Bacteriological and therapeutic profile of diabetic foot infection: a prospective study of 100 patients.

Profil bactériologique et thérapeutique du pied diabétique infecté: étude prospective de 100 patients.

Mohamed Ben Moussa, Mehdi Khalfallah, Ilhem Boutiba Ben Boubaker, Ramzi Nouira, Amine Slim, Hichem Jerraya, Chadli Dziri.

Service de chirurgie - Hôpital Charles Nicolle. Faculté de Médecine de Tunis. Université Tunis El Manar

RÉSUMÉ

Prérequis : L'identification de la flore bactérienne infectante constitue l'une des principales règles à suivre pour assurer le succès de l'antibiothérapie dans le traitement du pied diabétique. Le but du travail était de définir le profil bactériologique des germes responsables d'infection du pied diabétique au service de chirurgie B de l'hôpital Charles Nicolle de Tunis et de dégager les facteurs pronostiques de cette affection.

Méthodes : L'étude était prospective ouverte s'étendant sur 17 mois. Elle a intéressé 100 patients diabétiques opérés pour un pied diabétique infecté. Tous les patients ont eu un prélèvement bactériologique par curetage-écouvillonnage profond pratiqué au bloc opératoire.

Résultats: L'âge moyen des patients était de 59,5 ±11 ans, avec un sex-ratio de 2,4. L'infection du pied était une gangrène humide dans 82 % des cas. Les entérobactéries étaient les germes les plus fréquemment isolés (73%), suivies par les streptocoques (10%), Staphylococcus aureus (9%). L'antibiothérapie probabiliste qui a été instaurée (acide fusidique+amoxcilline/acide clavulanique) était inactive sur 44,1% des germes isolés. Le taux de bactéries multirésistantes était de 9,5 %. En comparant le groupe de malades ayant une évolution défavorable (ayant été réopérés) et le groupe de malades ayant une évolution favorable, nous avons dégagé deux facteurs de mauvais pronostic : L'artérite (p=0,018 ; OR=23,7) et la présence de bactéries multirésistantes (p=0,027 ; OR=5,8).

Conclusion: Les entérobactéries étaient les principaux germes responsables d'infection du pied diabétique. Les facteurs pronostiques, à savoir l'artérite et l'isolement de bactéries multirésistantes, soulignent l'importance de la prise en charge multidisciplinaire.

Mots-clés

Pied diabétique, infection, bactérie, résistance bactérienne, facteurs pronostiques

SUMMARY

Background: Identifying the infecting bacterial flora is one of the main rules to be followed to ensure the success of antibiotherapy in the treatment of the infected diabetic foot.

The aim of the work was to define the bacteriological profile of the bacteria causing the infection of the diabetic foot at the surgery unit B of Charles Nicolle's hospital in Tunis and determine the prognostic factors of this condition.

Methods: It was an open prospective study. It concerned 100 diabetic patients operated on for diabetic foot infection. All patients had bacteriological samples taken through deep scraping and swabing carried out in the operating room.

Results: The average age of patients was 59,5 ±11 years, with a sex-ratio of 2,4. The foot infection was represented in 82 % of cases by a wet gangrene. The enterobacteria were the most frequently isolated bacteria (73%), followed by streptococcus (10%), Staphylococcus aureus (9%). The rate of multidrug-resistant bacteria was of 9,5%. The empiric antibiotic therapy used (fusidic acid +amoxicillin/ clavulanic acid) was inactiveon 44,1% of the isolated bacteria. When we compared the group of patients with unfavourable development (who have been reoperated) and the group of patients with favourable development, we have found two poor prognosis factors : arteritis (p=0,018 ; OR=23,7) and presence of multidrug-resistant bacteria (p=0,027 ; OR=5,8).

Conclusion: The enterobacteria were the main bacteria causing the infection of diabetic foot. The prognostic factors found, arteritis and isolation of multidrug-resistant bacteria, outpoint the importance of multidisciplinary care.

