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Original article

Antibiosis of Antibiotics, Honey and Probiotics Related Bacteria to Diabetic Foot Infections

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Abstract
In addition to being the main factor associated with amputation, diabetic foot infections (DFIs) are associated with major morbidity, increased mortality, and reduced quality of life. Appropriate treatment choice is very important to reduce failure, antimicrobial resistance, adverse events, and costs. This study aimed to investigate the causative pathogens of DFIs in patients from Tripoli University Hospital, their profile of antimicrobial susceptibility towards commonly used antibiotics, two types of honey, and different strains of probiotics, including Lactobacillus acidophilus (L. acidophilus), Lactobacillus reuteri (L. reuteri), and Saccharomyces boulardii (S. boulardii). This prospective study included 50 patients admitted to Tripoli University Hospital. Bacteriological specimens were obtained and processed using standard procedures for microbiological culture and sensitivity testing; their antibiotic susceptibility pattern was determined using the Kirby-Bauer disk diffusion method. The agar diffusion method was applied to honey and probiotics. The most common location of ulceration was the toe (60%), plantar surface (26%), and dorsal portion (14%). A total of 88 bacterial isolates were obtained. Mono-microbial cultures were (34%) and (66%) were polymicrobial. Gram-positive bacteria represented 45.4%; the rest are gram-negative bacteria. The most common isolates were Staphylococcus aureus (S. aureus) (28.4%), Escherichia coli (E. coli) (9.1%), and Pseudomonas aeruginosa (P. aeruginosa) (6.8%). Most gram-negative isolates were sensitive to ciprofloxacin and gentamicin, followed by meropenem. Likewise, ciprofloxacin was the most effective against gram-positive isolates. Both L. acidophilus and L. reuteri inhibited the growth of all gram-negative isolates. In addition, there was no effect of S. boulardii on all isolates except P. aeruginosa and Klebsiella oxytoca. Both Enterobacter cloacae and Acinetobacter baumannii (A. baumannii) were resistant to all tested probiotic strains. Based on our results L. reuteri was more effective against all gram-positive isolates. Both types of honey inhibited the growth of all isolates, including multidrug-resistant E. cloacae and A. baumannii strains. On the contrary, Bacillus spp. were resistant to both honey types. The present study confirmed the high prevalence of multidrug-resistant pathogens in diabetic foot ulcers and that tested strains of probiotics and honey prevent the growth of a wide range of potential human pathogens, including 15 species of bacteria. This knowledge is crucial for planning treatment with new appropriate antimicrobials, reducing resistance patterns, and minimizing healthcare costs.
***Keywords**. Diabetic Foot Ulcer, Antibiotics, Probiotics, Honey, Multidrug Resistant Pathogens.*

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Introduction

Diabetic Foot Ulcer (DFU) is one of the diabetes mellitus complication where the foot is affected by ulceration as a result of neuropathy and/or peripheral vascular disease (1). The metabolic mechanisms in diabetes mellitus (DM) are diminished and leads to the decrease in the wound healings and increased the risk of infection (2). DFU has been increased significantly over the last years (3), which leads to a decrease the patient's quality of life, and in turn increases the percentage of morbidity and mortality (4). The number of cases with DM has increased by years, for example 30, 177 and 285 million cases have been recorded in 1985, 2000 and 2010 respectively. The number has been expected to be increased to more than 360 million cases by 2030 (3). The diabetic foot considers the important cause of non-traumatic lower extremity amputations and morbidity (5). Identifying the causes of diabetic foot infection (DFI) is very essential for achieving effective treatment including choosing the suitable antibiotic and as well as studying resistance in DFI (6). *S. aureus* and *P. aeruginosa* are most common causes for DFIs (7). Many microorganisms might be isolated from such

patients at the same time like gram positive cocci (e.g. *Peptostreptococcus* spp.), gram negative rods (e.g. *Escherichia, Proteus and Klebsiella* spp.), non-fermentative gram negative bacteria (*P. aeruginosa*) (6). Anaerobe bacteria have also been observed in DFU such as *Bacteroides fragilis*, *Peptostreptococcus* spp, *Clostridium perfringens*, and *Prevotella oralis* (8). Diabetic patients are frequently exposed to foot infections. The physician should select the suitable antibiotic as inappropriate treatment regimen as in the case of polymicrobial infection if a narrow spectrum antibiotic is chosen; there is a possibility of missing a pathogen and potentially leads to a poor therapeutic outcome. Likewise, choosing the unnecessarily broad-spectrum antibiotic leads to antibiotic resistance, and possibly increases the risk of drug toxicity and cost of therapy (9). The probiotic was defined by food and agriculture organization (FAO) and world health organization (WHO) as "Live microorganisms are given in a suitable quantity that are sufficient to deliberate a health benefit to the user."(10). The notable health effect of probiotics is due to the normalization of unbalanced microflora by probiotic strains. Probiotics can be administered for prophylaxis or treatment in order to control the epithelial, mucosal, intestinal, and systemic immune activity which in turn to obtain a beneficial health outcome (11).
In the past, people tried to use honey to treat wounds and ulcers because of its antibacterial and antimicrobial properties that originate from its components. The efficacy of honey has been revealed against methicillin-resistant *S. aureus* (MRSA), and *P. aeruginosa.* The use of honey in wound infection has an advantage in which it does not cause any drug resistance compared to long-term use of antibiotics (12). The antimicrobial activity of honey is due to acidity, osmosis, hydrogen peroxide, and nitric oxide (13).

