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RESEARCH ARTICLE

BACTERIOLOGICAL CHARACTERISTICS OF DIABETIC FOOT INFECTION AND SUSCEPTIBILITY OF MULTIRESISTANT ISOLATES TO HYDRAULIC EXTRACTS FROM *ALLIUM SATIVA*, *ALLIUM CEPA* AND *CANNABIS SATIVA*

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ABSTRACT

Background and objective: Diabetic foot infection is one of the most serious complications of diabetes and its persistence is the result of the ineffectiveness of antibiotic therapy due to the exponentially increasing of antibiotic resistant bacteria. The study aimed at investigating the antibacterial effect of the aqueous extract of some plants on the antibiotic resistant bacteria isolated from diabetic foot wound infections.

Methods: A 6-months cross-sectional study from July 2021 to January 2022 at the Yaoundé Central Hospital, was undertaken with diabetic foot wound patients. All samples were appraised to determine presence of infectious agents using standard methods for isolation and identification of bacteria. Subsequently, antibiotic resistance was done using Kirby-Bauer disc diffusion methods. Finally, extracts of *Cannabis sativa* leaves, *Allium cepa* and *Allium sativum* bulbs were obtained with water and their antibacterial activities were evaluated by the microdillution method on liquid medium.

Results: 20 patients whom 14 men were included, with a sex ratio of 2.33, and their mean age was 52.5±9.6 years. 60% of these patients presented wounds in grade III and were of several types : purulent (48.57%), moist (31.43%) and dry (20%). 35 strains were isolated. The predominant GPB were *S. aureus* (34.29%) followed by Coagulase-negative *Staphylococcus* (14.29%), and *Bacillus Spp* (2.86%). Among the GNB, *Pseudomonas aeruginosa* (11.46%), *Serratia Spp.* (8.56%), *Escherichia coli* (8.56%), *Enterobacter Spp*. (5.71%), *Proteus Spp*. (5.71%), *Klebsiella Spp*., *Yersinia Spp*. and *Salmonella Spp*. in proportions of 2.86% each. A high rate of antibiotic resistance was recorded for Oxacillin (100%), Vancomycin (83.34%) and Augmentin (55.56%). Sensitivity tests on liquid medium showed that MIC ranged between 3.12-25.00 mg/mL, 6.25-25.00 mg/mL and 1.86-25.00 mg/mL respectivelly for *A. sativa*, *A. cepa* and *C. sativa*. Alliums were much more active on GNB. Although these results are low, they could be an alternative for the diabetic foot infection treatment.

Conclusion: Alliums were much more active on GNB. Although these results are low, they could be an alternative for the diabetic foot infection treatment.

Keywords: *Allium cepa*, *Allium sativum*, antibacterial resistance, diabetic foot infection, *Cannabis sativa*, plant extracts.

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INTRODUCTION

Diabetes mellitus is a universal health problem, affecting about 171 million people worldwide in 2000 and estimated to affect 366 million people by 2030[1] and an estimated 15.5 (9.8-27.8) million adults 20-79 years old suffer from diabetes in the Africa area, which represents a regional prevalence of 2.1 (6%)[2]. Diabetic

foot complications, especially foot ulcers (DFU), constitute a major public health problem for diabetes patients in sub-Saharan Africa and are important causes of prolonged hospital admission and death in patients from this part of the continent. Diabetes foot infection due to gangrene is the most common cause of prolonged hospitalization and amputation of their limbs. Besides, 28%–51% of amputated diabetics will have a second amputation of the lower limb within five years of the first amputation. Along with increased morbidity, foot ulcers can lead to lifelong disability and substantially diminish the quality of life for these patients[3]. For these patients, the diabetic foot infection (DFI) is due to neuropathy, vascular insufficiency, and diminished neutrophil function[4]. The underlying tissues are exposed to colonization by pathogenic organisms. In this case, a superficial infection of soft tissues and bone associated with signs of inflammation and/or purulent discharge are put in evidence[5], but with delay in appropriate treatment, there is an increased risk of mortality for the amputees and increased number of bacterial resistances in survived patients. In the hope of reducing the risk of amputation and emergence of multi-resistant bacteria, it seems urgent to look for other ways to inhibit bacterial growth in these wounds and thus allow their healing. Recently, interest in the use of medicinal plants in the treatment of many diseases has increased. Herbal medicine is experiencing new success in more and more countries around the world. In addition, a large number of medicinal plants are now being used by traditional medicine to treat many conditions, including diabetes and its complications[6]. This study is designed to isolate and to identify bacterial involved in diabetic foot patients and to assess the susceptibility of antibiotics multi-resistant bacteria to hydraulic extracts of *Allium sativum* and *Allium cepa* bulbs and *Cannabis sativa* leaves.