Key-words

Diabetic foot, infection, bacterium, bacterial resistance, prognostic factors

Diabetic foot is a major public health problem both in developed and developing countries [1]. In France, the incidence of chronic foot wounds in diabetic patients has been estimated at 2,5 % per year in 2008 [2]. In Tunisia, the diabetic foot accounted for 7,35% of hospital admissions in an endocrinology department in 1997 [3]. The history of diabetic foot starts in most cases with ulceration following a trauma that often goes unnoticed, mainly caused by diabetic neuropathy and incidently by arteriopathy [4]. These ulcerations represent an identified source for infection in 40 to 80 % of cases [5]. These infections, when severe, represent the main cause of nontraumatic amputation in the United States and Europe [2]. The antibiotic treatment holds an important position in the treatment of these infections and this implies a perfect knowledge of the infecting bacteria. In Tunisia, no previous study has been carried out to identify the infecting bacteria.

Therefore, we intend, through this work, to define the clinical and bacteriological profile of the infected diabetic foot in patients admitted in the surgery unit B of Charles-Nicolle hospital so as to better guide first-line antibiotherapy and identify the prognostic factors of this condition.

METHODS

It was an open prospective study, extending over 17 months (01/09/2011 – 01/02/2013) and achieved in collaboration between the general surgery unit B and the microbiological laboratory of Charles-Nicolle hospital in Tunis. We decided to include100 diabetic patients operated on for infected diabetic foot. Each patient had one or several bacteriological samples taken aseptically in the operating room and the clinical data have been reported according to individual files.

Sampling for bacteriological examination:

After preparing the infected wound by surgical debridement, one or several bacteriological samples have been taken for each patient through deep scraping-scrubbing and the samples taken have been preserved in a T.G.VBio-Rad® transport medium until they reached the bacteriology laboratory.

Bacteriologicalanalysis:

In the laboratory, the samples have been inoculated on plain agar, desoxycholate lactoseagar, mannitol salt agar, blood agar and chocolate agar. For any positive culture, the identification of bacteria has been made by using conventional methods (morphological, cultural and biochemical characteristics). In case of positive culture for the same bacteria on several samples in the same patient, a single one has been recorded.

The study of antibiotic susceptibility has been carried out according to the MUELLER HINTON agar diffusion technique [6] and interpreted according to the recommendations of the antibiogram committee of the french society of microbiology [6]. The multiresistance of a bacterium (BMR) has been defined by the isolation of enterobacteria resistant to third generation cephalosporin antibiotics (C3G), of *Staphylococcus aureus* resistant to methicillin (MRSA), of *Acinetobacter bauamannii*or *Pseudomonas aeruginosa*resistant to ceftazidime or imipenem .In case of isolation of enterobacteria resistant to carbapenems, a search for the blaOXA 48 gene has been carried,out through simplex PCR assay.

Clinical analysis:

For each patient, we have noted, on the basis of a questionnaire, a physical examination and a foot X-ray, the demographic characteristics, the personal medical history, the personal surgical history of the infected foot, the risk factors for infection of the diabetic foot, the history of the infection, the data of the clinical examination of the wound at the time of admission to hospital, the grade of the infection according to the international consensus on the diabetic foot classification [2], the kind, doses and duration of the antibiotic treatment received after admission and the kind of surgical treatment undergone. Diabetes mellitus was defined by a fasting blood glycemia>1,26 g/l or a hyperglycemia > 2g/l. Neuropathy was defined by a decrease in superficial or deep sensibility and abolition of achille's tendon reflex. Arteritis was defined by an intermittent claudication, ischemic symptoms or decrease or absence of pulses in lower limbs. Osteitis was defined by an exposed foot's bone or radiologic signs as bone lysis, bone sequestrum or periosteal reaction.

Postoperative monitoring has been defined as favorable if the postoperative course was uneventful with a non-infected clean wound and systemic signs under control. The postoperative course was unfavorable if there was persistence or an extension of the local infection in spite of surgical treatment and/or if there was a worsening of systemic signs in the postoperative course requiring in both cases a return to the operating room.

Statistical analysis:

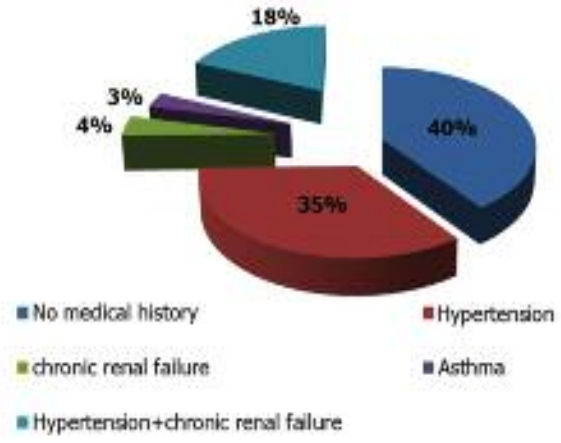
The data were analysed by means of the SPSS software version 19.0. Comparison of two means on independent series has been made by means of the Student's t test and percentages by Pearson's chi-squared test or the Fisher's exact bilateral test. Identification of the prognostic factors has been achieved by means of a multivariate analysis of logistic regression type. The materiality threshold has been set at 0,05 for all statistical tests.

