

Original Research Article

<http://dx.doi.org/10.20546/ijcmas.2016.507.047>

Microorganisms Responsible of Diabetic Foot Infection in Taiz City, Yemen

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ABSTRACT

Diabetic foot infections (DFI) are normally caused by microorganisms. They are usually inadequately managed due to misunderstanding of microbial prevalence and therapeutic approaches. This study aimed to detect pathogenic microorganisms in diabetic foot infection (DFI) of some Yemeni patients in Taiz City, and to investigate the sensitivity of isolated microorganisms to different antibiotics. Eighty samples were collected from (DFI) patients admitted in AL-Thowra hospital, and Al-Gomhory Hospital in Taiz City through study period. The rates of patients who got diabetic foot infection were 61% males and 39% females. Their ages range between 32 – 85 years. The ratio of patients who got amputation was 26%. Gram positive bacteria were responsible of 67% of DFI cases followed by Gram negative bacteria in 28% and yeasts in 5% of cases. The most commonly isolated microorganisms from the diabetic foot ulcers were *Pseudomonas aeruginosa* (37.8%) and *Staphylococcus aureus* (18.9%), followed by *Escherichia coli* (11.1%), *Klebsiella pneumoniae* (11.1%) and *Candida albicans* (5.6%). Other isolates were recorded in low frequencies.

Keywords

Diabetic foot infections, microorganisms, DFI, Taiz, Yemen.

Article Info

Accepted:

15 June 2016

Available Online:

10 July 2016

Introduction

Diabetes Mellitus (DM) is a progressive disease worldwide and remains an important cause of morbidity, mortality and major lower extremity amputation at some stage of life (Caputo *et al.*, 1997). The number of diabetic patients (DP) worldwide in 2007, about 246 million infected, and is expected to amount to more than 350 million in 2025 (Rubaiian, 2008).

Diabetes is a chronic disease that affects on about 14 million Americans and increasing in population to the point where public

health authorities are calling diabetes an "epidemic" that requires argent attention. Every year, 600,000 additional cases are diagnosed (Bennett, 1999). Diabetes drain 33% of health budgets in European countries and cost the United State of Americans (USA) 115 billion dollars (\$) annually. In kingdom Saudi Arabia, the rate of infection 24.7% a year and annual growth rate of DP are about 1% (Rubaiian, 2008).

Diabetic foot infection (DFI) defined as the disease caused by a microbial pathogen that

occurs when the presence of replicating organisms is associated with tissue damage (White, *et al.*, 2001). Other definition, DFI is the presence of multiplying bacteria in body tissues, resulting in spreading cellular injury due to competitive metabolism, toxins, intracellular replication, or antigen-antibody response (Ayton, 1985).

DFI causes a major public health problem and impose a heavy burden on health services and own family. It is responsible on patient hospital admission, drain of future earning power, major foot amputations, disability, loss work, family fragmentation, and eventually death (Caputo *et al.*, 1997). In USA in 2001, over one million amputations per year, every 30 seconds a leg is lost, 50% amputees undergo second amputation, and 50% amputees die 2-5 years after amputation (Johnson and Citron, 2002).

In 1990, over 54000 lower extremity amputations accrued among diabetics (8.3 per 1000). Medical cost of treatment of the DF \$85 billion (average of \$ 45,000 per patient) (Josef and Nesbit 1998).

In kingdom Saudi Arabia, the direct cost of DM is expected (5.9) billion riyals a year. DM is first cause for the foot amputations and depression, 20% of the mothers after the pregnancy is development of diabetes and 9% of patients die annually (Rubaiian, 2008).

Microorganisms can cause the ulcers of DM patients to become very inflamed, sore and delay healing include: aerobes bacteria such as *Streptococcus pyogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Escherichia coli*, *Klebsiella pneumoniae*. Anaerobes bacteria are *Clostridium perfringens*, *Bacteriodes fragilis* and yeast is *Candida albicans*.

Infections in patients with diabetes are difficult to treat because these patients have weakness in microvascular circulation, which limits the access of phagocytic cells to the infected area and results a poor concentration of antibiotics in the infected tissues (Marble and Alexander, 1985).

Detection of pathogenic microorganisms in diabetic foot infection (DFI) of some Yemeni patients.

