Identification and Drug Sensitivity of Aerobic Bacterial Isolates from Diabetic Foot Ulcers of Sudanese Patients: a Cross sectional study

saada Mohamed nour (saada.nori@yahoo.com)
University of Bahri College of Medicine https://orcid.org/0000-0002-1669-6108
Maowia Mukhtar
Bioscience Research Institute, Ibn Sina University
Mohamed Elmakk
University of Khartoum Faculty of Medicine
Elshibli Mohamed Elshibli
Tiabah University, Kindom Saudi Arabia
Walyeldin Elnour Elfakey
University of Bahri College of Medicine
Mahdi Shamad
University of Bahri College of Medicine
Nuha Ibrahim
National Tuberculosis reference Laboratory, National Public Health Laboratory
Mohamed yahia Fadul
Faculty of Medicine University of Khartoum, Fifth class student
Safia Mohamed
National Public Health Laboratory

Research article

Keywords: diabetic foot ulcer, aerobic bacteria, antibiotic sensitivity

Posted Date: January 29th, 2020

DOI: https://doi.org/10.21203/rs.2.22115/v1

Abstract

Background

Diabetic foot ulcer infection cause great morbidity and mortality among diabetic patients and is a major cause of lower extremity amputation worldwide. This study aimed to determine the profile of aerobic bacteria and their antibiotic sensitivity patterns in diabetic foot infections (DFI) among different Wagner's grades.

Methods

This study was conducted during December 2017 - March 2018 in a Diabetic Center, Sudan. A total of 152 diabetic patients with different grades of foot ulcers were randomly enrolled in the study. The patients were grouped using Wagner's classification. Tissue biopsies and deep swabs were collected from the ulcers for aerobic cultures. The cultured isolates were identified using phenotypic and biochemical properties and their sensitivity to commonly used antibiotics, Colistin, Aikacin, Ciprofloxacin, Augmentin, Ceftazideme, Gentamicin, Clindamycin, Ceftriaxone Meropenum. Cotrimoxazole, Erythomycin, Oxacillin and Vancomycin. Fusidic acid, Imepenem, and Piperacillin was tested using the Kirby Bauer disk diffusion method.

Results

The mean age of the patients was 54.31 (SD ± 12.1) years, male to female ratio of 8: 1. The mean duration of diabetes was 14 (SD ± 8) years. The ulcers varied in duration from 1 day to 10 years. of 152 samples 181 aerobic bacteria were isolated. Cultures yielded 1-3 isolate per culture. The maximum number was isolated from grade 3 group followed by long standing ulcer LSU group 50.8% and 28% respectively. Polymicrobial infection was higher in LSU (30.4%). The isolates were mostly Gram-negative bacteria. The most frequent were proteus spp. (35.3%), S. aureus MRSA 14.4% and Coliform 12.2% respectively. The most common isolates in grade 3 were *P. Mirablis*, *Staphylococcus* and *Coliform* and in long standing ulcers were *P. Mirablis*, *S. aureus MRSA* and Coagulase negative *staphylococcus* respectively.

Conclusion

Gram-negative bacteria were more prevalent and the most frequent pathogens were Proteus spp. The most common polymicrobial infections were due to *P. mirablis* with; *P. aerginosa*, *S. MRSA* and *Coliform* respectively . Gram negative rods were sensitive to Amikacin, (80.6) %) while the highest sensitivity of Gram positive was to Imepnem (85%). Most of the isolates were sensitive to Meropenem. No significant relation between Wagner grades and neuropathy was detected.

Background

Diabetic foot infection (DFI) is a serious complication of diabetes and a major cause of lower extremity amputation worldwide [1]. Approximately one-fourth of diabetic patients develop an ulcer during their lifetime, and about half of these ulcers become infected [2 3] Diabetic foot problems result in high economic cost and a large national economic burden[4]. Bacterial pathogens isolates from DFI vary with the grades and severity of infection. Early infections are generally monomicrobial, whereas advanced infections are mainly polymicrobial and low grades are generally infected with gram-positive organisms[4]. Several factors, such as inappropriate antibiotic treatment and frequent hospital admission, can predispose to infection with drug resistant bacteria [5].The identity of the bacteria infecting diabetic foot differs among patients and hospitals [6]. The effective antibiotic treatment is essential to control the infection [7]. The International Diabetes Federation has anticipated that the number of persons with diabetes will increase from 240 million in 2007 to 380 million in 2025 [8]. And this increment need more research programs. The aim of this study was to determine the aerobic bacterial profile and antibiotic sensitivity patterns in DFI in different Wagner's grades in order to provide more detailed suggestion to the clinician about the empirical antibiotic choice and to correlate the peripheral neuropathy with different ulcer grades in diabetic patients. A few studies identified the bacterial isolates and their sensitivity to common used anitbiotics in Sudan but to our knowledge this is report on bacterial isolates from different grades of ulcers considering the depth of the ulcers was not done in Sudan.