MATERIALS AND METHODS

Study design: It was a six months' translational study involving all diabetic patients with a DFU at the National Obesity Center of the Yaoundé Central Hospital from 25 July 2021 to 25 January 2022. An ethical clearance was obtained from the Hospital's Ethics Committee and each participant was enrolled after informed of the study and provided his informed consent.

Samples collection: After collecting, through a structured individual interview guide that included not only demographic data, but also clinical and therapeutic information (Table 1), biological samples were collected by methods that varied depending on the depth of the lesions and the presence or absence of pus: the collection by curettage (which consisted of collecting superficial tissue) and the swabbing for the collection of pus. The samples thus obtained were each placed in a screw tube containing the brain-heart media (BHM) and sent to the Microbiology Laboratory of the University of Yaoundé 1.

Isolation and identification: In the laboratory, these clinical samples were grown in an appropriate medium, in accordance with standard methods for bacterial isolation. Gram negative bacteria (GNB) were found on Eosine Methylene Blue agar (EMB) for enterobacteria isolation, Cetrimide for *Pseudomonas* species. Gram positive bacteria (GPB) were found on red blood agar for bacilli meanwhile Chapman and Colombia + blood was used for cocci bacteria. After 24 hours' incubation at 37°C, the bacterial isolates were subjected once again to Gram's staining and identified by conventional biochemical tests like Mannitol agar for motile bacteria, Kliger iron agar, oxidase disk for *Pseudomonas* species, catalase for *Streptococci* and coagulase for *Staphylococci*[7].

Antibiotic sensitivity test: The susceptibility test was performed using the Kirby Bauer disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. The bacterial inoculum was obtained by using isolated colonies on nutrient agar and homogenized in 5 ml of sterile distilled water. This suspension was then adjusted in comparison to the McFarland 0.5 standard. The test was carried out in series of three copies according to the CLSI protocol M2-A9[8,9]. The antimicrobial susceptibility of both positive and negative bacteria was determined using the antibiotic discs listed in Table 4 and Table 5. The research results were documented as sensitive (S), intermediate (I) and resistant (R). Multi resistant bacteria have been chosen to determine the inhibition parameters of plants extracts.

Plant sampling and extraction: Plants were identified at the Cameroon National Herbarium: *A. cepa* (25742/SRF), *A. sativum* (44810/HNC) et *C. sativa* (83664/SRF). After harvest, the leaves of *C. sativa* were dried in the shade at room temperature for 4 weeks then weighed and ground in a mill. The fresh bulbs of *A. cepa* and *A. sativum* were ground in a mill and the crushing were weighed, then the extraction continued by maceration in water for 72 hours, then the mixture filtered with Whatman paper n°1. The resulting filtrates were concentrated using 80°C water lyophilisation. Raw extracts collected were weighed and refrigerated at 4°C in labelled sterile jars.

Antibacterial assessment of plant extract: The microdilution in liquid medium using Alamar Blue enabled us to determine the MIC (Minimum Inhibitory Concentration). The MIC was defined as the lowest concentration of extract or antibiotic inhibiting visible bacterial growth after 24 h incubation at 37°C[9]. The stock solutions of extracts were prepared at a concentration of 100 mg/mL and those of gentamicin at a concentration of 1mg/ml. A 100 µL volume of the Mueller Hinton Broth medium was introduced into the microplate wells and 100 µL of the stock extract solution was introduced into the first column cups. Successive dilutions of geometric progression of reason 2 of the extracts were performed. This series of dilutions yielded concentrations ranging from 50 mg/mL to 0.04 mg/mL for plant extracts and 0.5 mg/mL to 0.0004 mg/mL for gentamycin.