Specific objective's

To study the relative frequency of bacterial isolates cultured from diabetic foot infections. And to determine the effect of some medicinal plant extracts collected from Taiz province and some chemical antiseptics on isolated microorganisms.

Materials and methods

Sample size: A total of 80 patients suffered from DFI were enrolled in this study. The age of the studied patients ranges from 32 to 85 years old.

Setting: This study was conducted in AL-Thowra, and Al-Gomhory Hospitals in Taiz city were the patient's admission to these Hospitals and the specimens were transported to Central Public Health Laboratory Taiz Branch - Yemen to isolate microorganisms.

Study design: A cross sectional descriptive study was conducted during the period from December 2007 till November 2008.

Sampling techniques: Both the ulcer and surrounding skin area were cleaned using swab soaked in 70% of ethanol alcohol. Two sterile dry cotton wool swabs were used to collect samples of purulent secretions from DF ulcer. The swabs were moistened with

sterile physiological saline and rubbed on the wound surface, then one of the swabs was placed into sterile bottle container contain Cooked Meat broth medium, labeled with the date, time of collection, the patient's name, number of sample, and incubated for 24-48h at 35-37 °C and inspected daily for growth. The other swab was placed into sterile plastic container, labeled with the date, time of collection, the patient's name, and number of sample. The specimens sent to the laboratory with accompanied request form that belongs to the patient. Specimens were cultivated as soon as possible (Cheesbrough, 2000 and Slater, *et al.*, 2004).

Culturing and isolation of the specimens

If found growth in Cooked Meat broth medium was inoculated on to two culture media as following: Neomycin blood agar plate for isolation of anaerobic bacteria such as *C. perfringens* and *B. fragilis*. Other blood agar plates for the isolation other anaerobic bacteria and incubated in anaerobic jar for 48h at 35-37 °C and inspected daily for growth (Vandepitte, 2003).

The other swab was cultivated as soon as possible onto a blood agar plate for the isolation of Gram-positive and Gram-negative bacteria and MacConkey agar plate for the isolation of Gram-negative bacteria and incubated aerobic for 24-48h at 35-37°C (Frykberg, 2003).. Microorganism that was isolated from (DFU) may belong to almost any group or species. The following steps will provide a framework within us able to identify any bacterial species.

Effect of some plant extracts on the growth of the isolated bacteria

Eleventh species of medicinal plants were collected from Taiz Government shows in Table 1.

The part of plants used were aerial parts in all plants, except *Juniperus procera* plant and material used was exudates of plant (Aldbai and Alkhalidi, 1996; Environmental Protection Authority, 2002 and Tomei, *et al.*, 2003).

Collected plants were air-dried at room temperature, ground into powder by using a sterile electric grinder. The powder was extracted by solubilization method as following:

Water extraction

Cool water extraction: ten grams of powder plant added to 100 ml of cool distilled water and extracted by shaking at room temperature for 8h daily to three day. Extract was dried by evaporator on slow heat.

Heat water extraction: ten grams of powder plant added to 100 ml of heat distilled water and extracted by shaking at 60 °C for 8h daily to three day. Extract was dried by evaporator on slow heat (Lazrek, *et al.*, 2005).

Methanol extraction: Ten grams of powder plant were dissolved with 100 ml methanol (99%) and extracted by shaking at 50 °C for 2h daily to three day. Extract was dried by evaporator at 60 °C. One Hundred disks were Prepared (5.25 mm diameter) of whatman filter paper by used borer. Put each 100 disk in test tub then closed it and sterilized by dry temperature at 140°C to 1h.

Dissolved 1g of extracted material in 10 ml of solvent (distilled water or methanol), then taken 1 ml of solution and input to disks in test tub. Each disk had 1g of extracted material.

Statically analysis

Analysis of the data was performed by SSPS

version 12. Statistical significance was set at Asymp. Sig. < 0.05 was considered significant.

Results and Discussion

Distribution of DFI cases according to the age and sex

The distribution of DFI cases according to the age and sex is shown in Table 2. In general, the patients were elderly their age ranged from 32 to 85 years old. The highest distribution of DFI cases were found in age group 61-70 years (30%) of both males and females equally, followed by age group 51-60 years (26.3%) were difference in ratio male (17.5%) to females (8.7%) and age group 41-50 years (23.7%) were similar in both gender.

