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Bacteriology

Bacteriological Profile and Frequency of Antibiotic Resistance of Diabetic Foot Infection in Marrakesh

Zohair Ait Ouzdi[1,3*], Lamiae Arsalane[1,3], Youssef El Kamouni[1,3], Mustapha Alaoui[2,3], Said Zouhair[1,3]

[1]Department of Bacteriology and Virology, Military Hospital Avicenna of Marrakech
[2]Department of Vascular Surgery, Military Hospital Avicenna of Marrakech
[3]Faculty of Medicine and Pharmacy, Cadi Ayyad University Marrakech

***Corresponding author:** Zohair Ait Ouzdi
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Abstract Original Research Article

Introduction: The diabetic foot infection is a frequent complication of diabetes. It is a major risk factor for amputation and remains among the leading causes of hospitalization of diabetics. Our work aims to determine the bacteriological profile of diabetic foot infection and to evaluate the resistance of the isolated bacteria to antibiotics. ***Patients and methods:*** We performed a prospective descriptive study from March 2016 to September 2017, including 170 patients hospitalized for diabetic foot infection at the military hospital in Marrakech. ***Results:*** We realized 170 samples of which 66% were deep and 33% were superficial. The isolation rate of Gram negative bacilli and Gram positive cocci were respectively 58.4% and 40.4%. The most common individual isolates were *Staphylococcus aureus* (20.2%), *Escherichia coli* (18%). Isolated enterobacterial strains were sensitive to amikacin and imipenem but insufficiently to ampicillin, ticarcillin, and amoxicillin-clavulanic acid. Gram-positive cocci expressed a high rate of resistance to penicillin G (92%). Vancomycin and fucidic acid were the most active antibiotics. The multi-drug resistant organism was representing 25.8% of isolates. Highly resistant bacteria have been isolated, consisting of 6 strains of carbapenemase-producing enterobacteria. ***Conclusion:*** The findings of this study demonstrated an alarming increase in the prevalence of antibiotic resistance of diabetic foot infection. Thus, it is imperative to rationalize the use of antibiotics, improve hygiene in hospitals and establish a system for continuous monitoring bacterial resistance.

Keywords: Antibiotics- bacterial resistance - diabetic foot – infection.

INTRODUCTION

The diabetic foot gathers all the pathological manifestations reaching the lower limb in the diabetic subject [1]. It is a real crossroads of the main neurological, vascular and infectious complications following diabetes. It represents a public health issue by its economic weight and its serious impact on the quality of life of patients [2]. Infection of the diabetic foot is a frequent and formidable complication [3]. It is a major risk factor for amputation and remains among the leading causes of hospitalization for diabetics [4]. Infection of the diabetic foot is also a non-negligible cause of unjustified antibiotic therapy and, as such, contributes to worsening bacterial resistance and extending it through care [2, 4]. It is therefore essential to know the bacterial ecology of diabetic foot infections in health institutions to allow adequate management and optimal use of antibiotics, with the hope of reducing the risk of amputation and emergence of multi-resistant bacteria.

MATERIALS AND METHODS

Our work is a descriptive prospective study carried out over a period of 18 months from March 2016 to September 2017 in 170 patients admitted for diabetic foot infection and admitted to the vascular surgery department of the Avicenne Military Hospital in Marrakech. In the case of infected wounds, we used a deep curettage sample, which consists of removing tissue by scraping the wound base with a sterile curette, or using a fine syringe during the observation of the wound a deep infection with a collection or swab with superficial pus. The bacteriological samples are then immediately sent to the microbiology laboratory. Microscopic analysis after Gram staining provided information on the morphology of the bacteria, their grouping and their dye affinity. In case of anaerobic infection, he showed an abundant and polymorphous bacterial flora. Culturing was done on mannitol agar (Chapman), columbia agar with 5% sheep blood and cooked horse blood agar supplemented with a vitamin mixture. Each of these media was seeded by framing

and then incubated at 37 ° C in an aerobic atmosphere at 5% for 24h to 48h in an oven. The precise identification of the bacteria (genus and species) and the antibiogram were performed by automated method on Phoenix 100 (Becton Dickinson). The detection of resistance phenotypes has been completed by the conventional method of diffusion of disks in agar medium. The reading and interpretation criteria are those of the antibiogram committee of the French microbiology association (CASFM / EUCAST 2017).