RESULTS

The average age was 59,5 ±11 years with a sex-ratio of

2,4 (71 men / 29 women). The medical history of patients was as shown in figure n°1.The patients suffered from type 2 diabetes in 85 % of cases and type 1 diabetes in 15 %.Median duration of the progression of diabetes was 15 ± 7,5years (extremes 1 - 40 years). Patients with type 1 diabetes were all treated with insulin before their admission. Patients with type 2 diabetes were taking oral antibiotics (ADO) in30 % of cases, insulin in 35 % of cases, ADO and insulin in 33 % of cases and no treatment in 2 % of cases. There was a bad treatment compliance in62 % of cases. The glycemic control has not been made. There was peripheral neuropathy in 18 % of cases and arteritis in 16 % of cases.

Figure 1: Medical history



As regards the history of diabetic foot, 39% of patients had already been operated for infected foot at least once and 15 % had had at least an amputation at the level of the same foot. For three patients, the median number of operations was 1,6 ± 1 operation (extremes : 1 - 7 operations). The median duration of the progression of the wound or the ulceration ahead of admission to hospital was 58,2 ± 47,7 days (extremes : 7 - 360 days) and the rate of chronic wounds (≥ 1month) stood at 80 % in our series . The infection complicating these wounds had been developing for 15,1 ± 7,8 days (extremes 2 - 45 days) ahead of admission to hospital. The infection had been treated ahead of admission to hospital with empirical antibiotic therapy in 63patients. This empirical antibiotic therapy was a combination of fusidic acid + [amoxicillin/clavulanic acid (AMC)] in 57 % of cases.

The clinical examination of the infected foot upon admission showed that the wet gangrene was the kind of infection most found and the toes were the most affected parts of the foot (Table 1). According to the international consensus classification of the diabetic foot, the infection was rated grade 4 in 86 patients and grade 3 in the remaining population. 28 patients had foot osteitis.

The treatment plan included a medical part based on antibiotic therapy and a surgical part which consisted in excision or amputation. A parenteral antimicriobial therapy represented by the empirical combination of fusidic acid + AMC was prescribed for 98 patients. Two patients had received upon admission ertapenem in view of a locally serious state of infection complicated by septicemia. The surgical management consisted in 85 % of cases in amputation of the foot, the leg or the thigh. Only 15 % of patients had a conservative treatment.

With deep curettage and scraping, we had obtained 124 samples, that is 1,2 samplings per patient (extremes :1 - 3 samples per patient). Culture was positive in 91 patients and negative in nine cases. We have isolated 136 bacteria, that is on average 1 ± 0,7bacteria per patient (extremes : 0 - 3 bacteria).The infection was monomicrobial in 50 patients and polymicrobial in 41 patients; with two organisms in 37 cases and three organisms in four patients.

The enterobacteria were the most frequently isolated bacteria (73%), followed by streptococcus (10%), *Staphylococcus aureus* (9%) and *Pseudomonas aerugionosa*(8%). The frequency of bacterial species isolated within family of Enterobacteriae and Streptococcaccae is shown in Table 2. The study of the distribution of the organisms isolated according to the different clinical data showed that Gram positive cocci were statistically more often isolated in patients with wounds developing since less than two months contrary to Gram negative bacilli which were statistically more frequent in patients with wounds dating back to more than two months (p=0,035).

P. aeruginosa was statistically more frequent in patients who had previously undergone foot operation (P=0,02) (Table 3).