The relationship occurrence of DFI with respect to age and gender of the patients was without statistical significant.

Microorganisms isolated from DFI

Out of 90 isolates, the Gram-negative bacteria was presented in 60 (66.7%) isolates, followed by gram-positive bacteria 25 (27.7%), and the lowest number found in yeast 5 (5.6%) isolates.

A total of 90 pathogenic microorganisms were isolated from 80 DFI cases. The organisms that were isolated from DFI are presented in Table 3. The most commonly isolated bacteria and fungi from the diabetic foot ulcers were *P. aeruginosa* 34 (37.8%), *S. aureus* 17 (18.9%), *E. coli* 10 (11.1%), *K. pneumoniae* 10 (11.1%), *C. albicans* 5 (5.6%), *P. mirabilis* 4 (4.4%) and Group β Streptococci 4 (4.4%). *B. fragilis*, *S. epidermidis* and *C. perfringens* were isolated in low frequency (2.2% for each). The relationship between microorganisms isolated was significant.

Microorganisms isolated from monomicrobial growth cultures

Table 4 shows 70 (87%) of patients had monomicrobial infection. The highest prevalent of microorganisms isolated from DFI patients with one isolated microorganisms were *P. aeruginosa* isolated from 31 patients (44.3%) which isolated equally in both gender or rise relatively in females than males followed by *S. aureus* isolated from 13 patients (18.6%) and rise relatively in males than females. *E. coli* recovered in 10 patients (14.3%), *K. pneumonia* in 9 patients (12.8%), and *C. albicans* in 5 patients (7.1%). The low frequency was Group β Streptococci recovered in 2 patients (2.9%). Distribution of monomicrobial growth according to gender was without statistical significant.

Microorganisms isolated from polymicrobial growth cultures

Polymicrobial growth cultures were found in 10 (13%) of patients as shows as in Table 5. The most commonly isolated organism from polymicrobial growth was *S. auerus* associated with *P. mirabilis* in two patients 20%, with *S. epidermidis* in one patient 10% and with *B. fragilis* in one patient 10%. *P. aeruginosa* associated with *P. mirabilis* in two patient 20% and with *B. fragilis* in one patient 10%. *K. pneumonia* associated with *S. epidermidis* in one patient 10%. Group β Streptococci associated with *C. perfringes* in two patients 20%.

Effects of some medicinal plant extracts on isolated microorganism

The effects of some medicinal plant extracts were studied against 4 common isolated bacteria from DFI patients (*P. aeruginosa*, *S. aureus*, *E. coli* and *K. pneumonia*). From 11 medicinal plants extracts, 9 plants extracts had no activity against the 4 isolated

bacteria. These plants are *Jatropha curcas*, *Calotropis procera*, *Ziziphus spina-christii*, *Copparis catilaginea – decne*, *Pulicaria undulate*, *Tribulus terrestris*, *Withania somnifera*, *Chenopodium murale* and *Pergularia tomentosa*.

Kleinia adora and *Juniperus procera* plants, aqueous and methanolic extracts showed antibacterial activity against tested bacteria. These extracts had activity against tested bacteria (inhibition zone was surrounded discs and measured in mm) when plant extracts were at concentration only. The cold water extract of *Kleinia adora* had high activity against isolated bacteria, while methanolic and hot water extracts had low activity on isolated bacteria.

The methanolic and hot water extracts of *Juniperus procera* plant were more effective against isolated bacteria from cold water extract.

The plants extracts that had no activity against the 4 isolated bacteria, Then did mixed of medicinal plant extracts together as following; Two medicinal plant extracts were mixed together then tested and mixed third extract with both then tested...etc respectively. These serials mixed were studied against microorganisms isolated. The results were also negative in all isolated microorganisms.

The big problem was that bacteria do not respond well to antibiotic treatment due to high numbers of microorganisms' colonization in foot. The type, and dose of antibiotic that used for treatment DFI, must be suitable with the microbial colonization (Lipske, *et al.*, 2005).