RESULTS

170 patients were admitted for diabetic foot during the period under consideration. The sex ratio was 5.07 for men. The average age was 61 years old with extremes ranging from 45 to 84 years old? The majority of patients had type 2 (94%) with an average duration of onset of 11 years (range 1 to 35 years). Of the 170 samples taken, 52 were superficial obtained by simple swabbing, and 118 were deep obtained by curettage and aspiration with a fine syringe. The bacteriological study of these samples showed the presence of 50% Gram-negative bacilli, 25% Gram-positive cocci and 14% anaerobes (appearance of abundant and polymorphic bacterial flora). The cultures made were monomicrobial, polymicrobial and sterile in (75%), (14%) and (11%) respectively.

The distribution of isolated bacteria by families is shown in (Figure 1). The distribution by species showed the predominance of *Staphylococcus aureus* which accounted for 20.2% of the isolates, followed by *Escherichia coli* (18%), *Acinetobacter baumannii* (7.9%) and *Pseudomonas aeruginosa* (Table 1). Concerning the resistance profile of the germs, the rate of methicillin-resistant *Staphylococcus aureus* (MRSA) was 28%. Fusidic acid, ciprofloxacin, trimethoprim-sulfamethoxazole, clindamycin and erythromycin showed good activity on *Staphylococcus aureus* isolates. All isolates were sensitive to vancomycin. High levels of resistance of isolates were noted for penicillin G and gentamicin (Figure 2). Enterobacterial isolates showed high resistance to ampicillin (87%), ticarcillin (79%), amoxicillin-clavulanic acid (71%), and ciprofloxacin (41%). Imipenem and amikacin were the most effective antibiotics on enterobacterial isolates (Figure 3). *Pseudomonas aeruginosa* isolates showed 67% resistance for ciprofloxacin and 17% resistance for ticarcillin and aztreonam. All of these isolates were sensitive to piperacillin, ticarcillin-clavulanic acid and piperacillin-tazobactam combinations, ceftazidime, cefepime, imipenem, tobramycin, amikacin and gentamicin. Isolates of *Acinetobacter baumannii* showed increased resistance to the majority of antibiotics tested. The resistance rate to imipenem was 57% and ceftazidime was 86%. We isolated 46 multidrug-resistant bacteria, representing 25.8% of the isolates (Table 2). Enterobacteria resistant to third generation cephalosporins were predominant representing 47.8% of multidrug-resistant bacteria and 28.9% of enterobacteria.

Methicillin-resistant *Staphylococcus aureus* (MRSA) accounted for 21.7% of multidrug-resistant bacteria and 27.8% of *Staphylococcus aureus*. All isolates of *Acinetobacter baumannii* were multiresistant to betalactamins; they accounted for 30.4% of multidrug-resistant bacteria. Highly resistant bacteria were isolated in our study. These are 6 strains of enterobacteria resistant to carbapenems, representing 7.9% of enterobacteria and 3.4% of all isolates.