Subsequently, 100 µL of inoculum was introduced into the cups, resulting in a concentration range of 25 mg/mL to 0.02 mg/mL for obtained plant extracts and 0.25 mg/mL to 0.0002 mg/mL for gentamicin. The microplates were incubated under standard conditions

for 24 hours at 37°C. After incubation, a 20µL volume of Alamar blue (50mg/ml) was introduced into each microplate well. The experiment was done in triplicate. A color change from blue to red or pink was indicative of bacterial growth[10].

Statistical analysis: Data was entered on Microsoft Excel 2016. Data analysis was performed with IBM SPSS Statistics Version 22.0. The descriptive data are presented in terms of numbers and percentages.

Ethical considerations: The study was performed after receiving approval from the Ethics Committee of the Yaoundé Central Hospital (N°2021/543/AR/ MINSANTE/SG/DHCY/UAF 21 October 2021) and obtaining informed consent from patients.

Table 1: Characterization of wounds.

Parameters		Frequency n (%)
Wounds origin	Wrong shoe	3 (15)
	Abscesses	12 (60)
	Bites from rodents	2 (10)
	Burns	3 (15)
Types of lesion	Neuropathic	6 (30)
	Ischemic	4 (20)
	Neither ischemic nor neuropathic	10 (50)
Grade of infection	II	8 (40)
	III	12 (60)
Infected foot	Left	9 (45)
	Right	11 (55)
Appearance of the wound	Purulent	9 (45)
	Wet	8 (40)
	Dry	3 (15)
Type of specimens	Pus	9 (45)
	Exudate	8 (40)
	Scab	3 (15)

RESULTS

Profile of sampled individuals: The present study included 20 participants, whom 6 (30%) were female and 14 (70%) were male, with a sex ratio of 2.33. The mean age of the series was 52.5±9.6 years (range 36-75 years). It was in majority patients from consultation, of whom 13 (65%) had type II diabetes. The origin of diabetic foot infection was for the majority of cases (50%) resulting from poor foot care (burns, abscesses, bites from rodents, poor footwear or in duration) with a common denominator deriving from infection (negligence) meanwhile 30% were neuropathic feet and 20% ischemic as presented by Table 1. The clinical description of lesions shown that 60% of participants presenting a high infection grade. Five (17%) patients had already undergone amputation. samples of 3 types were obtained, depending on the appearance of the wound: pus (45%) and exudates (40%) in majority (As shown in Table 1). Bacteria that were isolated from the diabetic foot infections are summarized in Table 2. From this table it appears that, Gram positive organisms were *Staphylococcus aureus* (34.29%), Coagulase-negative *Staphylococcus* CNS (14.29%), *Bacillus spp* (2.86%), and in the other hand, Gram negative bacteria were there such as: *Pseudomonas aeruginosa* (11,43%), *Serratia spp.* (8.57%), *Escherichia coli* (8.57%), *Enterobacter spp*. (5,71%), *Proteus spp.* (5,71%), *Klebsiella spp*., *Yersinia spp*., *salmonella spp* in proportions of 2.86% each.

Frequencies of identified species: Among the 20 studied participants, a total of 35 bacterial isolates was obtained from positive culture.

Table 2: **Frequency rate of isolated bacteria.**

Bacteria	Frequency n (%)
Bacillus spp.	1 (2.86)
Enterobacter spp.	2 (5.70)
Escherichia coli	3 (8.56)
Klebsiella spp	1 (2.86)
Proteus spp	2 (5.70)
P. aeruginosa	4 (11.43)
Salmonella	1 (2.87)
Serratia spp	3 (8.56)
Coagulase negative *Staphylococcus*	5 (14.29)
S. aureus	12 (34.29)
Yersinia spp	1 (2.86)
Total	35 (100)

It was also found that there could be several germs on a single wound (polybacterial). These bacteria were spread over the various wounds: 48.57% of the germs from the purulent wounds, 31.43% of the wet wounds and 20% of the dry wounds were identified (Table 3).