Materials and methods

Unless otherwise indicated, all chemicals in this study were supplied by Sigma Chemical Co. (Tripoli, Libya). Dehydrated growth media were supplied by Oxoid (Tripoli, Libya) and prepared according to the manufacturer's instructions. API 20E and API *Staph* were obtained from Biomerieux, France.

Study design

This prospective study included 50 consecutive patients with diabetic foot ulcers who were admitted to the Tripoli Medical Center, between February 2019 and October 2020. Demographic and lesion data were recorded for each case, including age, sex, duration of diabetes, duration of diabetic foot, diabetes medications used, features of the lesion and location of the lesion.

Collection, Isolation, and Identification of the Bacterial Samples

Pus samples (50) were collected using sterile disposable swabs. The superficial ulcers were excluded from the study to avoid the possibility of isolating colonizing bacteria. The wound area was first washed with saline, and the samples were collected aseptically from the wound, conditioned in nutrient broth medium. All the specimens were immediately taken to the microbiology laboratory and inoculated on different types of culture media. After inoculation, the media plates were then incubated at 37°C for 24 hours. The isolated bacteria are identified by gram staining of different types of culture. *Klebsiella* and *Bacillus* are identified by capsule and spore staining, respectively.
The following biochemical tests were applied (DNase test and coagulase test for *S. aureus,* indole test for *E. coli,* oxidase test for *P. aeruginosa,* and TSI test to differentiate members of enterobacteriaceae). According to previous tests, both API 20E and API staph were used.

Determination of antibiotics effect on isolated pathogens

Antibiotic susceptibility testing was performed by Kirby Bauer Disk Diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (14). The antibiotics tested for gram-positive isolates were (Amoxicillin/Clavulanic acid, cefoxitin, ciprofloxacin, meropenem, piperacillin, ceftriaxone, and vancomycin) and amoxicillin/clavulanic acid, gentamicin, ciprofloxacin, ceftriaxone, piperacillin, and meropenem for gram negative isolates.

Determining the probiotics and honey on isolated pathogens

Preparation of supernatants from the lactobacillus culture

L. reuteri and *L. acidophilus* were grown in Rogosa broth at 37°C for 72 hrs under microaerophilic conditions. These cultures were centrifuged at 10,000 g for 15 min at 4°C to obtain cell-free supernatant. Supernatants were then filter-

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sterilized by passing through a sterile 0.2 µl pore size filter. The *p*H of the supernatants was adjusted to 6.5 with NaOH. Two different *lactobacillus* supernatants were prepared while phenol 5% and Rogosa media were served as positive and negative controls, respectively (15).

Preparation of supernatant from Saccharomyces culture

S. boulardii were grown in yeast extract peptone dextrose (YPD) broth at 37˚C for 48 hrs. Supernatants were then used in agar diffusion cup-plate method (16).

Agar diffusion cup-plate method

The agar diffusion cup-plate method was applied for the detection of *lactobacillus*, *saccharomyces* supernatants, and honey inhibition activity. Muller-Hinton agar (MHA) was used to investigate the antibacterial activity. The MHA plates were streaked using a bacterial lawn from overnight bacterial cultures containing 1.5 x 10^8 CFUs. The plates were then allowed to dry for approximately 5 minutes. After that, a sterile cork borer was used to prepare five cups of 4 mm diameter in the medium of each Petri dish. An accurately measured 50 µl of the tested conditions (*lactobacillus, saccharomyces supernatants*), phenol 5%, and Rogosa agar were added to the cups on MHA plates, which were previously seeded with the respective bacteria. The study was performed in triplicate. All the plates were then kept at room temperature for effective diffusion of supernatants, and then they were incubated at 37 ± 1 °C for 24 hrs. The diameter of the zone of inhibition around the cup containing the tested conditions will then be measured (15). The same procedure was applied to the two types of honey, Sidr (SH) and Thyme (TH), respectively.

Results

Demographic characteristics

Fifty patients with diabetic foot ulcers were included in the study. Forty (80%) of them were males and 20% were females. The mean age of the patients was 60.80 ± 10.20 years (min 43, max 90). The most serious diabetic complications were neuropathy 40 (80%), retinopathy 22 (44%), and nephropathy 19 (38%). The localization of ulcers was commonly on the left toe (32%), with 28% on the right toes, 26% on the plantar area, and 14% on the dorsal portion. The diabetic foot ulcer was classified according to Wagner classification (Table 1), and the ulcers were found to be in grade 3, 2, and grade 4 in 27 (54%), 21 (42%), and 2 (4%) patients, respectively.