Results

A total of 152 diabetic patients were enrolled in this study, 135 patients (88.8%) were male and 17 patients (11.2%) were female. The mean age of the patients in the study group was 54.31 with SD ± 12.1years. The gender, ulcer grades and sensation loss of the participants are shown in table 1.

The mean duration of diabetes was 14 SD ± 8 years. The highest numer of patients 125 (82.2%) were in the age group of 40-65 years. The highest numer of patients 66 (43.7%) were in the group of duration 12-24 years. The ulcers varied in duration from 1 day to 10 years. The frequencies of age, diabetic duration, ulcer duration and sensation loss duration are shown in table 2.

Concerning patient's protective sensations, 82 patients (53.6%) lost Sensation and the sensation loss duration ranges from 7 days to 24 years among all patients. The percent of patients with peripheral neuropathy in each ulcer grade is illustrated in Figure 1.

Microbiological findings:

Bacterial growth was detected in 138 specimens (90.8%) and no growth was seen in 14 specimens (9.2%). Cultures yielded a total of 181 aerobic isolates from different ulcer grades (Figure 2), with a range of 1-3 organisms per sample, the average numbers of isolates was 1.2 per case. In present work, two types of bacterial infections were detected, monomicrobial cultures were 63.82% while the poly microbial cultures were 26.97% Figure 3. 30.4% of poly microbial were from long standing ulcers patients and 27% of poly microbial infections were from grade 3 patients. Frequency of bacteria isolated from the ulcers of

the participants is shown in table 3. The number of gram negative rods was 120 (66.3 %), higher than Gram-positive 61 (33.7 %), 55 of this number were cocci and 6 were coccobacilli. The percent of Gram negative to Gram positive bacteria in each grade is illustrated in table 4. The most frequent pathogens were Proteus 35.3%, [*P. mirablis* 48 (26.5%)+ *P. vulgaris* 16 (8.8%)] followed by *S. aureus* MRSA 14.4% and Coliform 12.2% respectively of all isolates. The maximum number of bacteria was isolated from grade 3 group followed by long standing ulcer group 50.8% and 28% respectively.

The most common isolates in grade 3 were *P. Mirablis, Staphylococcus* and Coliform. The most common isolates in long standing wounds were *P. Mirablis, S. aureus MRSA* and Coagulase negative *staphylococcus*. The most common associated bacteria in polymicrobial infection were *P. Mirablis* with *P. Aerginosa, S. aureos* MRSA and Coliform respectively.

Antibiotic sensitivity:

Frequency distribution of sensitivity of antibiotics among all patients is shown in Figure 3. The most sensitive antibiotics for Gram negative rods were Aikacin, 80.6 %, Imepinum 77.2 % and Meropenum 77%. For Gram positive the most sensitive antibiotics were Imepnem 85% and Vancomycin 69% . The most sensitive antibiotic among all isolates was Meropenem. The most sensitive antibiotic for each grade is illustrated in table 4.

Discussion

Foot infections in diabetes patients are a complex problem and a common cause of morbidity, ultimately leading to severe complications like gangrene and amputation [15]. Effective management of the infection requires isolation and identification of the bacteria and determining their sensitivity to antimicrobial agents [16]. The diabetes and diabetic foot infections are on the rise in Sudan with little data available to guide the doctor to achieve effective cure.

This study aimed to isolate and identify aerobic bacterial pathogens associated with diabetic foot infections in different grades of wounds and to determine their sensitivity to the commonly used antibiotic. As reported in other studies males were more represented in this study with the male to female ratio reaching 8:1. High prevalence of diabetic foot infection among males has been reported in other studies. [1,3,4] and was attributed to increased outdoor activities among males than females. To the contrary a study done in the J. D. C. 2012 reported the male to female ratio of 3:3.3 [17] . In our study, we found that the ulcers varied in duration from 1 day to 10 years which was a long duration range in comparison to previous study in the same center.[17]