Antibiotics susceptibility profile of isolated bacteria: The antibiotic resistance patterns of the isolated bacteria to commonly used antibiotics, obtained with the Kirby Bauer disk diffusion method, are shown in Figure 1. For Gram-negative bacteria, several strains were completely sensitive to certain antibiotics tested. E. *coli.*, *Enterobacter spp.*, *Proteus spp.*, *Klebsiella spp.* and *Yersinia spp* were completely sensitive to Imipenem and Amikacin, although some isolates such as *P. aeruginosa* (75%) and *Serratia spp* (40%) shown resistance to them. The sensitivity of *P. aeruginosa* to all types of antibiotics was less than 25% except for Amikacin (75%). The sensitivity of enterobacteria supports 64.29% for Fosfomycin and 57.14% for Ceftazidime (Table 4). Regarding the Gram positives bacteria, *S. aureus* and CNS were resistant at 16.67% for Imipenem, at 8.33% and 50% respectively for Amikacin and at 66.67 % and at 20 % for Amoxicillin + clavulanic acid. On the other hand, these isolates are shown to be completely resistant to Oxacillin for *S. aureus* and CNS and completely resistant to Vancomycin for CNS, on the other hand, *S. aureus* were resistant to 75% (Table 5).

Plant extracts sensitivity: After a set of processes consisting in obtaining the powders, pastes and filtrates of plants, the different mass yields obtained were calculated for each plant used. Table 6 below presents for each extract, the extraction yield (%) obtained by freeze-drying after maceration of the plants. The study obtained extract yield of 10.48% for *A. cepa*, 16.33% for *A. sativum* and 10.32% for *C. sativa*.

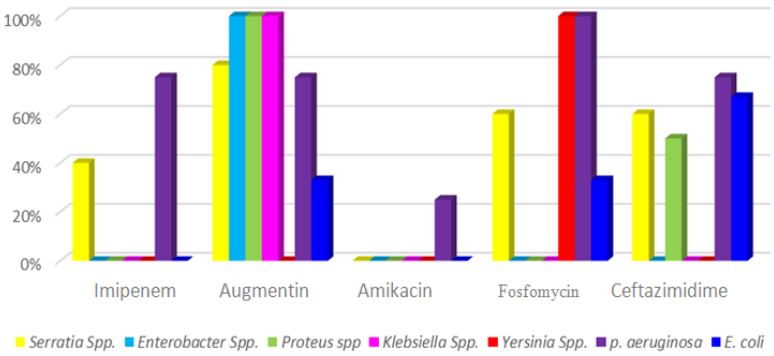

Figure 1: Antibiotic susceptibility testing of isolated bacteria.

The type of extraction was maceration and water was the solvent used.

Antibiotics susceptibility profile of isolated bacteria: The antibiotic resistance patterns of the isolated bacteria to commonly used antibiotics, obtained with the Kirby Bauer disk diffusion method, are shown in Figure 1.

MIC determination: With antibiotic resistant bacteria, MIC values of plant extracts were determined and reported as Table 7. The MICs of *A. cepa* are comprised between 6.25 mg/ml and 25.00 mg/ml of concentrations, exhibiting activity on strains of *Pseudomonas* and *Staphylococcus*.

Table 3: Bacteria distribution by type of wound.

Type of wound	*Ent.* Spp.	*E. coli*	*Kleb.* Spp.	*Prot.* Spp.	*P. aer.*	*Sal.* Spp.	CNS	*Ser.* Spp.	*S. aur.*	*Yer.* Spp.	Total	%
Wet	0	1	0	0	0	1	1	3	5	0	11	31.43
Purulent	1	2	0	1	4	0	2	1	5	1	17	48.57
Dry	1	0	1	0	0	0	2	1	2	0	7	20

Ent. Spp=*Enterobacter* Spp.*; E. coli=E. coli ; Kleb*. Spp=*Klebsiella* Spp.*; Prot*. Spp.=*Proteus Spp. ; P. aer.=P. aeruginosa ;* Sal. Spp.=*Salmonella* Spp*;* SCN=*Staphylocoque à coagulase négative; Ser*. Spp=*Serratia* Spp. *; S. aur.=S. aureus ; Yer*. Spp= *Yersinia* Spp.