This study showed that patients were elderly and their ages ranged from 32 to 85 years. Similar observation was also reported in Osztyal, Saudi Arabia, Kuwait and in Nepal

by Kajetan, *et al.*, (1995); El-Tahawy, (2000); Abdulrazak, *et al.*, (2005) and Sharma, *et al.*, (2006). 49 (61%) of patients were males and 31(39%) were females. The males were more infected with DFU due to males don't care in their foot and more travel than females. These findings consistent with previous study in Osztyal (Hungary) in ratio of males to females and their ages (Kajetan *et al.*, 1995).

Concerning age groups, the ratio of males to females were similar in the age group 61-70 year, and age group 41-50 year, and difference in age group 31-40 year and age group >71 year (Table 2). This similar and difference between rates males to females in several age groups of DF patients may be refer to number of males to females, found previous fungal skin infection among fingers or on back of the foot and debilitated patients.

The general objective in this study was to detection of pathogenic microorganisms in diabetic foot infection (DFI) of some Yemen patients. In this study bacteria and fungi that cause DFI were studied and the results showed that all patients have one or more pathogenic microorganisms. Similar finding were also reported in Saudi Arabia (El-Tahawy, 2000), in Kuwait (Abdulrazak, *et al.*, 2005), and in Nepal (Sharma, *et al.*, 2006) who found that DFI patients had one or more pathogenic microorganisms. Refer to elevated of carbohydrates level in blood and body cells (hyperglycemia) and foot complications were the main reasons for these infections. The microorganism that isolated from DFI were *P. aeruginosa*, *S. aureus*, *E. coli*, *K. pneumonia*, *C. albicans*, *P. mirabilis*, Group β Streptococci, *B. fragilis*, *S. epidermidis* and *C. perfringes* (Table 5).

DF ulcers were exposed to skin commensals, and there microflora

represented the surrounding environment. The contaminated microbes can quickly become established within a wound, reaching a state of colonization (Palumbo and Melton, 1985).

Aerobic Gram-positive bacteria including *S. aureus*, *S. pyogenes*, strict anaerobic bacteria and aerobic Gram-negative bacilli frequently cause infections in patients with diabetes (Joshi, 1999). Some bacteria (such as *Enterococcus* spp.) were reported in Kuwait study (Abdulrazak, *et al.*, 2005), but in this study this bacteria was not isolated from DFI.

The first goal in this study was to study the relative frequency of bacterial isolates cultured from diabetic foot infections. In this study, Gram-negative bacilli were the dominant pathogens found in 66.7% of DFU cases, while Gram-positive bacteria were isolated from 27.7% of the cases and yeasts were isolated from 5.6% of the cases as shown as in Figure 6. These rates of isolated pathogens were contrasted compared with previous studies in Osztaly, Saudi Arabia, Kuwait and in Nepal (Kajetan, *et al.*, 1995; El-Tahawy, 2000; Abdulrazak, *et al.*, 2005 and Sharma, *et al.*, 2006).

Table.1 Medicinal plants used in this investigation against isolated bacteria

Scientific name	Family name	Arabic name
<i>Jatropha curcas</i>	Euphorbiaceae	Sharpa
<i>Calotropis procera</i>	Asclepiadaceae	Ushar
<i>Ziziphus spina-christii</i>	Rhamnaceae	Sidr
<i>Copparis catilaginea – decne</i>	Copparidaceae	Rusaf
<i>Pulicaria undulate</i>	Asteraceae	Jeth Jath
<i>Tribulus terrestris</i>	Zygophyllaceae	Hasak or Qutiba
<i>Withania somnifera</i>	Solanaceae	Ubab
<i>Chenopodium murale</i>	Chenopiaceae	Zurpikh
<i>Pergularia tomentosa</i>	Asclepiadaceae	Ghulqa
<i>Juniperus procera</i>	Cupreffaceae	Ferwish
<i>Kleinia adora</i>	Asteraceae	Adhkher

Table.2 Distribution of DFI cases according to the age groups and sex.

Age groups (years)	Male (n= 49)		Female (n= 31)		Total of cases (n= 80)		P Value	df	Asymp. Sig.
	N.	%	N.	%	N.	%			
< 30	0	0	0	0	0	0	6.122	5	.294
31-40	5	6.2	2	2.6	7	8.7			
41-50	10	12.5	9	11.3	19	23.7			
51-60	14	17.5	7	8.7	21	26.3			
61-70	12	15	12	15	24	30.0			
>71	8	10	1	1.2	9	11.2			
Total	49	61.2	31	38.8	80	100			

Table.3 Frequency of pathogenic microorganism isolated from 80 DFU patients.