Two patterns of bacterial infections were detected in present work; monomicrobial infection – which was the most 63.82% while polymicrobial infections was 26.97%. The average numbers of isolates was 1.2 per case which was similar to study done by Eithar [18] in the same diabetic Centre reported similar findings. In addition other study reported a similar number of isolates per case 1.39 [19]. Polymicrobial

nature of DFIs has been reported in several studies conducted both in Sudan and abroad. [20] . A study in India reported the majority of DFIs were polymicrobial nature with aerobic Gram-positive cocci, and especially staphylococci as the most common causative agents [21]. This disagreement with our result could be due to the hospital environment and the use of different antibiotics in the two studies. In a study in Manipal- India, of the 108 specimens from the diabetic foot lesions, culture showed polymicrobial growth in 44.4% which was equal to monomicrobial growth in 44.4% [13]. These discrepancies suggest differences in diabetic foot infections, with severe infections usually having polymicrobial isolates and mild infections usually having monomicrobial isolates [14, 22] To our research group these discrepancies may attributed to differences in the hygiene, hospital practice and usage of antibiotics. Also study done in USA reported variation of the bacterial pathogens encountered with the Wagner grade and severity of infection [4] and early infections are generally monomicrobial, whereas advanced infections tend to be polymicrobial. Low grades are generally infected with gram-positive organism [4] and these findings were highly consistent with the present work, in which Gram positive bacteria found in grade 1, 2 and decreased in grade 3 where the ratio of Gram negative to Gram positive was 5:1 then disappeared towards grade 4 and reappeared in long standing ulcers which were in maturation phase. Like-wise Widatalla [23] found that the most common pathogens were *Staphylococcus aureus* (33.3%), *Pseudomonas aeruginosa* (32.2%), and *Escherichia coli* (22.2%). Other study in J. D. C [18], also found the commonest isolated organism was *S. aureus* (46%). Those 2 studies [18 23] were in agreement with most international reports [24 25 26 27] where *S. aureus* was found as the most predominant and not agrees with our findings. Recent study in Sudan; identified *proteus* spp. (mirabils and vulgaris) as the most frequent bacteria in diabetic wounds (37.5%) and it was in agreement with the findings of this study. [28]

In present report, microbiological investigation revealed the number of Gram-negative organisms 66.3 % higher than Gram-positive which was 33.7 %. Almost similar results were obtained by two Indian studies [3,4] where Gram-negative organisms were found to be more than Gram positive. Also study conducted in North India found Gram-negative aerobes were most frequent organisms 63.8% than Gram-positive aerobes 36.1% [29]. Other study reported Gram-negatives were more prevalent, but predominant organisms isolated were members of the Enterobacteriaceae. [30]

In the present study the percentage of diabetic patients with peripheral neuropathy was in range 36.8% - 65.1% with average 51% and this was in agreement with study estimated that 45% to 60% of all ulcerations in diabetic patients were mainly due to neuropathy [31]. Diabetic peripheral neuropathy (DPN) is the most frequent neurological complication of diabetes mellitus (DM). Peripheral neuropathy along with foot deformities and acute or repetitive trauma are the triad of factors that contribute ultimately to diabetic foot ulcers. [32]. In this study, No significant association between Wagner grades and neuropathy was detected suggesting no impact of peripheral neuropathy on DFU healing Although the association between peripheral neuropathy and non-healing diabetic ulcers was reported from developing countries [33 34 35 36]

Limitations of the study

This study didn't include the anaerobic bacteria due to the limitation in the laboratory facilities.

Conclusion

Gram-negative bacteria were more prevalent and the most frequent pathogens were Proteus spp. The most common polymicrobial infection were *P. mirablis* with; *P. aerginosa, S. MRSA* and *Coliform* respectively. Polymicrobial infection was higher in long standing ulcers (30.4%). Most of the isolates were sensitive to Meropenem. No significant relation between Wagner grades and neuropathy was detected suggesting no impact of peripheral neuropathy on DFU healing.

Therefore, based on the findings of this study early diagnosis, proper microbiological cultures and antimicrobial resistance screen are essential for effective management of diabetic wound infection.

Methods

Study area and setting

The study was conducted in Jaber Abu Eliz diabetes Center (J.D.C) in the capital Khartoum. The center is the largest diabetes clinic specialized in treatment and care of diabetic foot and it receives patients referred from different region of the country. J.D.C is a multidisciplinary specialized Diabetic Centre in the Sudan. It caters for 70, 000 registered diabetic patients, utilized by people from Khartoum state, its surrounding areas and from other states.

Study design and period

Across-sectional study was conducted from December 2017 to March 2018.

Sample size and sampling technique

152 diabetes patients with foot ulcers were enrolled in the study. The patients were attending the outpatient clinic in the Surgery Unit at J. D. C. They were enrolled using systemic random sampling.