Table 1: Distribution of patients (%) according to the Wagner Classification Systems

Wagner classification	Number of patients (%)
Grade 0	0
Grade 1	0
Grade 2	21 (42%)
Grade 3	27 (54%)
Grade 4	2 (4%)
Grade 5	0

Microbiology

The total number of bacterial isolates was 88. Data summarized in Table 2, 17 (34%) patients' cultures were mono-microbial, and 66% were polymicrobial. Of these 33 patients, 28 (56%) were infected with 2 pathogens, and 5 (10%) with 3 pathogens. Gram-positive bacteria represented 40 (45.4%) while gram-negative bacteria represented 54.5% of the isolates. In mono-microbial infections, gram-positive bacteria 10 (11.3%) were more common than gram-negative bacteria 7 (7.9%), whereas in polymicrobial infections, both gram-positive 30 (34%) and gram-negative bacteria 41(46.5%) were common. The most frequent organisms isolated were *Enterobacteriaceae* (36.5%), followed by *S. aureus* (28.4%), Coagulase negative staphylococci (CoNS) (9%), *P. aeruginosa* (6.8%), *Acinetobacter baumanii* (*A. baumannii*) (4.5%), *Micrococcus luteus* (*M. luteus*) (4.5%) and *Bacillus* spp. (4.5%).

Microbial susceptibilities toward tested antibiotics

The antibiotic sensitivity pattern of the gram-negative bacteria isolated from diabetic foot ulcers is summarized in Table 3. The majority of isolates of *E. coli* were susceptible to ciprofloxacin and gentamicin, but less susceptible to other antibiotics tested, except amoxicillin-clavulanic acid, for which they showed resistance. Similarly, most of the *Proteus* spp. was susceptible to gentamicin, ciprofloxacin, ceftriaxone, piperacillin, and meropenem, while being less susceptible to amoxicillin-clavulanic acid. Similarly, *Klebsiella* spp. *E. cloacae* and *M. morganii* were susceptible to ciprofloxacin, ceftriaxone, gentamicin, and meropenem, while being less susceptible to amoxicillin-clavulanic acid and piperacillin. *Citrobacter* spp. was susceptible to ciprofloxacin, gentamicin, and meropenem, but resistant to other antibiotics tested, except ceftriaxone, in which they were showing susceptibility with *C. freundii*.

Most of the *P. aeruginosa* were showing varying susceptibility to ciprofloxacin, gentamicin, piperacillin, and meropenem, but resistance to amoxicillin-clavulanic acid and ceftriaxone. The majority of *A. baumannii* were susceptible to ceftriaxone, ciprofloxacin, meropenem, and gentamicin, while being resistant to amoxicillin-clavulanic acid and piperacillin. The antibiotic susceptibility patterns of the gram-positive bacteria isolated from diabetic ulcers are shown in Table 4.

Table 2. Bacteria isolated from diabetic foot ulcers

Bacteria	Number of isolates (%)
Gram negative bacteria	
E. coli	8 (9.1%)
E. cloacae	5 (5.7%)
P. vulgaris	5 (5.7%)
P. mirabilis	4 (4.5%)
Morganella morganii	4 (4.5%)
K. pneumonia	4 (4.5%)
K. oxytoca	3 (3.4%)
Citrobacter koseri	2 (2.2%)
Citrobacter freundii	3 (3.4%)
P. aeruginosa	6 (6.8%)
A. baumannii	4 (4.5%)
Gram-positive bacteria	
S. aureus	25 (28.4%)
Staphylococcus lentus (CoNS)	4 (4.5%)
S. saprophyticus (CoNS)	2 (2.2%)
S. epidermidis (CoNS)	2 (2.2%)
M. luteus.	4 (4.5%)
Bacillus spp.	3 (3.4%)

Cons; Coagulase-Negative Staphylococci.

Table 3. Antibiotic sensitivity pattern of Gram-negative bacteria isolated from diabetic foot ulcer.

Bacteria (no of isolates)	Sensitivity pattern (%)					
	CIP	A/C	CTR	PIP	GEN	MRP
E. coli (8)	87.5	0	50	37.5	75	50
E. cloacae (5)	100	20	60	20	80	60
P. vulgaris (5)	60	40	60	80	80	60
P. mirabilis (4)	100	25	50	50	100	75
M. morganii (4)	100	25	50	25	75	100
K. pneumonia (4)	100	50	75	25	100	75
K. oxytoca (3)	100	66.6	100	66. 6	100	100
C. koseri (2)	100	0	0	0	100	50
C. freundii (3)	66. 6	0	66. 6	0	100	66. 6
P. aeruginosa (6)	50	0	0	66. 6	66. 6	50
A. baumannii (4)	25	0	50	0	75	75