Table 4: Antibiotic resistance of Gram-negative bacteria.

Antibiotic	CASFM/ EUCAST Recommendation	*Enterobacter* Spp.			*E. coli*		*Klebsiella* Spp.	*Proteus* Spp.	
		St1	St2	St3	St4	St5	St6	St7	St8
Amikacine	S≥18 ; R<18	22	23	24	25	11	21	20	20
Augmentin	S≥19 ; R<19	0	10	20	20	13	10	12	17
Ceftazidime	S≥22 ; R<19	23	30	18	34	13	30	19	30
Fosfomycine	S≥21 ; R<21	26	25	24	12	22	24	24	28
Imipenème	S≥22 ; R<19	30	28	34	28	30	28	27	30
	CASFM/ EUCAST Recommendation	*P. aeruginosa*				*Salmonella* Spp.	*Serratia Spp.*		*Yersinia* Spp.
		St9	St10	St11	St12	St13	St14	St15	St16	St17
Amikacine	S≥18 ; R<18	22	20	15	12	0	21	0	25	21
Augmentin	S≥19 ; R<19	11	9	0	13	23	10	0	22	10
Ceftazidime	S≥22 ; R<19	30	12	24	12	10	30	0	20	22
Fosfomycine	S≥21 ; R<21	22	25	0	26	0	24	0	23	0
Imipenème	S≥22 ; R<19	30	30	25	31	26	28	17	22	14

S=senstivity ; R= resistance ; St= strain

The *A. sativum* MICs comprised between 3.12 mg/ml and 25.00 mg/ml of concentration, exhibit activity against strains of *Pseudomonas*, *Serratia* and *Staphylococcus*. *C. sativa* with a MIC ranged between 1.86 mg/ml and 25.00 mg/ml, had a higher activity than the other extracts on the majority of strains. The reference antibiotic (gentamicin) shown a MIC ranging from 0.001 mg/ml to 0.25 mg/ml concentrations lower than the extracts.

DISCUSSION

The predominance of the male sex in the studied population is a phenomenon confirmed by several authors, with a *sex ratio* of 2.5 for Amoussou-Guenou *et al.*, in Cotonou[11], 1.6 for Loukro *et al.*, in Ivory Coast[12], 4.3 with Zemmouri *et al.*, in Morocco[13], and 2.33 in current study. This could be explained by the fact that women are more diligent and thorough in their care, and the generally recognized poor adherence to therapy in men who have a pattern of foot care and hygiene neglected. In current survey, the average age was 52.5±9.6 years with ranging from 34 to 65 years, including 13 patients over 50 years of age. Similar age averages were observed with Amoussou-Guenou *et al.*, (median age of 57 years), and Loukro *et al.*, (56.8±18.7 years)[11,12].

Table 5: Antibiotic resistance of Gram-positive bacteria.

Antibiotique	CASFM/EUCAST Recommendation	*S. aureus*											
		St18	St19	St20	St21	St22	St23	St24	St25	St26	St27	St28	St29
Amikacine	S≥18 ; R<18	23	24	19	12	20	20	25	0	28	20	20	21
Amoxi + Clav. Ac.	S≥20 ; R<19	9	10	0	22	40	15	22	23	30	16	13	14
Imipenème	S≥22 ; R<22	30	30	0	30	25	23	22	29	38	28	24	30
Oxacilline	S≥22 ; R<22	0	0	0	0	0	0	0	0	0	0	0	0
Spiramycine	S≥22 ; R<19	0	0	0	0	0	0	0	0	0	0	0	0
Vancomycine	S≥22 ; R<22	0	0	0	0	20	0	0	15	24	0	0	0

	CASFM/EUCAST Recommendation	Coagulase negative *Staphylococcus*				
		St30	St31	St32	St33	St34
Amikacine	S≥18 ; R<18	23	14	12	23	25
Amoxi + Clav. Ac.	S≥20 ; R<19	22	16	22	10	9
Imipenème	S≥22 ; R<22	28	30	30	27	10
Oxacilline	S≥22 ; R<22	0	0	0	0	0
Spiramycine	S≥22 ; R<19	0	0	0	0	0
Vancomycine	S≥22 ; R<22	0	0	0	0	0

S=senstivity; R= resistance; St= strain ; Amoxi + Clav Ac.= Amoxilline + Clavulanic Acid

Table 6 : Summary of extract yield characteristics.

