Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 5, 2021, Pages. 187-192
Received 15 April 2021; Accepted 05 May 2021.

Isolation of Bacteria from Diabetic Foot Patients in Hospital of Al-Dewaniyah City, Iraq

ThurayaAamerHabeeb[1], Laila JasimShaebth[2], and Nawres Adnan Abdulameer[3]

[1,2,3]Technical Institute of Al-Diwaniyah, Al-Furat Al-Awsat Technical University (ATU), Iraq.
[1]dw.thr@atu.edu.iq
[2]laila84@atu.edu.iq
[3]dw.noras@atu.edu.iq

Abstract.
The World Health Organization defines the diabetic foot as a "situation of infection, ulceration or also destruction of tissues depths of the feet, associated with abnormalities neurological and varying degrees of vascular disease peripheral in the lower limbs of patients with Diabetes Mellitus. The objective of the work was identify the prevalence of microorganisms that caused infections in diagnosed patients with diabetic foot treated at the Regional Al-Dewaniyah Hospital, Iraq, during the year 2020. Descriptive, cross-sectional and retrospective study. The sampling was non-probabilistic, for convenience, and 115 samples corresponding to 94 patients. Of the 94 patients with diabetic foot, 52% were mens. 25% of infections occurred in patients aged 51 to 60 years. 21 different microorganisms were isolated in the 115 samples. 80% (75) were monomicrobial, and 20% (40) polymicrobial. Among the Gram positives, the most frequently isolated microorganism was the Staphylococcus aureus 19% (22) and Enterococcus spp. 6% (6) and among the Gram negative were the Klebsiella pneumoniae13% (16) and Acinetobacter spp. 12% (14). The results of sensitivity tests antimicrobial agents showed that 100% of the S. aureus strains were resistant to Oxacillin and high resistance of K. pneumoniae strains to Cephalosporins. Acinetobacter Strains spp. were 100% resistant to cephalosporins and Piperacillin. Isolated microorganisms and antimicrobial resistance profile as the same present coincide with the bibliography, and it is very important to implement prevention programs this pathology in order to avoid amputations in this type of patients.

Keywords: Bacteria; Foot; Diabetic.

INTRODUCTION:

Diabetes Mellitus (DM) is one of th emajor public health problems due to its high prevalence, morbidity, mortality and high healthcare costs that represent[1]. Condition where the body is unable to controlblood sugar, which can be defined as "A chronic condition that is triggered when the body loses its ability to make enough insulin or to use it effectively " [2,3]. A diabetic does not absorb glucose properly, sothis remains circulating in the blood (hyperglycemia),damaging tissues over time. Eastdeterioration causes health complications that they can be potentially lethal[3]. It is classified into 3 main types; DM type 1 (DM1),type 2 (DM2) and gestational, which present with hyperglycemia, causing acute complications andsevere, chronic, macrovascular, microvascular, can cause myocardial infarction, accident vascular brain, kidney failure, blindness, injury peripheral nerves (diabetic neuropathy) and amputations [2].Amputations and foot ulcers are frequent complications in diabetics, where the risk of lower limb amputation is approximately 40 times greater than in the population general. Mortality related to amputation immediate is estimated at 19% and survival is 65%in three years and 41% in five years. This complication is known as "diabetic foot", occupying one ofthe first places among the main problemshealth, and it is estimated that by the year 2025 the total of affected with this disease will amount to 300millions of people around the world [4]. The World Health Organization (WHO) definesthe diabetic foot as a "situation of infection, ulceration or also destruction of tissues depths of the feet, associated with abnormalitiesneurological and varying degrees of vascular diseaseperipheral in the lower limbs of patientswith DM "[5]. Diabetic foot infections aremost frequently due to microorganisms from the genus Staphylococcus spp. and to a lesser extentby Streptococcus spp. Most infections are polymicrobial, and more than 50% of ulcersinfected contain Gram negative rodsaerobic and anaerobic, promoting the development ofa rapid and progressive wet gangrene that does notprompt treatment can be fatal[6]. pathognomonic sign of fulminant infection may be subcutaneous emphysema, although this alsocan occur in diabetics with infectionscaused by less virulent microorganisms,such as Escherichia coli and other coliforms[6].Thedisease is

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 5, 2021, Pages. 187-192
Received 15 April 2021; Accepted 05 May 2021.

mainly concentrated in the segment of the elderly, although currently there are also young people and even children whosuffer, which is due to the little lifestyle healthy that are maintained, in which sedentary lifestyle and poor diet (7). In the kids and adolescents is more common type 1 that has a incidence of 1.8 per 100,000 inhabitants, which represents between 28 and 30 new cases each year (7).This indicates that there is a high probability that these patients could develop diabetic foot, such as consequence of DM.