Base line data

The demographic data including age, sex, duration of diabetes, duration of the ulcer, and duration of the sensation loss was collected by face to face interview using a predesigned questionnaire. All the questionnaires were checked for accuracy and completeness. The questionnaire was prepared in English version and translated to Arabic version which is the language of the study participants.

Patients

The patients were grouped according to Wagner diabetic foot ulcer classification into 5 groups [grade 1 , grade 2, grade 3, grade 4 and long standing ulcers (maturation stage)]. The number of patients in each group were as following: 8 patients were grade 1, 19 patient were grade 2, 70 patients were grade 3, 12 patients were grade 4, and 43 patients were long standing ulcer (maturation phase). Specimens used in this study were obtained from every patient groups.The ulcer duration was determined verbally based on the patient response. While the sensation of patients was examined clinically, osteomyelitis was diagnosed by probe to bone test and bone biopsy for microbiological investigation. The gender, ulcer grades and sensation loss of the participants are shown in Table 1.

Collection of microbiological samples

The ulcers were cleaned vigorously with saline and extensively debrided first to avoid the isolation of colonizing flora. Specimens were collected by 2 methods, tissue biopsy (soft tissue and bone) from the central region of the ulcer bed using a 6-mm disposable sterile punch biopsy (Stiefel Laboratories, Ltd., Sligo, Ireland) and placed immediately into a sterile vial containing 2 ml of sterile normal saline. Some samples were collected by deep swab technique from patients with new wound (grade 1) and long standing wound (maturation phase). All specimens were taken from patients on dressing table. The specimens were transferred within 1 hour to Bacteriology Department at the National Public Health Laboratory, where optimal microbiological culture techniques were used.

Bacterial isolation and identification

The specimens were inoculated on blood agar and MacConkey agar plates for the isolation of aerobic bacteria. Inoculated blood agar was put into candle jar with carbon dioxide and incubate for 18-24 h at 35 °C -37°C. Inoculated mac agar was incubated for 18-24 h at 35 °C -37°C. Gram stain was done from bacterial colonies. Selected isolates from Mac and blood agar subcultured into nutrient agar and incubated to refresh the sample. Additionally, mannitol salt agar were inoculated and were incubated at 37°C for 24 hours. The isolates were identified based on colony morphology, colour change, gram-staining results, Urea and Sulfide indole motility (SIM), and biochemical reactions for catalase, oxidase, coagulase, and other biochemical tests [9 10 11]. In this study, anaerobic bacteria were not investigated due to the limited laboratory facilities. The investigations were designed to identify aerobic bacteria in each ulcer grade.

Antibiotic susceptability test

Antibiotic susceptibility test was performed using the Kirby Bauer disk diffusion method according to the guidelines of the Clinical and Laboratory Standards Institute guidelines (CLSI) [12] . In brief, isolated colonies were suspended in sterile normal saline 0.5% concentration and matched to the 0.5 McFarland standard then a sterile cotton wool swab was dipped into the suspension and used to streak Mueller Hinton agar plates. (Oxoid, Basingstock, Hampshire, UK) this was for gram positive bacilli, staphylococci and enterococci. The inoculated plates were incubated at 35-37°C for 18-20 h. Then the diameter of the zone of inhibition was measured and interpreted according to the criteria recommended by the CLSI. *S.*

aureus was tested for methicillin resistance using 1 µg oxacillin disc. Reference strains of *E. coli* (ATCC 25922) and *pseudomonas aeruginosa* (ATCC 27853) were used as controls for Gram-negative bacteria. *S. aureus* (ATCC 25923) was used as Gram-positive control. The antibiotics tested for Gram-positive bacteria (G+ve cocci) were Colistin, Aikacin, Ciprofloxacin, Augmentin, Ceftazideme, Gentamicin, Clindamycin, Ceftriaxone and Meropenum. For S. aureus, Augmentin, Amikacin, Cotrimoxazole, Ceftazidime, Ciprofloxacin, Erythomycin, Gentamicin, Meropenem, Oxacillin and Vancomycin. For Gram positive rod, the antibiotic tested were Augmentin, Cotrimoxazole, Erythromycin, Fusidic acid, Gentamicin and Oxacillin. For Gram negative rod, the antibiotic tested were Augmentin, Amikacin, Cotrimoxazole, Ceftazidime, Ceftriaxon, Ciprofloxacin, Clindamycin, Imepenem, Meropenem and Piperacillin. Multidrug-resistant organisms (MDROs) were defined as bacteria that were resistant to more than one or all classes of antibiotics [13 14] .