CIP- ciprofloxacin, A/C- amoxicillin-clavulanic acid, CTR-ceftriaxone, PIP- piperacillin, GEN- gentamicin, MRP- meropenem

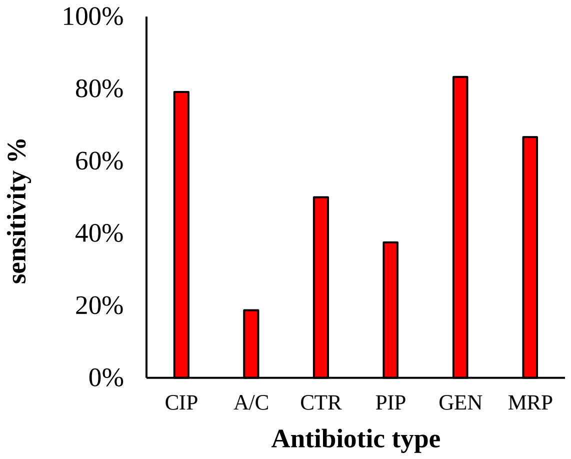

Figure 1. Antibiotic sensitivity patterns of gram-negative bacteria.

Table 4. Antibiotic sensitivity pattern of Gram-positive bacteria isolated from diabetic foot ulcer.

Bacteria (No. of isolates)	Sensitivity pattern (%)						
	CXT	A/C	CTR	MRP	PIP	CIP	VA
S. aureus (25)	40	40	40	40	20	84	48
Staphylococcus epidermidis (2)	0	0	0	0	0	50	0
Staphylococcus saprophyticus (2)	0	0	0	0	0	100	50
Staphylococcus lentus (4)	0	0	0	0	0	75	50

CXT-cefoxitin, A/C- amoxicillin-clavulanic acid, CTR-ceftriaxone, MRP- meropenem, PIP- piperacillin, CIP- ciprofloxacin. VA-vancomycin

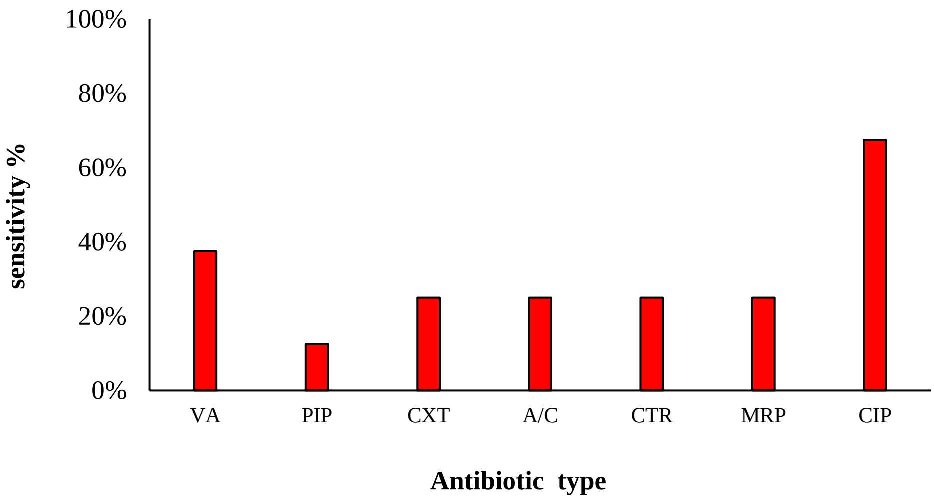

Figure 2: Antibiotic sensitivity patterns of gram-positive bacteria.

The maximum susceptibility of *S. aureus* was shown against ciprofloxacin, intermediate to vancomycin, amoxicillin-clavulanic acid, ceftriaxone, cefoxitin, meropenem, and minimum susceptibility to piperacillin. CON was susceptible to ciprofloxacin and vancomycin, and resistant to other antibiotics tested.

Microbial susceptibilities toward probiotics were tested

The antimicrobial capability of probiotic strains was evaluated using the well-diffusion assay. Zone of inhibition was observed clearly when *E. coli, P. mirabilis, P. vulgaris, M. morganii, K. pneumoniae,* and *C. freundii* were treated with *L. acidophilus* and *L. reuteri.* In contrast, no effect was observed with *S. boulardii* (Figure 3). The three types of probiotics (*L. acidophilus, L. reuteri,* and *S. boulardii*) have been shown to have an inhibitory effect on the growth of *P. aeruginosa* as shown in Figure 3. Conversely, no zone of inhibition was observed with either *E. cloacae or A. baumannii,* which indicated

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their resistance to all of the studied probiotic strains. *K. oxytoca* is affected by *L. reuteri* and *S. boulardii*, whereas *C. koseri* was only affected by *L. reuteri* as presented in Figure 3.