MATERIALS AND METHODS

Descriptive, cross-sectional and retrospective study from January 1 to December 31 of the year2020. Non-probability sampling, for convenience.115 samples were included corresponding to 94diabetic patients, reported as carriers standing diabetic. The samples were grown on Mac Conkey agar, 5% Sheep Blood Agar and Chocolate Agar, the sowing method used was by depletion, Identification of genus and species was carried out by conventional biochemical tests. The bacteria Gram negatives were identified using the Oxidase, TSI, Citrate, Orinithine, Lysine, SIM, Urea and Phenylalanine and Gram positives were identified from tests for catalase, coagulase, bileesculin, specific latex for Staphylococcus aureus, Orinithine and Polymyxin B. For the susceptibility tests, the technique was used Kirby-Bauer. The antibiogram for Gram germspositive was performed using antibiotics Ciprofloxacin, Clindamycin, Erythromycin,Cefoxitin, Rifampicin and Vancomycin. In the group of Gram negatives, the antibiotics used were Ampicillin, Ceftazidime, Amoxicillin / Ac.clavulanate, Cefotaxime, Cefepime, Ciprofloxacin,Imipenem, Meropenem, Piperacillin and Colistin.

RESULTS

115 samples were analyzed from 94 diabetic patients.25% (23) of the patients were aged 51 to60 years, being the most common age group, and of the 94 patients with DM, 52% (49) were men and 48% (45)they were women. In both sexes, the highest frequencyof bacterial infections occurred in the group ofage 51 to 60 years. (Table 1).

Table 1. Percentage distribution by age category and sex of bacterial infections in patients with diabetic foot.

Ages	Men		Women		Total	
	n	**%**	**n**	**%**	**n**	**%**
21-30	**4**	**8.2**	**4**	**8.9**	**8**	**9.0**
31-40	**4**	**8.2**	**2**	**4.4**	**6**	**6.0**
41-50	**9**	**18.4**	**11**	**24.4**	**20**	**21.0**
51-60	**12**	**24.5**	**11**	**42.4**	**23**	**25.0**
61-70	**11**	**22.4**	**4**	**8.9**	**15**	**15.0**
71-80	**3**	**6.1**	**7**	**15.5**	**10**	**11.0**
81-90	**6**	**12.2**	**4**	**8.9**	**10**	**11.0**
> 91	**-**	**-**	**2**	**4.4**	**2**	**2.0**
Total	**49**	**100**	**45**	**100**	**94**	**100**

In the 115 samples analyzed, 21 types were isolated of different microorganisms. It was infections monomicrobial 80% (75) and polymicrobial the 20% (40). Of the polymicrobial cultures, 17% (16)presented 2 concomitant microorganisms and a3% (3) presented 3 infecting microorganisms.58% (12) of the microorganisms were classified as Gram negative bacteria (GNB), and 42% (9) asGram positive bacteria (BGP). The microorganism most frequently isolated was S. aureus 19% (22) followed by Klebsiellapneumoniae 13% (19) (Table 2).

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 5, 2021, Pages. 187-192
Received 15 April 2021; Accepted 05 May 2021.

Table2. Percentage distribution of isolated microorganisms of bacterial infections in patients with diabetic foot.

Microorganism	FrequencyPer Sample	Percentage (%)
Staphylococcus aureus	22	30.1
Klebsiellapneumoniae	16	21.9
Acinetobacter spp.	14	19.1
Pseudomonas aeruginosa	11	15.0
Enterococcus spp.	6	8.2
Group A Streptococcus	4	5.5
Total	73	100

Among the CGPs, the three most common microorganismsfrequently isolated were Staphylococcus aureus,19% (22), Enterococcus spp. 6% (6) and Streptococcusof group A 4% (4). Gram negative rodsmost frequently isolated were Klebsiellappneumoniae13% (16), Acinetobacter spp. 12% (14) andPseudomonas aeruginosa 11% (13). Table 3.

Table3. Percentage distribution of Gram bacteria positive and Gram negative isolates from patients with diabetic foot.