Data quality control

Aseptic technique was used throughout sampling and handling procedures by using sterile materials, flaming and icebox. For remarkable studies of microorganism, pure culture was used. Solutions and equipments containing water were autoclaved at 121°C for 15 to 20 minutes. The sterility of the media were detected by incubating 5% of the batch at 37°C for 18-24h

Statistical methods

The data analyzed using SPSS 21 with reference significance level .05 and CLs 95%, descriptive statistics (mean±SD) obtained for quantitative variables, while qualitative variables described using frequency and percent difference between frequencies in (table 3) tested by goodness of fit test using chi-square test or Fisher Exact test when needed, the relationships between qualitative variables tested by independence test using chi-square or Fisher Exact test when needed, to obtain the p-value.

Abbreviations

ATCC:

American type cell culture

BPW:

Buffered peptone water

D:

Diabetic

DFI:

Diabetic foot infection

DFU:

Diabetic foot ulcer

Mac:

MacConkey

MDROs:

Multidrug-resistant organisms

MHA:

Mueller Hinton Agar

MRSA:

Methicillin Resistant *Staphylococcus aureus*

MSA:

Manitol Salt Agar

SPSS:

Statistical package for social sciences

SIM:

Sulfide indole motility

U:

Ulcer

LSU:

Long standing ulcer

Declarations

Ethics approval and consent to participate

The ethical approval for this study was obtained from the ethics committee of research department at the Ministry of health Khartoum state- Sudan on 17.8 2017. Written consent were obtained from all

participants before their enrolment after explaining the aim of the study. The youngest patient was nineteen years old so was no need to obtain informed consent from parent or guardian.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Competing interests

The authors state that they have no competing interests.

Funding

This study was fully funded by the Ministry of higher education and Scientific Research, Sudan which provided the financial support.

Authors' contributions

SM, designed the study, collected the samples and analyzed the samples and the data, wrote the manuscript. The principal investigator and receiver of the grant. MM, Designed the study, contributed to the analysis of the samples, wrote the manuscript. ME, supervision of the clinical component, management of the patients and wrote the manuscript. EM, carried out statistical analysis. WE, gave valuable ideas of the manuscript and revised the manuscript . MS, gave valuable ideas of the manuscript and revised the manuscript. NY, designed the sample collection and check the sterilization of all materials and equipment. MY, collected the data, SA, isolation and identification of bacteria. All authors have read and approved the manuscript.

Acknowledgement

We express our profound and sincere gratitude to Ministry of higher education the sponsor of this study. To all doctors and nurses in Jabir Abueliz Diabetic Centre for valuable help.

References

1. Sivanmaliappan TS, Sevanan M. Antimicrobial susceptibility patterns of *Pseudomonas aeruginosa* from diabetes patients with foot ulcers. Int J Microbiol 2011; 2011:605195. (Accessed 2014 January 14). Available at http://www.hindawi.com/journals/ijmicro/2011/605195/.
2. Lipsky BA, Berendt AR, Deery HG, Embil JM, Joseph WS, Karchmer AW et al. Diagnosis and treatment of diabetic foot infections. Clin Infect Dis 2004; 39:885-910.