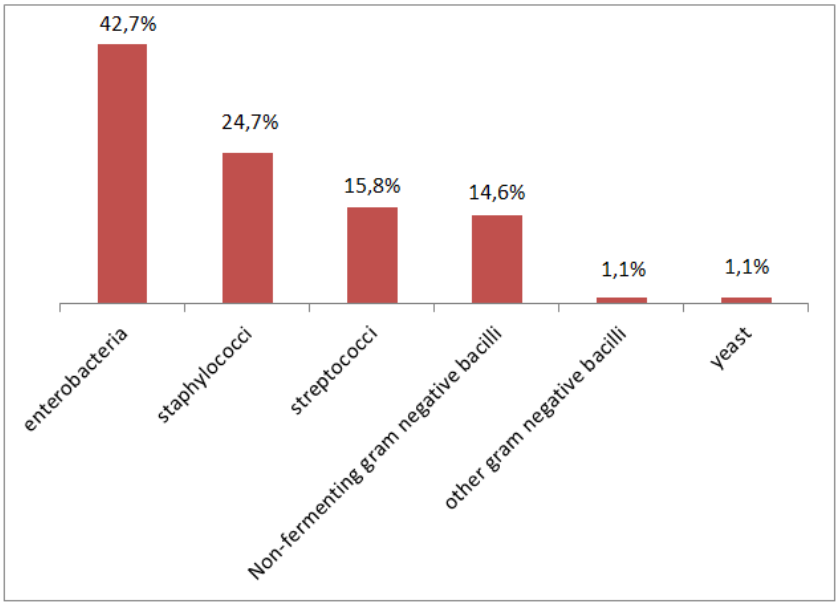

Fig-1: Distribution of isolated germs by family

Table-1: Distribution of isolated germs by family and species

seeds	Numbers	Percentage (%)
Gram positive cocci	72	40,4
staphylococci	44	24,7
Staphylococcus aureus	36	20,2
Staphylococcus haemolyticus	4	2,2
Staphylococcus hominis	2	1,1
Staphylococcus lugdunensis	2	1,1
streptococci	28	15,8
Streptococcus pyogenes	2	1,1
Streptococcus bovis	4	2,2
Streptococcus agalactiae	8	4,5
Enterococcus faecalis	8	4,5
Enterococcus species	4	2,2
Enterococcus faecium	2	1,1
Gram-negative bacilli	104	58,4
Enterobacteriaceae	76	42,7
Escherichia coli	32	18,0
Klebsiella pneumoniae	10	5,6
Enterobacter cloacae	8	4,5
Enterobacter aerogenes	4	2,2
Proteus mirabilis	8	4,5
Proteus vulgaris	6	3,4
Providencia rettgeri	2	1,1
Citrobacter koseri	2	1,1
Morganella morganii	2	1,1
Serratia marcescens	2	1,1
Non fermenting gram negative bacilli	26	14,6
Pseudomonas aeruginosa	12	6,7
Acinetobacter baumannii	14	7,9
Other Gram-negative bacilli	2	1,1
Aeromonas hydrophila	2	1,1
yeasts	2	1,1
Candida albicans	2	1,1
Total	180	100