Gram Bacils	Positive FrequencyPer Sample	Percentage
Staphylococcus aureus	22	48,9
Enterococcus spp.	6	13,3
Staphylococcus coagulase (-)	4	8,9
Streptococcus group "A"	4	8,9
Streptococcus viridians	3	6,7
Enterococcus faecalis	2	4,4
Streptococcus spp.	2	4,4
Staphylococcus epidermidis	1	2,2
Streptococcus agalactiae	1	2,2
Total	45	100
GRAM BACILSNEGATIVES		
Klebsiellappneumoniae	16	23,2
Acinetobacter spp.	14	20,2
Pseudomonas aaeruginosa	13	18,8
Pseudomona spp.	7	10,1
Escherichia coli	5	7,2
Proteus mirabilis	4	5,8
Proteus vulgaris	4	5,8
Enterobactercloacoe	2	2,9
Klebsiellaoxytoca	2	2,9
Enterobacter spp.	1	1,4
Providenciaalcalifaciens	1	1,4
Total	69	100

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 5, 2021, Pages. 187-192
Received 15 April 2021; Accepted 05 May 2021.

The results of sensitivity tests antimicrobial agents showed that 100% of the S. aureus strains were resistant to Oxacillin. The genus Enterococcus spp. was sensitive to Ampicillin, Ciprofloxacin and Vancomycin. Streptococcus group. A were sensitive to Ampicillin, Ciprofloxacin and Clindamycin, but resistant to Erythromycin. The antimicrobial susceptibility profile for K. pneumonia proved to be sensitive to quinolones and carbapenems, but resistant to the others. The Acinetobacter spp. turned outbe sensitive to carbapenems and resistant to others antibiotics. The Pseudomonas spp. they were sensitive tofourth generation cephalosporins, quinolones and carbapenems. Table 4.

Table 4. Antimicrobial sensitivity of Gram bacteria positives and negatives isolated from patients with foot diabetic.

Antibiotics	Staphylococcus aureus				Enterococcus spp.				Streptococcus Group A			
	S		R		S		R		S		R	
	N	%	N	%	N	%	N	%	N	%	N	%
Ampicicline	-	-	-	-	6	100	0	0	4	100	0	0
Ciprofloxacin	20	90,8	2	9,1	4	66,6	2	33,3	4	100	0	0
Clindamycin	17	77,2	5	22,7	-	-	-	-	4	100	0	0
Erythromycin	18	81,8	4	18,1	-	-	-	-	0	0	4	100
Levofloxacin	34	100	0	0	-	-	-	-	-	-	-	-
Oxacycline	0	0	22	100	-	-	-	-	-	-	-	-
Rifampicin	22	100	0	0	-	-	-	-	-	-	-	-
Vancomycin	22	100	0	0	6	100	-	0	-	-	-	-
Antibiotics	***Klebsiellapneumoniae***				***Acinetobacter spp.***				***Pseudomona aeruginosa***			
	S		R		S		R		S		R	
	N	%	N	%	N	%	N	%	N	%	N	%
Ampicicline	0	0	16	100	0	0	14	100	-	-	-	-
Cefotaxime	5,8	36,3	10,1	63,3	0	0	14	100	-	-	-	-
Cefepime	5	31,2	11	68,7	4	28,5	10	71,4	7,4	57,1	5,5	42,8
Ciprofloxacin	9,8	61,5	6,1	38,4	3	21,4	11	78,6	9,2	71,4	3,7	28,5
Imipenem	16	100	0	0	8	57,1	6	42,8	8,3	64,2	4,6	35,7
Meropenem	16	100	0	0	10	71,4	4	28,5	13	100	0	0
Piperacycline	3	18,7	13	81,2	0	0	14	100	9,2	71,4	3,7	28,5
Piperazilin / Tazo	5	31,2	11	68,7	3	21,4	11	78,5	6,5	50	6,5	50

Regarding the distribution of microorganisms by sex, the highest number of infections byPseudomonas spp. and S. aureus occurred in the sexfemale, while in males, the majorityof the infectious ones were produced by S. aureus, K.pneumoniae and Pseudomonas spp. Table 5.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 5, 2021, Pages. 187-192
Received 15 April 2021; Accepted 05 May 2021.

Table5. Percentage distribution of microorganisms by sex isolated from patients with diabetic foot.