Table 1 : distribution of patients according to the type and site of foot infection

Type of infection	WG	Phlegmon	WG+ Phlegmon	FU	WG+FU	FU+leg ulcer	Complex
%	77	6	4	5	1	1	6

Site of infection	Toe	Toe+Transmetatarsal	Amputation stumb	Transmetatarsal	Leg+thigh	Other
%	47	17	15	14	3	3

WG : wet gangrene, FU : superinfected foot ulcer

Table 2 : Frequency of the isolated bacterial species within the family of enterobacteriaceae and Streptococcaccae

Enterobacteriaceae	Number	Percentage (%)
Citrobacter freundii	4	4
Escherichia coli	10	11
Enterobacter spp	8	8
Klebsiella spp	22	23
Morganella morganii	6	6
Proteus mirabilis	33	34
Proteus vulgaris	3	3
Providencia spp	7	7
Serratia spp	4	4
Total	97	100
Streptococcaccae	**Number**	**Percentage (%)**
S. agalactiae	7	52
Group C ,G streptococci	4	32
Alpha-hemolytic streptococci	2	16
Total	13	100

Table 3 : distribution of bacteria isolated according to the various clinical parameters

	S. aureus	*Enterobacteriaceae*	Streptococci	*P. aeruginosa*	p
Type of infection					
Wet Gangrene	8,5 %	73,9 %	9,5 %	9,5 %	NI
Phlegmon	14 %	56 %	30 %	9,5 %	
Foot ulcer	17 %	68 %	15 %	0 %	
Osteitis					
Osteitis (+)	4 %	75 %	8 %	10 %	0,29
Osteitis (-)	11 %	69 %	13 %	7 %	
Duration of wound					
< 2months	11 %	65 %	17 %	7 %	0,21
≥ 2 months	7 %	70 %	6 %	8 %	
History of foot surgery					
Yes	7 %	70 %	9 %	13 %	0,17
No	10 %	72 %	13 %	3 %	
Ambulatory ATB					
Yes	9 %	71 %	10 %	9 %	0,78
No	8 %	72 %	14 %	6 %	

NI : Test non advisable because poor number of phlegmon and foot ulcer ; ATB: antibiotic

The study of sensitivity to antibiotics showed that 48 %of enterobacteriaceae were resistant to the combination amoxicillin+clavulanicacid(AMC). Resistance to third generation cephalosporins among the enterobacteriaceae concerned 11%of the bacteria of this family. This resistance to third generation cephalosporins was more frequently found in *Enterobacter cloacae.Resistance to carbapenems was detected in a strain of K. pneumoniae. This strain resistant to ertapenem, to* 3GC, and sensitive to imipenem produces carbapenemase and extended-spectrum beta -lactamase (ESBL).

Resistance to fluoroquinolone was noticed in 18 % of enterobacteriaceae and among aminoglycosides, amikacin kept the best activity against the isolated enterobacteria with only

3% of resistance. Among the non fermentative gram negative bacilli**,** we had isolated two imipenem-resistant strains (*P. aeruginosa and A. baumannii*) and a single one resistant to ceftazidime (*A. baumannii)*. *S. aureus* was sensitive to various antibiotics. No meticillin-resistant *S. aureus* had been detected during the study period. As regards *S. aureus,* the combination amoxicillin+clavulanicacid, fluoroquinolone, fusidic acid, lincosamides, pristinamycin, rifampicin, glycopeptides and tigecycline were active on all strains.

The rates of resistance to antibiotics of the isolated bacteria are summed up in tables 4.The overall rate of MDR among the organisms found stood at 9,5 %. Infection by at least one MDRB was found in 12 patients. Two MDRB were found in a single patient.

About 91 % of the isolated bacteria were naturally resistant to fusidic acid and the combination amoxicillin-clavulanic acid (AMC) was inactive in vitro on 44,1 % of the isolated bacteria. Therefore, the empiric antibiotic therapy used for our patients was inadequate in 45 % of patients.

The evolution was considered favorable in 56 % of cases and unfavorable in 44 % of cases. The search for prognostic factors by means of an univariate comparative

Table 4 : Gram negative bacilli and Gram positive cocci rate of resistance to antibiotics