Microorganism	Frequency	Percent	P_ Value	df	Asymp. Sig.
Gram-negative bacteria	60	66.7	117.29	9	.00
<i>Pseudomonas aeruginosa</i>	34	37.8			
<i>Escherichia coli</i>	10	11.1			
<i>Klebsiella pneumonia</i>	10	11.1			
<i>Proteus mirabilis</i>	4	4.4			
<i>Bacteriodes fragilis</i> (anaerobic)	2	2.2			
Gram-positive bacteria	25	27.7			
<i>Staphylococcus aureus</i>	17	18.9			
Group β Streptococci	4	4.4			
<i>Clostridium perfringes</i> (anaerobic)	2	2.2			
<i>Staphylococcus epidermidis</i>	2	2.2			
Yeasts: <i>Candida albicans</i>	5	5.6			
Total	90	100			

Table.4 The frequency of microorganisms isolated from 70 patients had monomicrobial culture growth.

Microorganism	Females		Males		Total		P_ Value	df	Asymp. Sig.
	N.	%	N.	%	N.	%			
<i>P. aeruginosa</i>	16	22.9	15	21.4	31	44.3	4.59	5	.47
<i>S. aureus</i>	6	8.6	7	10	13	18.6			
<i>E.coli</i>	2	2.9	8	11.4	10	14.3			
<i>K. pneumonia</i>	3	4.3	6	8.6	9	12.8			
<i>C. albicans</i>	1	1.4	4	5.7	5	7.1			
Group β Streptococci	1	1.4	1	1.4	2	2.9			
Total	29	41.4	41	58.6	70	100			

Table.5 Frequency of microorganisms isolated from 10 patients had Polymicrobial growth cultures

Polymicrobial infection	No. of cases	%	P_ Value	df	Asymp. Sig.
<i>S. aureus</i> + <i>P. mirabilis</i>	2	20	1.200	6	.977
<i>P. aeruginosa</i> + <i>P. mirabilis</i>	2	20			
Group β Streptococci + <i>C. perfringens</i>	2	20			
<i>S. epidermidis</i> + <i>K. pneumonia</i>	1	10			
<i>P. aeruginosa</i> + <i>B. fragilis</i>	1	10			
<i>S. aureus</i> + <i>B. fragilis</i>	1	10			
<i>S. aureus</i> + <i>S. epidermidis</i>	1	10			
Total	10	100			

Table.6 Effect of *Kleinia adora* and *Juniperus procera* plant extracts on some isolated bacteria (inhibition zone in mm)

Bacteria	<i>Kleinia adora</i>			<i>Juniperus procera</i>		
	C.W extract	H.W extract	Methanolic extract	C.W extract	H.W extract	Methanolic extract
<i>P. aeruginosa</i>	29	15	14	10	28	30
<i>S. aureus</i>	30	12	13	12	31	30
<i>E. coli</i>	28	17	16	9	29	29
<i>K. pneumonia</i>	29	14	14	13	28	29

Increase in ratio of Gram-negative in this study may be refer to immunocompromised were highly susceptible to hospital-acquired infections, either after colonization with environmental strains or followed invasive surgical, and exert their pathogenic effects by producing endotoxin (Hunt, 2003).

This study showed that the most commonly isolated microorganisms among DFI patients were *P. aeruginosa* 37.8% of patients followed by *S. aureus* in 18.9% of patients. Patients who had received prolonged or

inappropriate or broad-spectrum antibiotics or had long hospitalization, chronic wound or surgical procedure were most likely to have infection and bacterial colonization such as *P. aeruginosa* and multiple resistant *S. aureus* (Vandepitte, 2003). These *S. aureus* infections were able to develop due to exposure to the contaminated hands of caregivers rather than to the overuse of antibiotics (Hartemann, *et al.*, 2004).