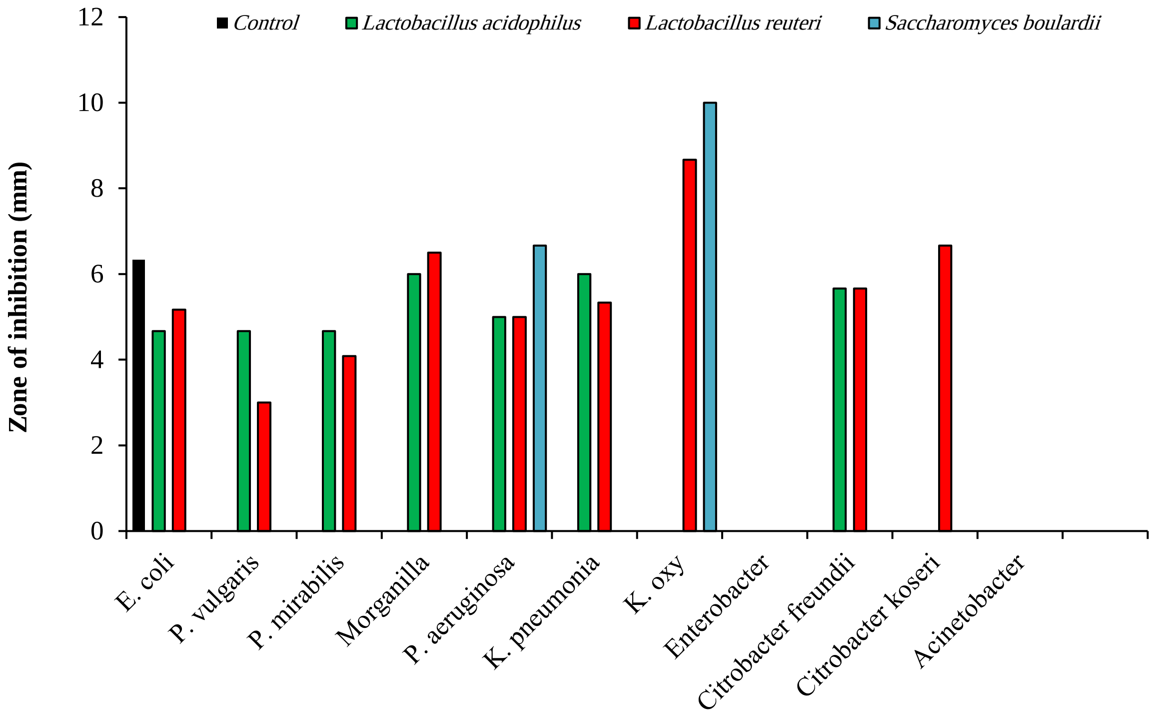

Figure 3*: *Zone of inhibition of different gram-negative isolated strains by bacterial supernatants of tested probiotics by well-diffusion assay. (n=9, p≤0.05).

The three types of probiotics studied have been shown to have a great effect on S. aureus, where large zones of inhibition were observed as shown in Figure 3. (CoNS) Staphylococcus was also inhibited by probiotics except S. boulardii. Both M. leutus and Bacillus spp. were sensitive to only L. reuteri, as clearly explained in Figure 4, respectively.

Microbial susceptibilities toward the honey types tested

The effect of Thyme (TH) and Sidr (SH) honey on different types of Gram-negative bacteria was examined using the well-diffusion assay (Figure 5). The greatest effect of inhibition was noticed on E. coli, P. mirabilis, M. morganii, E. cloacae, *C. freundii*, and *C. koseri*. whereas the least effect was on *P. aeruginosa*, *P. vulgaris*, *A. baumanii*, *K. pneumonia*, and *K. oxytoca*.

It has been shown that both TH and SH had an approximately a similar effect on S. aureus, CONS staphylococci, and M. luteus as presented in Figure 6, respectively. Conversely, Bacillus spp. bacteria were not affected by either of the two honey types.

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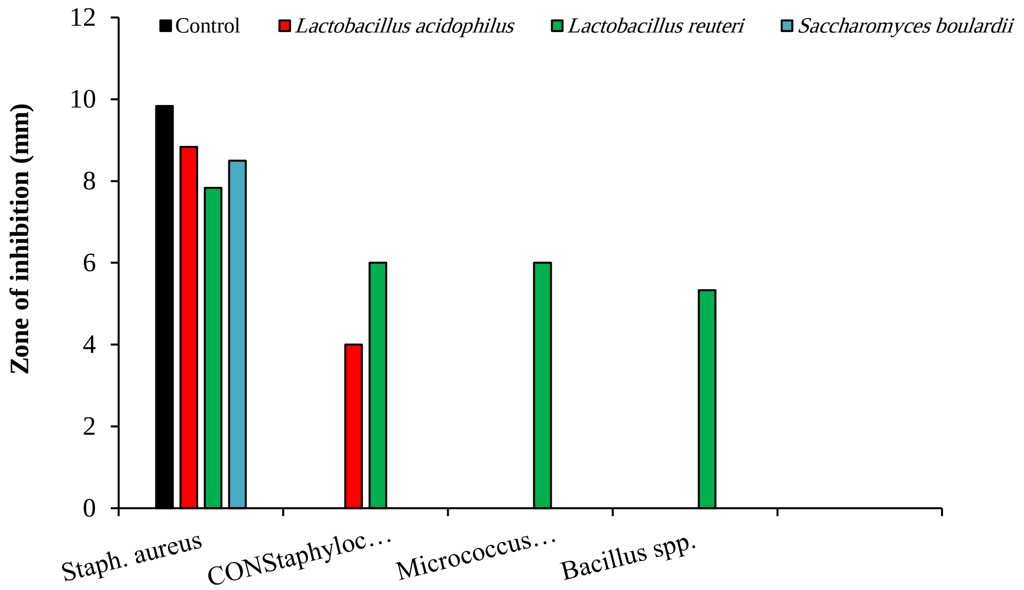

