetic patients are more susceptible for foot intertrigo infections, especially fungal infection correlates with previous study. fungal infection (Tinea Pedis, interdigital type) was seen in 52% of patients [12]. Multiple risk factors had been reported for Tinea Pedis and tinea unguium. These include age, male sex, climate (high temperature and humidity), footwear, exercise, use of public facilities, home infections, and incidence of certain diseases such as obesity, diabetes, and vascular disorders [17-19]. The majority of patients in current study belong to the age group of 35-65 years old which correlates with the previous studies that foot intertrigo was common in age group of 36-81 years [12,17,20,21]. These finding suggest that interdigital infection common disorder that can affect any stage of life. In diabetic patients interdigital Tinea Pedis must be treated, clear, and prevent recurrence because Tinea Pedis is potential risk for diabetic foot ulcers (DFU). If neglected this minor infection or not treated well would cause small fissures and breaks in the skin causing a portal of entry for bacteria which called complex dermatophytosis (as in current study all cases n=50 interdigital Tinea Pedis have secondary bacterial infection), the condition of Tinea Pedis without bacterial infection was called simplex dermatophytosis [22,23].

The most common bacterial culture results in this study were gram- negative bacterial infections were with 76.8 % of patients as compared with gram- positive bacterial infections which found with 23.2%. Gram-negative *Bacillus* is the predominant pathogen in diabetic infections. *Escherichia coli* among gram- negative bacilli and *Staphylococcus aureus* among gram- positive cocci and these results correlates to other studies in India [24, 25], in Ethiopia [26], in China [27], in Lebanon [28] and in Saudi Arabia [17]. However, in Another study was against to current result, it found the most common infections were gram-positive *Staphylococcus* (41.1%) and gram- negative *Bacillus* (*Escherichia coli* (12.8%)) [23]. In addition, another study also found secondary bacterial infection in dermatophytosis with *Staphylococcus* followed by *Escherichia coli* [29].

Increased resistance of pathogenic bacteria is an important problem in the treatment of diabetic foot infection. Another finding of this study was culture sensitivity test recorded that in all cases found. highly resistance to Erythromycin in 42% of cases and highly sensitivity to ciprofloxacin 84% of cases. It also found that the most prescribed antibiotic Augmentin was less sensitive and less effective in 32% of cases.

Long-term (more than 6 months) antibiotics, long foot wound duration, high blood pressure, anemia, chronic osteomyelitis, and history of infection have been recognized as the predictive risk factors [30]. This study provides a reference for the local bacterial distribution, antimicrobial sensitivity and resistant. In order to improve the clinical outcomes of diabetic foot infection patients, a timely microbiology examination and the appropriate selection of antibiotics based on the severity of ulcers and infections, drug-resistant bacteria risk factors, and antimicrobial susceptibility are necessary.

## CONCLUSION

Patients who don't respond to topical and/or systemic antifungal therapy should undergo screening for bacterial infection to prevent deeper bacterial infection. For all Tinea Pedis (diagnosis clinically and KOH or culture) patients, especially diabetic patients, if they have not responded to antifungal therapy, it is mandatory to do culture sensitivity for bacterial infection to prevent serious complications.

## Acknowledgments

We are heartily thankful to the diabetic center director Abdalhaleem AL-jayash for his encouragement and support. Our thanks are conveyed to the patients who accepted to participate in our research, as well as to all staff and nurses of the Diabetic Center in Al-Jabal Al-Akhdar in El-Beyda City (Libya) for their great assistance and cooperation during collecting samples.

## Conflict of interest

There are no financial, personal, or professional conflicts of interest to declare.

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## الصورة الميكروبيولوجية وأنماط الحساسية للمضادات الميكروبية لدى مرضى سعة القدم بمركز السكر بمدينة البيضاء

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### المستخلص

الغرض من هذه الدراسة هو تحديد المظهر الميكروبيولوجي وأنماط الحساسية المضادة للميكروبات لدى المرضى الذين يعانون من سعة القدم في مركز مرض السكري في مدينة البيضاء (ليبيا). تم جمع العينات من مرضى السكري الذين يعانون من ثنية القدم المترددين على قسم العيادات الخارجية بمركز السكري في الفترة من ديسمبر 2020 إلى فبراير 2021 باستخدام مسحات معقمة. تم تنمية العينات في الاوساط الغذائية التالية الدم وأجار ماكونكي لعزل البكتيريا. تم إجراء اختبارات العزلة الميكروبيولوجية واختبارات الحساسية المضادة للميكروبات وفقاً للطرق الميكروبيولوجية القياسية. تم تحديد أنماط الحساسية للمضادات الحيوية لجميع العزلات للمضادات الحيوية المختلفة عن طريق اختبار الحساسية للمضادات الحيوية. بشكل عام، كان متوسط عمر 50 مريضاً (50% ذكور و50% إناث)  $9.4 \pm 58.4$  سنة ومتوسط مدة الإصابة بالسكري  $6.6 \pm 14.6$  سنة. معظم العزلات (76.8%) كانت عصيات سلبية الجرام (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella Spp* and *Proteus Spp*) تليها المكورات موجبة الجرام بنسبة (23.2%) (*Streptococci Spp* and *Staphylococcus aureus*) كانت معظم البكتيريا حساسة للسيبروفلوكساسين بنسبة (84.0%) ومقاومة للإريثروميسين بنسبة (42.0%). وتبين أن غالبية البكتيريا المعزولة من المرضى مقاومة لمضادات متعددة. توضح النتائج أهمية الوقت في تحديد جمع عينات لتحديد مسببات الأمراض ونمط حساسيتها للمضادات الحيوية قبل البدء في العلاج المضاد للميكروبات في الحالات المصابة.

**الكلمات الدالة:** داء السكري، سعة القدم، العدوى البكتيرية، حساسية المضادات الحيوية.