	Amx	Amc	Tic	Ctx	Caz	Fep	Imp	Etp	An	Net	Cs	Ofx	Cip	Sxt	Fos
Citrobacter freundii (n=4)	RN	RN	3	0	0	0	0	0	0	0	0	1	0	2	0
Escherichia coli (n=10)	9	5	9	2	1	2	0	0	0	3	0	4	4	7	0
Enterobacter spp (n=8)	RN	RN	4	4	4	3	0	0	1	3	0	4	4	4	0
Klebsiella spp (n=22)	RN	23	RN	9	9	5	0	5	9	23	0	14	14	27	0
Proteus mirabilis (n=33)	73	21	70	0	0	0	0	0	0	12	RN	6	6	19	0
Proteus indole+ (n=16)	RN	RN	13	0	0	0	0	0	0	38	RN	19	13	38	13
Serratia spp (n=4)	RN	RN	3	3	3	3	0	0	0	1	RN	1	1	1	0
P. aeruginosa (n=11)	RN	RN	10	RN	0	0	10	RN	0	0	0	-	0	RN	-
A. baumannii (n=1)	RN	RN	1	RN	1	1	1	RN	1	0	0	-	1	1	-

*resistance is expressed under the form of simple frequency (number of resistant strains) for bacteria whose number is less than 10 and in the form of percentage for bacteria whose number is over 10.

Amx : amoxicillin, Amc : amoxicillin- clavulanic acid , Tic : ticarcillin, Caz : ceftazidime, Ctx :cefotaxime, Fep : cefepime, Imp :imipenem , Etp : ertapenem, An : amikacin, Net : netilmicin, Cs : colistin, Cip : ciprofloxacin, Ofx : ofloxacin, Sxt : cotrimoxazole, Fos : fosfomycin, RN :natural resistance

study has shown that arteritis (p<0,001), the site of the infection located at the level of the amputation stump (p=0,013), osteitis (p=0,01), enterobacterial infection (p=0,04), MDRB infection (p=0,021) were associated with an unfavorable prognosis. The multivariate study has allowed to bring out two risk factors connected in an independent way with unfavorable evolution: chronic arteritis of lower limbs (adjusted p =0,018 ; adjusted OR =23,7) and the presence of multidrug resistant bacteria (adjusted p =0,027 ; adjusted OR = 5,8) (Table 5).

Table 5 : Comparison of patients outcome (prognostic factors)

	Unfavourable outcome (N=56)	Favourable outcome (N=44)	P
Complications of diabetes			
Arteritis	69%	98%	<0,001
Site of infection			
Transmetatarsal	48%	16%	0,001
Amputation stumb	7%	25%	0,013
Type of infection			
Leg ulcer	2%	0%	0,037
Frequency of MDRB			
Isolated bacteria	5%	20%	0,021
S. aureus	18%	4%	0,04
Enterobacteria	70%	86%	0,04

MDRB : multidrug-resistant bacteria

DISCUSSION

Our study represents the first epidemiological survey which studied the bacterial flora causing diabetic foot infection and the prognostic factors of this pathology in surgical environment in Tunisia.The importance of bacteriological documentation is a key element to ensure the success of any antibiotic treatment especially as the literature data are very variable and sometimes even contradictory as regards the diabetic foot bacteriology [5]. The bacteriological study carried out has allowed concluding that gram negative bacilli of the enterobacteria family were the main bacteria causing diabetic foot infection in our patients (73%). Numerous authors have reported the prevalence of gram negative bacilli of the enterobacteria family in recent publications [7-13]. However, the data of western literature stressed the prevalence and the pathogenic role of *S. aureus* [14-17]. The prevalence of enterobacteria in our series could be explained by the fact that our patients have been recruited in surgical setting where patients cared for are often suffering from chronic foot wounds that have been treated with one or several antibiotics in ambulatory care, admitted to hospital in almost all cases for severe damage such as wet gangrene, often complicated by osteitis and septicemia. Parvez et al say that chronic foot ulcers and taking antibiotics that deteriorate the foot skin flora account for the prevalence of enterobacteria [13]. Toumihas reported in a french-tunisian work that enterobacteria were bacteria isolated from chronic wounds and lesions of wet gangrene type previously treated with broad-spectrum antibiotics [1]. The difference of foot hygiene practices between the two populations could also account for the inconsistency between western and eastern studies [13]. The same inconsistency has been noticed between western and eastern studies on the survey of bacteria causing peritonitis in patients receiving peritoneal dialysis [18].

After the enterobacteria, the streptococci and especially beta-hemolytic streptococcus group B were frequently isolated. Most authors acknowledge the pathogenic role of *S. agalactiae* in diabetic foot infections [14,19]. In our work, the latter represented almost half the bacteria of this family and this is in tune with the work of Citron who found 190 streptococci out of 454 isolated bacteria, among which 78 bacteria (41 %) were beta-hemolytic streptococci group B [13]. The isolation of streptococci in our series was statistically higher in patients with wounds dating back to less than two months. This result is consistent with the data of literature [1, 20]. Diabetic foot infections resulting from recent wounds are caused by gram-positive cocci (*S. aureus*and streptococcis) according to Lipsky [20].