Figure 4: Zone of inhibition of different gram-positive isolated strains by bacterial supernatants of tested probiotics by well- diffusion assay. (n=9, p≤0.05).

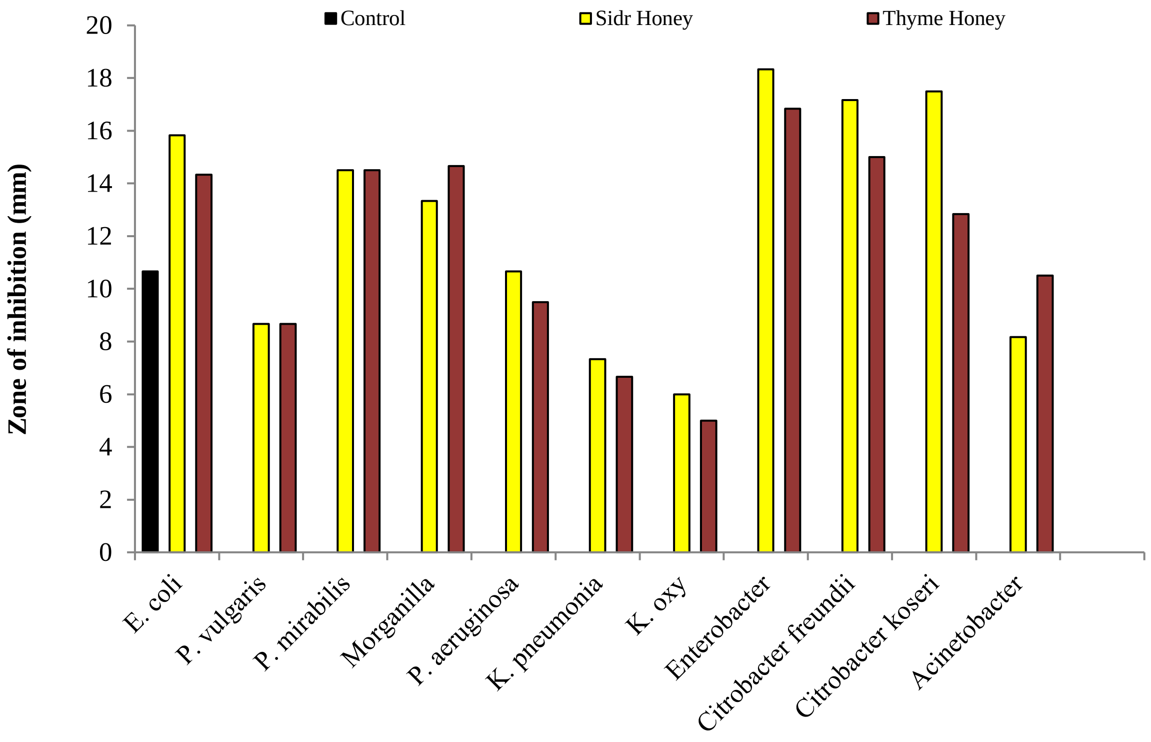

Figure 5: Zone of inhibition of different gram-negative isolated strains by different types of honey using well-diffusion assay (n=9, p≤0.05).