3. Lavery LA, Armstrong DG, Murdoch DP, Peters EJ, Lipsky BA. Validation of the Infectious Diseases Society of America's diabetic foot infection classification system. Clin Infect Dis2007; 44:562-565.
4. Charles, A Adams, Jr., M.D. and Edwin A Deitch, M.D. Diabetic foot infections, surgical treatment-NCBI Bookshelf 2001.
5. El-Tahawy AT. Bacteriology of diabetic foot infections. Saudi Medical J 2000; 21:344-347.
6. Markakis K., Bowling F. L., Boulton A. J. The diabetic foot in 2015: an overview. *Diabetes/Metabolism Research and Reviews*. 2016;32(Supplement 1):169–178. doi: 10.1002/dmrr.2740.
7. Chan JC, Malik V, Jia W, Kadowaki T, Yajnik CS, Yoon KH, Hu FB. Diabetes in Asia: epidemiology, risk factors, and pathophysiology. JAMA. 2009 May; 301(20):2129–2140. doi: 10.1001/jama.2009.726. Available from: http://dx.doi.org/10.1001/jama.2009.726.
8. International Diabetes Federation: Diabetes Atlas 3rd Edition, Brussels, Belgium: International Diabetes Federation: 2006 [Google Scholar]
9. Cheesbrough M. District laboratory practice in tropical countries: Cambridge university press; 2006.
10. UK Standards. UK Standards for microbiology investigations identification of Enterobacteriaceae: bacteriology test procedures Standards UNIT. Public Health England. 2014;4: 18–20.Google Scholar
11. Mackie TJ. Mackie & McCartney practical medical microbiology: Churchill living stone. New York, NY: USA; 1989.Google Scholar
12. Clinical and Laboratory Standards Institute (2010). Performance Standards for Antimicrobial Susceptibility Testing-Nineteenth Informational Supplement. CLSI document M100-S20. Clinical and Laboratory Standards Institute, Wayne, Pennsylvania, USA.
13. Sekhar SM, Vyas N1, Unnikrishnan MK, Rodrigues GS2, Mukhopadhyay C3 Antimicrobial Susceptibility Pattern in Diabetic Foot Ulcer: A Pilot Study http://www. amhsr.org, Sep 28, 2016
14. Citron DM, Goldstein EJC, Merriam VC, Lipsky BA. Bacteriology of moderate to severe diabetic foot infections and invitro activity of antimicrobial agents. J Clin Microbiol 2007; 45:2819-2828.
15. Gadepalli R, Dhawan B, Sreenivas V, Kapil A, Ammini AC, Chaudhry R: A clinicomicrobiological study of diabetic foot ulcers in an Indian tertiary care hospital. Diabetes Care. 2006, 29:1727-1732. 10.2337/dc06-0116.
16. Hassan Gubara Musa, MD1 and Mohamed ElMakki Ahmed, MS, FRCSI1,2* Associated risk factors and management of chronic diabetic foot ulcers exceeding 6 months' duration. Clinical Research Article. Oct. 2012.
17. Eithar M. M.*, Mohammed Elfatih A. Omer. Aerobic bacteria isolated from diabetic septic wounds. American Journal of Research Communication, 2015, 3(10): 91-99} usa-journals.com, ISSN: 2325-4076.
18. Abdalrahman, H. M. (1999). Antibacterial activity of some antibiotics against clinical isolates taken from diabetic septic wound. Thesis submitted for a degree of Master, University of Khartoum, Faculty of Pharmacy.

19. Bansal E, Garg A, Bhatia S, Attri AK, Chander J. Spectrum of microbial flora in diabetic foot ulcers. Indian J Pathol Microbiol. 2008; 51:204–8. [PubMed.
20. Lipsky BA, Berendt AR, Cornia PB, Pile JC, Peters EJ, Armstrong DG, et al. 2012 Infectious Diseases Societyof America clinical practice guideline for the diagnosisand treatment of diabetic foot infections. Clin Infect Dis2012; 54:e132-7.
21. 18.Anandi C, Aaguraja D, Natarajan V, Ramanatham M, Subramaniam CS, Thulasiram M et al. Bacteriology of diabetic foot lesions. Ind J Med Microbiol2004; 22:175-178.
22. AbuBakr H. Widatalla ,Seif ElDin I. Mahadi, Mohamed A. Shawer, Shadad M. Mahmoud, A.E. Abdelmageed and Mohamed ElMakki Ahmed, Diabetic foot infections with osteomyelitis: efficacy of combined surgical and medical treatment. Journal List Diabet Foot Ankle v.3; 2012 PMC3464066.
23. Hartemann-Heurtier A, Senneville E. Diabetic foot osteomye-litis. Diabetes Metab 2008; 34: 87–95.
24. Rao N, Lipsky BA. Optimising antimicrobial therapy in diabetic foot infections. Drugs 2007; 67: 195–214.
25. Venkatesan P, Lawn S, Macfarlane RM, Fletcher EM, Finch RG, Jeffcoate Conservative management of osteomyelitis in the feet of diabetic patients. Diabet Med 1997; 14: 487 90.
26. Wheat J. Diagnostic strategies in osteomyelitis. Am J Med 1985; 78: 218–24.
27. Ahmed Ibrahim Hashim, Amal Mubarak Hassan Mubarak, Nosiba Ahmed Hassan Hamed, and Ahmed Bakheet Abd Alla. Isolation of Aerobic Bacteria from Wounds of Diabetic Patients Undergoing Hyperbaric Oxygen Therapy in Khartoum State. Global Advanced Research Journal of Microbiology (GARJM) ISSN: 2315-5116 June 2018 Vol. 7(4): pp. 064-072
28. Zubair M,Malik A, Ahmad J. Clinico-microbiological study And antimicrobial drug resistance profile of diabetic foot infections in North India. Foot (Edinb) 2011; 21: 6-14.
29. Benwan K, Al Mulla A, Rotimi VO. A study of the microbiology of diabetic foot infections in a teaching hospital in Kuwait. J Infect Public Health 2012; 5:1-8
30. Role of Peripheral Neuropathy in the Development of Foot Ulceration and Impaired Wound Healing in Diabetes Mellitus. Nutritional and therapeutic intervention for diabetes and metabolic syndrome (second Edition) 2018 pages 95-104,
31. Reiber GE, Vileikyte L, Boyko EJ, del Aguila M, Smith DG, Lavery LA, Boulton AJ. Causal pathways for incident lower-extremity ulcers in patients with diabetes from two settings. Diabetes Care. 1999; 22:157–162. [PubMed] [Google Scholar]
32. Parisi MC, Zantut-Wittmann DE, Pavin EJ, Machado H, NeryM, Jeffcoate WJ. Comparison of three systems of classification in predicting the outcome of diabetic foot ulcers in Brazilian population. Eur J Endocrinol 2008; 159: 41722.
33. Abbas ZG, Lutale JK, Game FL, Jeffcoate WJ. Comparison off our systems of classification of diabetic foot ulcers in Tanzania. Diabet Med 2008; 25: 1347.
34. Citron DM, Goldstein EJC, Merriam VC, Lipsky BA. Bacteriology of moderate to severe diabetic foot infections and invitro activity of antimicrobial agents. J Clin Microbiol 2007; 45:2819-2828.