As regards P*. aeruginosa,* the frequency of isolation of this organism was statistically higher in the group of patients who had previously had foot operations. Al Benwan explained the high prevalence of isolation of *P. aeruginosa* in a series gathering 440 diabetic feet by the different surgical procedures used for treatment as well as the long hospitalization stays [21]. The isolation of *P. aeruginosa* from post-operative wounds has also been found by Mutluoglu [22].

The rates of resistance to antibiotics of the bacteria isolated from diabetic foot lesions vary according to studies and this is due to the different habits in matters of antibiotics prescription despite the existence of recommendations that have tried to standardize prescriptions [23]. So, 11 % of the enterobacteria isolated in our series were resistant to 3GC, mainly by the production of ESBL. The frequency of isolation of enterobacteria producing ESBL and causing this infection ranges between 6 % and 67 % according to the authors [24].

We have reported the isolation in our work of a *K.pneumonia* strain producing OXA 48-type carbapenemase. This is probably a nosocomial strain especially as the patient in whom it has been isolated, had been hospitalized seven times for the same pathology and had suffered several amputations. The emergence of carbapenemase in surgery and intensive care units is well documented in Tunisia [25]. Khan et al have managed to isolate NDM-1 carbapenemase-producing *K. pneumonia* from a foot ulcer in a diabetic patient [26].

Taking into account the susceptibility of the bacteria found to antibiotics, the most active antibiotics found in our work were imipenem and amikacin. The US infectious pathology society suggested in its last recommendations in 2012, imipenem for the empiric therapy of severe foot infections especially in case of isolation of ESBLs-producing bacteria [27].

Ertapenem was active in vitro against most isolated bacteria except for *P. aeruginosa and A. baumannii. The interest of this drug in the treatment of foot* suppurations in diabetic patienrts is well proven in literature [27, 28].

The study of the prognostic factors of this pathology has allowed concluding that chronic arteritis of the lower limbs was associated in a significant and independent way with a bad prognosis. According to the results of a European multicentric study, arteritis was a major prognostic factor of unfavorable evolution and non-healing of infections and foot ulcers [29]. The same results have been found by Leese and Ince [30, 31]. Besides, we found, thanks to the multivariate analysis that the presence of MDRB was associated in a significant and independent way with unfavorable evolution. According to a retrospective study of Wagner et al, the infection of foot ulcers by MRSA was associated with a slower recovery [32]. Zubair has compared the death rate between a group suffering from MDRB infection and another group infected with drug-susceptible bacteria in a series gathering 342 diabetic feet and found that the death rate was twice higher in the group infected with MDRB (p=0,002, OR=3,2)[7].

However, some critics can be made to our work: the study of bacteria did not allow us to isolate anaerobic germs in spite of the fact that their involvement in the diabetic foot infections has been shown by numerous authors [19,33].No MRSA has been found during our work. We have no explanation about the absence of anaerobic germs and MRSA. The MSRA rate in patients with infected diabetic foot varies according to studies. It stands at 19,7 % for Richard and 60 % for Cabete [34,35]. Bacteriological monitoring of these patients to look for MSRA should be carried out because this germ is frequently isolated in surgery units [25].

The association of MDRB with a bad prognosis remains a subject for debate [36]. Numerous authors have shown that infection by these microorganisms has no impact on the prognosis [23,36].

The bacterial flora found in our patients relates to most cases of severe infections, all cases of surgical treatment and this does not allow to extrapolate our results to the whole diabetic patients with foot infection. In order to better assess the infecting bacterial flora, multicentric studies including the units of diabetology, internal medicine and infectious disease in which are admitted less severe infections, should be carried out.

The large number of bacteriological, prognostic and clinical factors stresses the importance of multidisciplinary care in order to improve the prognosis of this disease. «Diabetic foot units» should be set up in our country.

CONCLUSION

The enterobacteria were the main bacteria causing diabetic foot infection (73%). The bacteriological and clinical prognostic factors found (arteritis p=0,018 ;Isolation of multidrug-resistant bacteria p=0,027) outpoint the importance of multidisciplinary care in the treatment of diabetic foot.

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