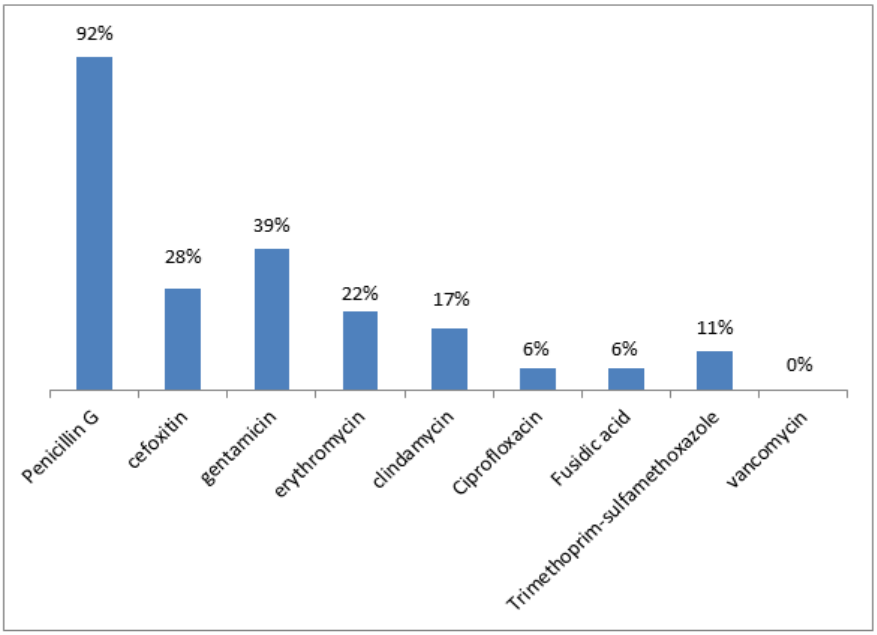

Fig-2: Resistance rate of Staphylococcus aureus isolates

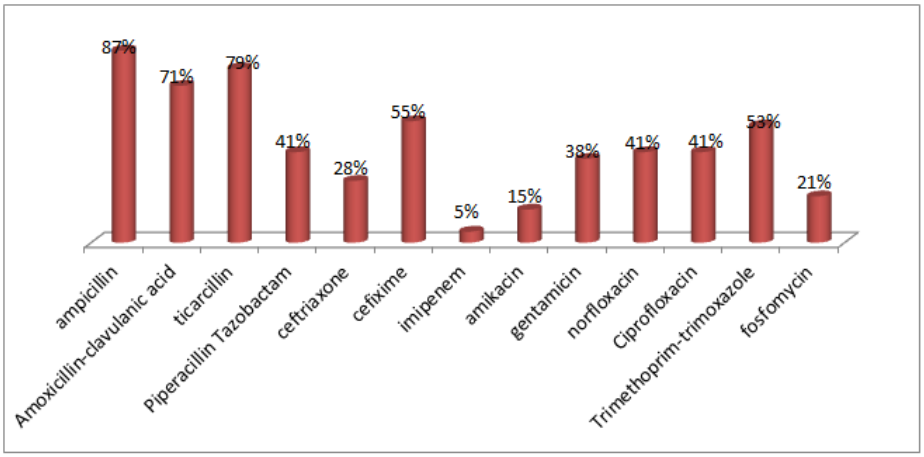

Fig-3: Resistance rate of enterobacterial isolates

Table-2: Distribution of multidrug-resistant bacteria

Multidrug-resistant bacteria	Number	Percentage
Methicillin-resistant Staphylococcus aureus (MRSA)	10	22%
MRSA resistant to glycopeptides	0	0%
Enterococcus faecium resistant to glycopeptides	0	0%
Enterobacteria resistant to third-generation cephalosporins	22	48%
- Enterobacteria producing ESBL	18	39%
- Enterobacteria producing cephalosporinases	4	9%
Enterobacteria resistant to carbapenems	6	13%
Pseudomonas aeruginosa resistant to ceftazidime and / or carbapenems	0	0%
Acinetobacter baumannii multiresistant to betalactamines	14	30%

DISCUSSION

Collection is a critical step in the microbiological documentation of diabetic foot infections. Its quality conditions the results of analysis and therefore the therapeutic attitude adopted by the clinician. Currently, there is no consensus on the best sampling technique because none has an ideal sensitivity [5]. However, learned societies advocate deep sampling by curettage, by fine syringe aspiration of purulent secretions or by preoperative tissue or bone biopsy [6]. In our study, three methods of sampling were applied; deep sampling by curettage, aspiration with a fine syringe and superficial swabbing. The majority of our samples were deep representing 64% of all. The particular requirements of anaerobic cultures led us to devote our study to the isolation of strict aerobic germs. Although strict anaerobes are widely indicted in diabetic foot infections, they nevertheless remain sensitive to the antibiotics conventionally used in this pathology and therefore in practice their isolation is of little use [7, 8]. In 14% of our samples, their presence was strongly suspected on direct examination. Although most studies on this subject report that diabetic foot infection is polymicrobial [9, 10]. In our study, cultures were monomicrobial in 75% of cases and polymicrobial in 14% of cases. Similar results have been reported by Turhan *et al.* and Richard *et al*. [11, 12]. Medical literature reports that diabetic foot infections are dominated by Gram-positive bacteria [12, 13]. This predominance, however, remains non-universal since recent studies in countries in Africa and Asia have reported the prevalence of Gram-negative bacteria in diabetic foot infections [11, 14, 15]. This geographical disparity is linked to climatic environmental factors, to the previous taking of antibiotics or to the technical factors of sampling or cultivation [16]. Our study showed the predominance of Gram-negative bacilli with an isolation rate of 58.4%. The Gram-positive bacteria isolation rate was 40.4%. The most frequently isolated species was *Staphylococcus aureus* which accounted for 20.2% of the isolates. Indeed, several studies carried out on this subject show that *Staphylococcus aureus* is the most frequently isolated pathogen in diabetic foot infections [12, 13, 17]. Among the Gram-negative bacilli, we noted the prevalence of enterobacteria representing 47.2% of isolates. *Escherichia coli* were the most commonly found species and the second most common among all germs. It accounted for 18% of the isolates. These results are similar to those reported by Zemmouri and al where *Escherichia coli* was the second most frequently isolated pathogen after *Staphylococcus aureus*, with an isolation rate of 20% [18]. In our study, Gram-positive bacteria expressed a high rate of resistance to penicillin G (92%). Vancomycin and fusidic acid were the most active antibiotics; vancomycin was active on all Gram-positive bacteria and fusidic acid was active on 95% of staphylococci. In the Turhan and al study, vancomycin was active on all BGPs. Fusidic acid was active on all staphylococci,