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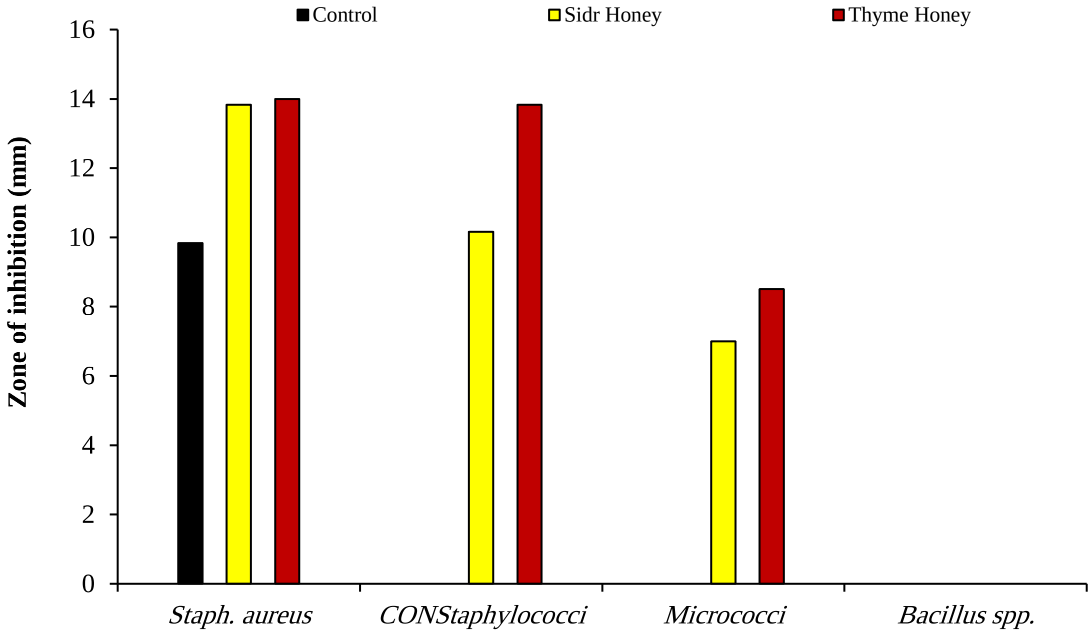

Figure 6: Zone of inhibition of different gram-positive isolated strains by different types of honey using well-diffusion assay (n=9, p≤0.05).

Discussion

Diabetic foot ulcers are considered a global issue affecting most diabetic patients as a result of poor control and incomplete care. Several factors, such as peripheral arterial disease and neuropathy, can cause a delay in wound healing, and thus, patients are more prone to infection (4). In this study, 50 patients with DFUs were included, of whom 80% were males. Although the age group was between 43-90 years, the mean age group was 60 years, with a ratio of 4:1 of males to females. The same result was obtained in another study (Samant *et al*.). (1) in which they explained that the longer nerve length in men leads to twice the possibility of having sensory neuropathy compared to women.

Monomicrobial infections can be treated easily compared to polymicrobial ones, where the treatment is harder as the bacteria form biofilms that hinder the antibiotic activity (1). Our results showed that 34 % of isolates were mono-microbial, whereas 66 % were polymicrobial. The results agreed with those obtained by Perim *et al*., 2015 (17), where the polymicrobial infection rate was 70 %. Conversely, this conflicted with the results obtained by Ozer *et al*., (18) and Hefni *et al*., (8), where 19.2% and 40% of cultures were polymicrobial, respectively. The reasons behind that could be owing to the history of the therapy regimen or the ignoring of anaerobic bacteria. Another possible explanation of the lower infection rate was related to the superficial subcutaneous infections (8, 18). It has been reported that the severe infections are usually connected to polymicrobial cultures (17). Accordingly, in this study, the higher infection rate (polymicrobial infection) could be related to the exclusion of the superficial infection (Grade 1) where the study was concentrated on Grades 2, 3, and 4.

There were slightly more gram-negative bacteria in comparison with gram-positive bacteria, with the percentage of 54.5 and 45.4 %, respectively. This was in agreement with a number of studies where gram-negative bacteria were the predominant pathogens (1, 8, 19). In contrast, a study conducted by Perim *et al* (2015) showed more gram-positive bacteria compared with gram-negative bacteria (17). The results showed a difference in the sensitivity of the tested microorganisms to the used antibiotics, as shown in Tables 3 and 4. Such variation may suggest the superiority of the combination therapy over mono therapy regimen, so that based on the results shown in Tables 4 and 5; we could also assume that monotherapy may not be the best management for causal microbes. Thus, the choice of empiric antibiotic therapy for diabetic foot infections can be based on a number of conditions: (a) the severity of infection, (b) the seriousness and duration of an infection, and (c) the local configuration of bacterial etiology and their antibiotic sensitivity (8). The main therapy to treat DFU is the use of antibiotics to inhibit bacterial infection. However, this kind of treatment is not always effective due to several factors such as the pathophysiological condition of the patient, and the developing of resistance by bacteria. However, number of studies has shown that the use of topical and oral probiotics either before or after surgery can decrease the wound infection and also leads to shorter duration of the course of antibiotic treatment. Furthermore, topical application of probiotics for chronic ulcers and burn infections and can reduce the number of pathogenic bacteria. Therefore, it is worthy to study the use of probiotics for wound infections (20). In this study it is suggested to use probiotics for the treatment of DFUs as an alternative to the use of antibiotic in the future. The antimicrobial activity of probiotics is owing to the metabolic compounds that are produced by lactic acid such as fatty acids, organic acids, hydrogen peroxide, and bacteriocins, which act nonspecifically against broad ranges of pathogens (21, 22).