Basset, *et al.*, found that *P. aeruginosa* infection is especially more prevalent among

patients with ulcers and intravenous drug addiction (Basset, *et al.*, 1973). Whereas sneakers to ulcer and produce several substances that are thought to enhance the colonization and infection of host tissue. These substances together with a variety of virulence factors, including lipopolysaccharide, exotoxin A, leukocidin, extracellular slime, proteases, phospholipase, and several other enzymes make *P. aeruginosa* the most clinically significant bacteria among DFU patients (Bodey, *et al.*, 1983).

In Saudi Arabia, El-Tahawy, (2000) found that *S. aureus* was the commonest isolate being recovered from 28% of cases and *P. aeruginosa* 22% of cases. Low *P. mirabilis* and *S. aureus* isolated rates in this study may refer to patients who had received antibiotics before specimen collection.

In this study found that rate *E. coli* isolated of DFI was in concurrence with Kuwait study with little different in number of study patients (Abdulrazak, *et al.*, 2005), and in contrast, in rate *K. pneumonia* isolated was low in Kuwait study may be attributable to used antibiotics, number of patients, site of specimens collection, samples collected methods, media used for isolation and identification criteria.

The results of the present study showed that two anaerobic bacteria were isolated during this investigation; *B. fragilis* 2.2% and *C. perfringens* 2.2%. This finding is in agreement with (Kajetan, *et al.*, 1995; Calhoun, *et al.*, 2002; Slater, *et al.*, 2004 and Hartemann, *et al.*, 2004) who found that the isolation rate of anaerobic bacteria was very low. Low isolation rates of anaerobic bacteria may be due to the depth of infection and previous treatment of patients with multiple antibiotics (Hartemann, *et al.*, 2004).

The results of the present study found that 5.6% of patients had fungal infection. The only isolated fungi was *C. albicans*. Similar observations were also reported by (Kajetan, *et al.*, 1995; El-Tahawy, 2000 and Abdulrazak, *et al.*, 2005).

Our study showed that the polymicrobial infections were low 13% than monomicrobial infections 87% as shown as in Table 6 and 7. Low rate of polymicrobial isolates may be due to competitive metabolism, toxins, intracellular replication, or antigen-antibody response (Ayton, 1985).

In this study it was found that most of the polymicrobial infections were caused by *P. aeruginosa* with *B. fragilis* and *S. aureus* with *B. fragilis* and Group β Streptococci with *C. perfringens*. The bacteria are thought to be synergistic and form biofilms on the surface of chronic wounds (Gerding, 1995). This allows anaerobes to survive on wound surfaces and supports growth of bacteria not normally considered pathogenic (Bowler and Davies, 1999). Decrease rates of polymicrobial infection and isolated pathogens in DFI patients may be attributable to the lack of severity of some infections and the low virulence of isolated microorganisms (Abdulrazak, *et al.*, 2005).

The second goal in this study was to determine the effect of some medicinal plant extracts collected from Taiz province and some chemical antiseptics used in Taiz Hospitals against some isolated microorganisms. The medicinal plant extracts of *Jatropha curcas*, *Calotropis procera*, *Ziziphus spina-christii*, *Copparis catilaginea – decne*, *Pulicaria undulate*, *Tribulus terrestris*, *Withania somnifera*, *Chenopodium murale* and *Pergularia tomentosa* were inactive against most common isolated bacteria *P. aeruginosa*, *S. aureus*, *E. coli* and *K. pneumoniae*.

These findings may be attribute to these plants don't have any active compounds which effect on tested bacteria, used methods in extraction, and time period of plants collection. While water plant extracts of *Kleinia adora* and *Juniperus procera* had activity against tested bacteria.

The cold water plant extracts of *Kleinia adora* was active against isolated bacteria, while methanolic and hot water extracts had slight affect on isolated bacteria. This may be referring to insoluble active materials with methanol or hot water.

In contrast the methanolic and hot water extracts of *Juniperus procera* plant were more effective against isolated bacteria from cold water extract (Tomei, *et al.*, 2003; Lazrek, *et al.*, 2005 and Rustaiyan, *et al.*, 2006).

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How to cite this article:

Mohammed A. Al-Hegami, Saeed M.S. Alghalibi and Abdullah Al-Mamari. 2016. Microorganisms Responsible of Diabetic Foot Infection in Taiz City, Yemen. *Int.J.Curr.Microbiol.App.Sci.* 5(7): 431-441. doi: <http://dx.doi.org/10.20546/ijcmas.2016.507.047>