35. Anandi C, Aaguraja D, Natarajan V, Ramanatham M, Subramaniam CS, Thulasiram M et al. Bacteriology of diabetic foot lesions. Ind J Med Microbiol2004; 22:175-178.

Tables

Table (1) The gender, ulcer grades and sensation loss of the participants

		Count	Column N %
Sex	Female	17	11.2%
	Male	135	88.8%
Ulcer grade	Grade 1	8	5.3%
	Grade 2	19	12.5%
	Grade 3	70	46%
	Grade 4	12	7.9%
	Long standing ulcer	43	28.3%
Sensation Loss	Yes	82	53.9%
	No loss	70	46.1%
Sample	Swab	45	37.16%
	Tissue	103	62.84%

Table (2) Frequency of age groups, diabetes duration, ulcer duration and Sensation loss among participants

		Count	Percentage
Groups of age	15 to 40 years	12	7.9%
	40 to 65	125	82.2%
	65 to 90	15	9.9%
Groups of D. Duration	Less than 12 months	10	6.6%
	1 to 12	59	38.8%
	12 to 24	66	43.4%
	24 to 36	17	11.2%
Groups of U-Duration	Less than 12 months	135	88.8%
	12 to 36	14	9.2%
	More than 36	3	2.0%
Groups of loss of sensation	Less than 12 months	30	36.6%
	12 to 60	29	35.4%
	60 to 120	15	18.3%
	More than 120	8	9.7%

Table (3) Frequency of bacteria isolated from the ulcers of the participants

		Count	N %	P-value
Gram	G(-ve) rods	120	66.3%	.000
	G(+ve) Cocobacilli	6	3.3%	
	G(+ve)Coci	55	30.4%	
Isolates	Acinobacter	6	3.1%	.000
	Coliforms	22	11.3%	
	E.coli	8	4.1%	
	K .oxytoca	3	1.5%	
	K. pneumonia.	5	2.6%	
	P. mirables	48	24.7%	
	P. vulgaris	16	8.2%	
	P.aeruginosa	19	9.8%	
	S.aureas	8	4.1%	
	S.aureas (MRSA)	26	13.4%	
	S.aureas (VRSA)	4	2.1%	
	Saph. Coagulase –ve	16	8.2%	

Table (4) Wagner classification and associated aerobic bacteria and peripheral neuropathy

Wound grade	No of patients	No of patients with Pripheral neuropathy	% of patients with Pripheral neuropathy	Typical pathogenes	The most sensitive antibiotic for each grade
Zero				Normal skin flora	
One	8	4	(50%)	The percent of Gram negative to Gram positive 2:1 the most common speicies are E.coli and staph.coagulase –ve	Meropenem Gentamicin
Two	19	7	(36.8%)	The percent of Gram negative to Gram positive 1:1 the most common speicies are Staphylococcus and different spp. of Gram negative	Vancomycine Gentamicin Oxacillin Amikacin
Three	70	38	(54.3%)	The percent of Gram negative to Gram positive 5:1 the most common speicies are *P. Mirablis, Staphylococcus* and Coliform	Meropenem Amikacin Gentamicin Oxacillin
Four	12	5	(41.6%)	No gram positive bacteria isolated in this grade, the most common Gram negative are *P. aeruginosa* and *P. Mirablis*	Amikacin Meropenum
Long standing ulcers (maturation phase)	43	28	65.1%	The percent of Gram negative to Gram positive 4:3 the most common speicies are *Proteus, s. sureus* MRSA, Co agulase negative Staph. And *P. aeruginosa*	Amikacin Getamicin Vancomycine