In addition, *L. reuteri* produces an antimicrobial substance called reuterin, which has activity against both Gram-positive and Gram-negative enteric pathogens (23). The probiotics efficacy was determined by the well diffusion assay method, and inhibition zone diameters were measured against the tested strains. Cell-free supernatants of *L. acidophilus* and *L. reuteri* showed antimicrobial activity against the bacterial strains, including *E. coli, Proteus* spp., *M. morganii*, *K. pneumoniae,* and *C. freundii* isolated from DFU patients. However, the *S. boulardii* does not affect all the previously tested bacteria. The antimicrobial effect of *L. acidophilus*, *L. reuteri* and *S. boulardii* was clearly seen against *P. aeruginosa*. Some studies showed that *P. aeruginosa* had resistance to several drugs due to rapid adaptation and irrational use of antibiotics during therapy. In addition, it has the capability of the formation of biofilm that restricts the efficacy of antibiotic treatment (24, 25). Lactobacillus has the capability to interfere with the invasive Pseudomonas biology. The exact mechanism of probiotics against Pseudomonas pathogens is unclear; however, several possible explanations can clarify this action. The injured tissues may be occupied by probiotic bacteria, which in turn would be a host to pathogenic bacteria, and therefore, prevent them from taking a place in the injured tissue. Another possible reason is that the occurrence of Lactobacillus causes the acidification of the injured tissue environment, which is considered unfavorable media to the growth to *P. aeruginosa* (25). Both *E. cloacae and A. baumannii* showed resistance to all of the studied probiotic strains. It has been reported that these bacteria are the most common cause of nosocomial infection and resist various types of antimicrobial agents. The results showed that *L. reuteri* was more effective against all gram-positive isolates *S. aureus*, CoN *staphylococci*, *M. luteus,* and *Bacillus* spp. The results of this study were in agreement to those obtained by Alatery et al 2015 where they found that *L. acidophilus* had antimicrobial activity against *Proteus* spp., *S. aureus, P. aeruginosa*, and MRSA, which were collected from DFU patients (15). Another study conducted by Abdulla 2014 showed a similar results to those obtained from the present study, where it was found that cell-free supernatants of *L. acidophilus* had varying inhibitory effects against *E. coli, P. aeruginosa, P. vulgaris, S. aureus, S. epidermidis*, and *B. subtilis* (26).

Honey is a natural, cheap product used for the treatment of infected wounds, such as those related to diabetic ulcers. It has antimicrobial activity and does not interfere with wound healing. Honey was also investigated in this study as a probiotic to treat wound infection of DFU. The mechanism of action of the antibacterial activity of honey is due to its high osmolality, low water content, and pH. In addition, it is composed of phytochemical components such as hydrogen peroxide and non-peroxide. The treatment of wounds of diabetic foot ulcers is challenging because of the biofilm that is produced by bacteria. Honey was reported to be effective in treating wound infections resistant to antibiotics as it antagonizes the action of biofilm (27). In this study, the effect of both Thyme and Sidr honey on various types of Gram-negative bacteria was investigated using the well-diffusion assay. It has been noticed that both types of honey had maximum effect of inhibition on *E. cloacae, M. morganii, E. coli, P. mirabilis,* and *Citrobacter* spp. whereas the minimum effect was on *P. vulgaris, P. aeruginosa, A. baumanii and Klebsiella* spp. Also, a similar effect was seen on *S. aureus*, CoNS staphylococci, and *M. luteus*. However, it was found that either of the two honey types did not affect *Bacillus* spp. bacteria. The result of this study was in agreement with that obtained by Shubar et al., 2018, where the honey had a great zone of inhibition against tested bacteria isolated from food ulcer of diabetic patients, namely *S. aureus, MRSA, E. coli, Citrobacter, Proteus, S. epidermis, P. aeruginosa, and Enterobacter* bacteria (28)

Conclusion

DFU is considered one of the complications of DM that interrupts the body's normal healing process, which lead to serious psychological and financial impacts on the patient and the healthcare practice, respectively. Microbial infection is an important factor that causes tissue disturbances, which can lead to unsuccessful wound healing. Our study has showed that 66% of diabetic foot infections were polymicrobial. The most generally identified Gram-negative and Gram-positive microorganisms were *E. coli* and *S. aureus,* respectively. Ciprofloxacin was the most effective treatment against both Gram-positive and Gram-negative bacteria. Effective antibiotic treatment should depend on the severity of the infection, etiology of the disease, and it is antibiogram. It was concluded that both probiotics and honey had antibacterial activity against the tested bacteria isolated from DFU patients, with a greater effect observed with honey compared to the probiotics. Both types of honey (Thyme and Sidr) have similar findings in terms of their antibacterial effect. Although probiotics had less activity compared to honey, it was better to use probiotics in conjunction with honey, as probiotics were reported to have an immunomodulation effect. The results were optimistic in terms of using honey to treat DFU, as it is available and cheap compared to antibiotics since the latter has the additional problem of drug resistance and therapy failure.

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