WOMEN			MAN		
GRAM NEGATIVE BACTERIA	**FREQUENCY**	**%**	**GRAM NEGATIVE BACTERIA**	**FREQUENCY**	**%**
Pseudomonas spp.	**11**	**22**	**Klebsiella spp.**	**10**	**16,4**
Acinetobacter spp.	**9**	**18**	**Pseudomonas spp.**	**10**	**16,4**
Klebsiella spp.	**7**	**14**	**Proteus spp.**	**6**	**9,8**
Proteus spp.	**2**	**4**	**Acinetobacter spp.**	**5**	**8,2**
Providencia spp.	**1**	**2**	**Enterobacter spp.**	**2**	**3,3**
E. coli	**1**	**2**	**E. coli**	**2**	**3,3**
			Citrobacter spp.	**1**	**1,6**
GRAM BACTERIA POSITIVE			**GRAM BACTERIA POSITIVE**		
Sthapylococcus spp.	**10**	**20**	**Staphylococcus spp.**	**16**	**26,2**
Streptococcus spp.	**4**	**8**	**Streptococcus spp.**	**5**	**8,2**
Enterococcus spp.	**4**	**8**	**Enterococcus spp.**	**4**	**6,6**
Enterobacter spp.	**1**	**2**			
TOTAL	**50**	**100**	**TOTAL**	**61**	**100**

DISCUSSION

The prevalence of bacterial infections in thisThe study presented figures of 52% in men with a25% distribution in the age group 51 to60 years. These values coincide with other studies,where the percentage of infection in men is26.9%. (8)In our study, 20% of the infections were polymicrobial. These results are to be expected, asthat in a review conducted in Wales by Howell-Jones et al. in 2005, he mentions that the microflora of diabetic foot ulcers are almost always polymicrobial, presenting from 2 to 4Concomitant bacteria in infection (9).studies using molecular techniques emphasized thecomplex ecology of these wounds and using techniquesconventional the mean number of bacteria perulcer has a range of 1.6 to 4.4, observing thatulcers that do not show signs of infection containmore than one bacterial species. In an investigationStaphylococcus epidermidis was isolated in 20.6%of diabetic foot ulcers, Pseudomonasaeruginosa in a range of 7 to 33%, other species isolated were E. coli, Enterobacter cloacae, Klebsiellaspp., Streptococcus spp., Enterococcus spp. and Proteus spp. The most frequent anaerobic bacteria were Bacteroides spp. in 12% and Peptostreptococcus spp. On 8% (9). The most frequently isolated microorganism was the S. aureus, which is part of the normal microbiota of the human body, which can cause diseases opportunists. Although the mucous membranes of the skin favor adherence to S. aureus, offer a mechanical barrier very effective against tissue invasion. When is barrier is disrupted, microorganisms gain access to the underlying tissue creating a lesion with characteristic local obsessive, as occurs in the footdiabetic. S. aureus is believed to be responsible formore than 80% of suppurative diseases, since they constitute 80% of clinical isolates (10). On the General University Hospital "José María Morales Meseguer ", in Spain, 55% of germs isolated with more frequent were Gram microorganisms positive and of these, S. aureus was the most common (33%). Pseudomonas frequently followed aeruginosa (12%) and Enterococcus spp. (9%), which coincide with the results presented in this research (11). In another study by the National Toxicology Center of Cuba, 63 samples, 33 were confirmed positive for S. aureus, 29 of these being Methicillin Resistant (MRSA) (12). Regarding the antimicrobial susceptibility profile, most of the BGP were sensitive to Ciprofloxacin, generally used to treatment of urinary tract infections, diarrhea bacterial and prostate infections, however, these same bacteria were for the most part resistant to Oxacillin and Erythromycin.Oxacillin belongs to the group of penicillins resistant to beta-lactamase, and resistance is increasingly common from Staphylococcus to these antibiotics. In these cases, combined treatment between two antibiotics of different classes that are sensitive according to the antibiogram

(13). In the Spanish study, S. aureus, MRSA was also detected (12). In the study by Macias AE et al, eight strains were isolated of S. aureus, of which three (38%) were MRSA (14). In the analysis of gram-negative bacteria, the majority of them were sensitive to carbapenems, currently, some bacteria are presenting resistance to this group of antibiotics, making more treatment of these infections is difficult (15). The resistance of the strains tobeta-lactams are known as strains. Extended Spectrum Betalactamases (ESBL), and are microorganisms capable of producing beta-lactamaseand hydrolyze the betalactam ring of penicillins and cephalosporins. In the Spanish study citedpreviously, E. coli presented almost 30% ofresistance to the combination of Amoxicillin withClavulanic Acid and Ciprofloxacin. Inotherresearch, of the 68 Gram negative rodsisolates, 24 were resistant to Ciprofloxacin (35%),and in the 55 isolated Enterobacteriaceae and 4 (7%) wereESBL (16).