Used plant part	Extract yield (%)	Extract physical properties	
		Color	Texture
A. cepa bulbs	10.48	Brown	Pasty
A. sativum bulbs	16.33	Brown	Crystalline
C. sativa leaves	10.32	Greenish	Powdery

Aging is a natural evolutionary phenomenon that, according to the literature, is a leading risk factor for the occurrence of diabetes and these complications. Indeed, the risk of occurrence and superinfection of trophic lesions of the diabetic foot increases with age. Type II diabetes was the majority in current study.

These findings are consistent with other African authors who found type II diabetes to be the majority in their studies[11,12]. After isolation and identification, the most common bacterium was *S. aureus* (34.29%) followed by CNS (14.29%). These results are in accordance with those of Zemmouri *et al.*, (34%)[13], and Al-Joufi *et al.*,(28.72%)[14], regarding the strain of *S. aureus* in Morocco. Any streptococci was not found, this could be justified by the sampling method: since streptococci are anaerobic bacteria, swabbing was not the appropriate sampling method for sampling these pathogens; on the other hand, the culture of these bacteria required anaerobic conditions not being at our disposal did not allow us to find these pathogens. The presence of *Bacillus spp.* can be explained by contamination of used sample during laboratory manipulations or when passing the petri dishes in the incubator.

Table 7: Plant extracts MIC values.

Strain	Plant extracts MIC (mg/mL)			Gentamicin
	A. cepa	*A. sativum*	*C. sativa*	
E. coli	ND	ND	25	0.0312
Proteus Spp	6.25	6.25	12.5	0.0004
P. aeruginosa	ND	25	ND	0.003
P. aeruginosa	6.25	ND	25	0.0312
P. aeruginosa	ND	ND	ND	0.0625
Serratia Spp	ND	25	25	ND
Yersinia Spp	ND	ND	25	0.001
S. aureus	12.5	ND	ND	0.007
S. aureus	12.5	25	25	0.007
S. aureus	12.5	12.5	1.86	0.0009
S. aureus	ND	ND	25	0.0625
S. aureus	25	6.25	6.25	0.003
S. aureus	6.25	3.12	3.12	0.0004
S. aureus	ND	ND	ND	0.25
S. aureus	ND	25	6.25	0.0312
S. aureus	ND	ND	ND	0.0625
S. aureus	ND	ND	ND	0.0312
Coagulase negative *Staphylococcus*	12.5	ND	ND	0.0009
Coagulase negative *Staphylococcus*	25	ND	ND	0.25
Coagulase negative *Staphylococcus*	ND	ND	ND	0.25

Gram negative bacteria accounted for 50% of all pathogens isolated from the wounds, these pathogens *P. aeruginosa* (11.43%), *Serratia spp*. (8.56%), *E. coli* (8.57%), *Enterobacter spp.* (5.70%), *Proteus spp.* (5.70%), *Klebsiella spp., Yersinia spp., salmonella spp* in proportions of 2.86% are mostly enterobacteriaceae. Several studies have revealed that Gram positive bacteria are the main culprits of diabetic foot wound infections such as Velasco *et al.*, and Stappers *et al.*,[15,16]. Nevertheless, there are studies conducted on patients living in warm climates that have reported that Gram negative organisms are the most common organisms in diabetic foot infections[17]. Due to microbial infections, healing of diabetic foot wounds is clinically difficult[18]. Staphylococci were found in the current study and some species were sensitive to Imipenem and others to Imipenem and Amikacin. This trend was also found in Douala, Cameroon, with Okalla *et al.*,[19]. At the same time, these isolates showed complete resistance to Oxacillin, 83.34% to Vancomycin and 55.56% to Augmentin as reported by the study by Velasco *et al.*,[15]. The high resistance to Vancomycin is contrary to the study by Al-Joufi *et al.*,[14] where 100% of the strains tested were sensitive to Vancomycin. This can be explained by the poor quality of used antibiotic due to the different chain hot and cold changes.