including methicillin-resistant strains [11]. Fusidic acid could therefore be a good alternative in the treatment of diabetic foot infections. For Gram negative bacilli, the enterobacterial strains isolated in our study expressed a high level of resistance to ampicillin (87%), ticarcillin (79%), amoxicillin-clavulanic acid (71%) and ciprofloxacin (41%). Imipenem and amikacin were the most active antibiotics. The resistance rate to these antibiotics was respectively 5% and 15%. In the studies of Turhan and al and Al Benwan *et al*. imipenem, amikacin and piperacillin-tazobactam were the most active antibiotics on Gram negative bacilli. These bacteria expressed a high level of resistance to ampicillin, amoxicillin-clavulanic acid and ciprofloxacin [9, 11]. Based on these results, it would be preferable to avoid prescribing amoxicillin-clavulanic acid and ciprofloxacin in the probabilistic antibiotic treatment of diabetic foot infection. The resistance rate for these antibiotics is high especially for Gram negative bacilli. This could be the source of emergence and diffusion of multidrug-resistant bacteria. In our study, we isolated 46 multidrug-resistant bacteria representing 25.8% of the isolates. In the Djahmi and al study, the multidrug-resistant bacteria level was higher representing 58.5% of the isolates [19]. In our study, MRSA accounted for 21.7% of BMRs and 28.8% of *Staphylococcus aureus* isolates. This is consistent with Richard and al's study, where MRSA accounted for 25% of *Staphylococcus aureus* isolates [12]. Djahmi and al reported a higher rate of MRSA; they accounted for 85.9% of *Staphylococcus aureus* isolates [19]. All isolated MRSA in our study were sensitive to vancomycin. The same result was reported by Durgad and al and Djahmi *et al*. [14, 19]. In contrast, vancomycin-resistant strains of *Staphylococcus aureus* have been described during diabetic foot infection, particularly in the United States [20, 21]. Third-generation cephalosporins resistant enterobacteria accounted for 47.8% of multidrug-resistant bacteria and 28.9% of enterobacteria in our study. Djahmi and al showed a higher rate of resistance of enterobacteria to third-generation cephalosporins; they accounted for 57% of enterobacteria [19]. Richard and al reported a lower rate of 6% [12]. In our study, ESBL-producing enterobacteria accounted for 81.8% of enterobacteria resistant to third-generation cephalosporins and 23.7% of all enterobacteria. Durgad *et al*. found a similar rate; 23% of enterobacteria isolated in their study produced ESBL [14]. Our isolates of *Pseudomonas aeruginosa* were multisensitive to antibiotics. This result is similar to that reported by Durgad *et al*. [14]. In contrast, multiresistant strains have been reported by Gadepali *et al*. [22]. The strains of *Acinetobacter baumannii* isolated in our study were 14 in number. They were all multiresistant to betalactamines thus representing 30.4% of multidrug-resistant bacteria. Of these strains 8 (57%) were resistant to imipenem. Gadepali *et al*. Turhan *et al*. And Mendes and al Reported *Acinetobacter baumannii* strains multidrug-resistant to betalactamines and carbapenems in their studies [11, 22, 23]. Among the multidrug-resistant bacteria isolates in our study, highly resistant bacteria were isolated, which are enterobacteria resistant to carbapenems. Six strains were isolated representing 7.9% enterobacteria and 3.4% of all isolates; these are two strains of *Klebsiella pneumoniae* and one strain of *Enterobacter cloacae*. These bacteria are involved in nosocomial infections, they have a high resistance potential linked to the multiplicity of resistance mechanisms they develop.

CONCLUSION

Optimal antibiotic therapy is one of the key elements in the management of diabetic foot infections. It requires a surveillance of the bacterial epidemiology and a precise documentation of the infection using quality bacteriological samples. In our study, isolated bacteria had high levels of antibiotic resistance. In addition, a high prevalence of multidrug-resistant bacteria was found. The emergence of multidrug-resistant bacteria is a global public health problem. In the absence of new antibacterial agents, this may lead to therapeutic impasses. The fight against this phenomenon requires a multidisciplinary approach that should integrate the rationalization of the prescription of antibiotics and strict compliance with hygiene measures.

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