In conclusion, most foot infections diabetic were monomicrobial, being S. aureus,K. pneumaniae and Acinetobacter spp. with a profilevery high antimicrobial resistance. It would be veryimportant to implement prevention programsthis pathology in order to avoid amputations in this typeof patients.

REFERENCES

1. [1] Isla Pera P. Diabetes Mellitus, the pandemic of the centuryXXI. Recently Scientific Journal of Nursing.2012; (5): 2-13.
2. [2] Mandal A. Diabetes and gangrene. News medical:2015- (accessed May 24, 2017). Available inhttp://www.news-medical.net/health/Diabetesand-gangrene- (Spanish) .aspx
3. [3] International Diabetes Federation. Whatis diabetes.Brussels: International Diabetes Federation. 2015(accessed May 15, 2017). Available at: https: //www.idf.org/about-diabetes/what-is-diabetes.
4. [4] Valencian Institute of the Foot. Diabetic foot.Valencia, Spain: Valencian Institute of the foot. 2016; Accessed March 18, 2017 . Availablein:http://institutovalencianodelpie.es/podologiaavanzada/wound-healing / diabetic-foot /
5. [5] Ribeiro Parisi M.C. –Chapter 05 - A syndromediabetic disease, pathophysiology and practical aspects(Diabetes.org.br). Sao Paulo Brazil: SociedadeBrazilian diabetes. 2015-) updated 23 ofJanuary 2017: accessed March 18, 2017 (Availablehttp://www.diabetes.org.br/ebook/component/k2 / item / 42-a-pe-diabetic-physiopathology-syndromee-aspects-praticosituo
6. [6] Cañarte-Alcívar J, Intriago-Ganchozo J, Romero-Santillán B. Prevalence of diabetic footin patients treated at Hospital SantoSunday of the Tsáchilas. Mastery of Sciences.2016; 2 (3): 201–12.
7. [7] Flores-Moreno R, Cárcamo-Mejía S, Pavón-Núñez D, Avilés CF A, M-Díaz C, Giacaman-Abudoj L, et al. Bacteriological Profile in Patientswith Diabetic Foot, who attend the National Instituteof Diabetic Tegucigalpa, Honduras, January2013-December 2015. Arch Med. 2016; 12 (3): 1–8.
8. [8] Arango Montes G., Diabetic foot Mexico: Faculty of Medical Sciences; 2015. (accessed May 15, 2017).Availablefrom: http://www.facmed.unam.mx/apartments / family / af8 (3) /pie-diabetico.html.
9. [9] Soriano Pereira P, De PablosVelazco P.Epidemiology of diabetes mellitus. Rev Endo andNutri. 2007; 54 (3): 2-7.
10. [11] Ruiz Mercado H, Miranda Sosa SA, GonzálezHiguera JA, Ochoa González FJ. Microorganismsmore frequent bacteriological and resistance inDiabetic Foot Infections in HospitalRegional “Dr. Valentín Gómez Farías ”from ISSSTE,Zapopan, Jalisco. Rev Mex Angiol. 2007; 35 (4): 177–84.
11. [12] Basualdo JA. Biomedical Microbiology. 2nd ed.Argentina: Atlante; 2006.
12. [13] Rang HP, Ritter JM. Flower RJ, Henderson G. Rangand Dale Pharmacology. 8th ed. Barcelona: Elsevier,2016.
13. [14] Hernandez Pedrozo W, Ramos GodinezA, Nodarse Hernández R, Padron SánchezA. Bacterial resistance in bacteriaproducers of extended beta-lactamases (Blee).RevCubMedIntEmerg 2006; 5 (1): 256-264
14. [15] Martínez-Gómez DA, RamírezAlmagroCristóbal,Campillo Soto A. Diabetic foot infections.Prevalence of different microorganisms andsensitivity to antimicrobials.MicrobiolClin. 2009; 27 (6): 317–32
15. [16] Pérez Rodríguez S, Díaz Machado A, GónzalezDelgado CA, García González Y.
16. [17] Bacterial growth in diabetic foot ulcer prior toHeberprot-P. Rev Cubana Med Mil. 2014; 43 (2)
17. [18] Head-of-Cow A; MacíasE; Álvarez JA;Aurora Cuevas, Ramírez AJ; Ramírez WA et al.Microbiology of the diabetic foot determined bybiopsy study. Rev InvClin. 2009; 61 (4): 281-285