In the current study, antibiotic resistance among isolates of *P. aeruginosa*, *E. coli*., *Enterobacter spp.*, *Proteus spp.*, *Klebsiella spp.* and *Yersinia spp* was similar to previous studies conducted in other parts of the world, with a resistance of 37.5% to beta-lactams[20]. Imipenem and Amikacin showed bactericidal activities against most of these isolates compared to other antibiotics. One of the most important observations was the high resistance rate of *P. aeruginosa* to Imipenem and Ceftazidime. These isolates were taken for most patients who underwent surgery for the removal of the toe. Therefore, we suspect an infection of nosocomial origin due to the long hospitalization of patients that may derive from multi-resistant antibiotics[21].

The study of the antibacterial activity of *A. sativum* extracts on the antibiotic resistant bacteria strains, showed different inhibitory concentrations ranging from 3.12 mg/ml up to 25.00 mg/ml for the most part is active on used bacteria. These concentrations are low compared to the results of Magryś *et al.*, which obtained on all its strains a very high extract activity[22]. This could be explained first by the type of solvents used which, unlike current study, was a mixture of water and ethanol which makes it possible to extract compounds much more active than water, but also the concentration range used which is much higher than ours 6000 mg/ml. In current study, *A. cepa* showed MICs ranging from 6.25 mg/ml to 25.00 mg/ml, and a low antibacterial activity in contrary of the study conducted by Zhou *et al.*, in which the aqueous extracts from storey onion have the strongest inhibitory effect on all the tested strains (MIC 31.3-125 mg/mL)[23]. Alliums are a family made up of ajoene, an organosulfur component, which is very little present in onions but very present in garlic. This would explain the low activity of *A. cepa* compared to that of *A. sativum*. In addition, according to Kyung's studies in 2012, GNBs are more sensitive to *Alliums* than GPBs; which would explain why more than 60% resistance to garlic and onion extracts was observed for *S. aureus* and CNS strains. The activity of *C. sativa* also ranges between 1.86 and 25 mg/ml, out of used 20 strains, it is active on 12 strains and even shows bactericidal activity on *S. aureus* which compared to that of Schofs *et al.*, remains low, which would be explained by the concentration range used, which is here greater than 100mg/ml[24]. Several studies showed that these three plants have the ability not only to boost the immune system, but also to fight against diabetes through their components such as ajoene and allicin for alliums and cannabitiol for cannabis. This can explain the decrease in the rate of infection and the more or less rapid healing of wounds.

CONCLUSION

The current study revealed that the most frequent pathogens were: *Staphylococcus aureus* followed by coagulasse-negative staphylococci and *Pseudomonas aeruginosa*. Although most of the isolates were sensitive to Imipenem and Amikacin, it was observed that a high rate of multi-resistant bacteria with Augmentin, Vancomycin and Oxacillin. Plant extracts of *A. cepa*, *A. sativum* and *C. sativa* exhibited antibacterial activities. Although their antibacterial activities seem weak, it could be a new way, an alternative for the treatment of diabetic foot wound infections.

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CONFLICT OF INTEREST

There is no conflict of interest associated with this work.

AUTHOR'S CONTRIBUTION

J Njiki Bikoï, SL Koko-Ta and EDF Moni Ndedi Conceptualize, J Njiki Bikoï, MY Dehayem and SL Koko-Ta recruited patients and collected samples; SL Koko-Ta, E Nguiffo Makue, AE Membangbi and EDF Moni Ndedi took care of the laboratory work, designed the experiment and interpreted data obtained. All authors contributed to data analysis, draft and revised the paper, gave final approval of the version to be published and agreed to be accountable for all aspects of the work, SH Riwom Essama was here for the supervision.

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