Figures

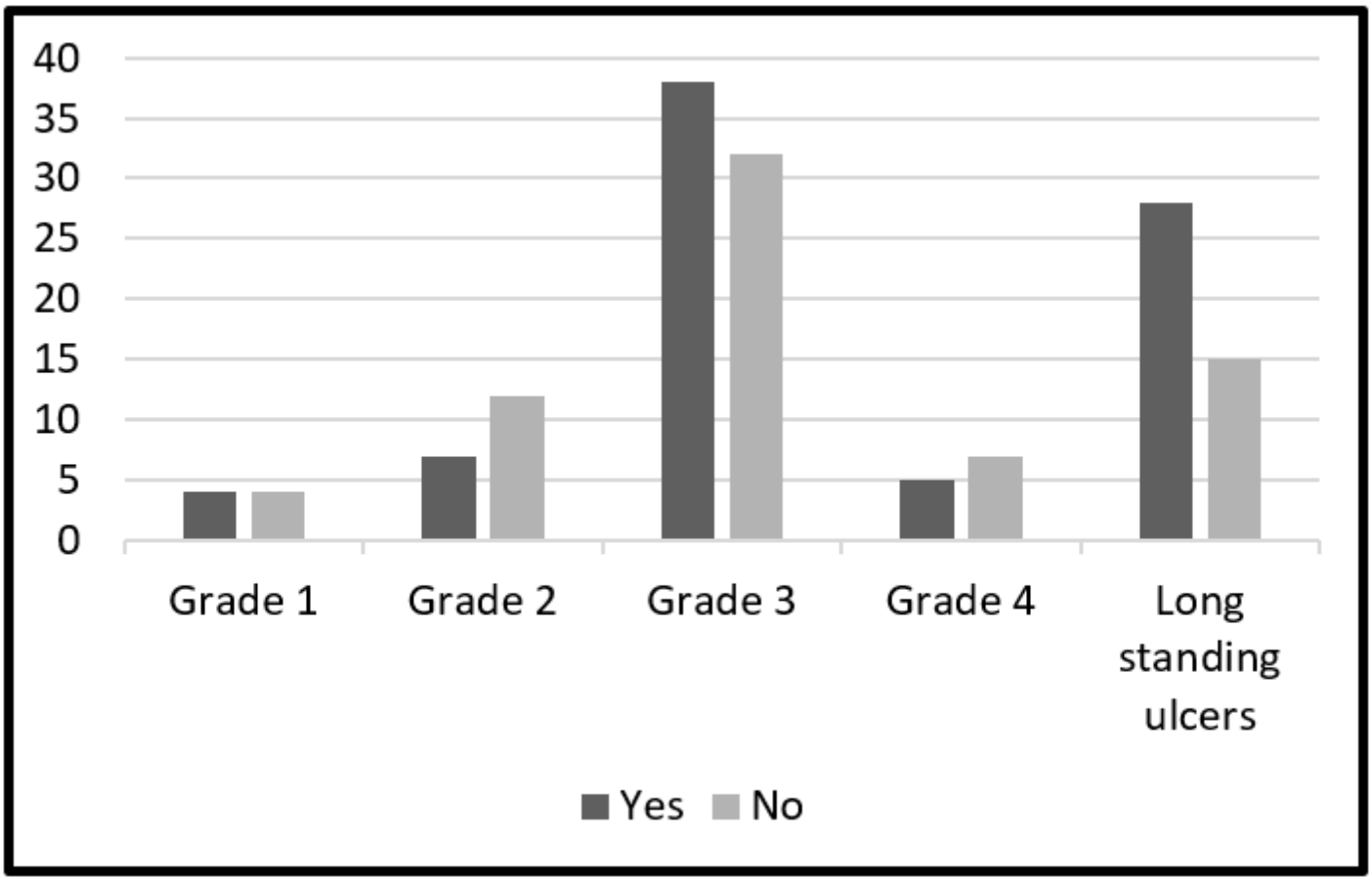

Figure 1

Ulcer grades and associated peripheral neuropathy

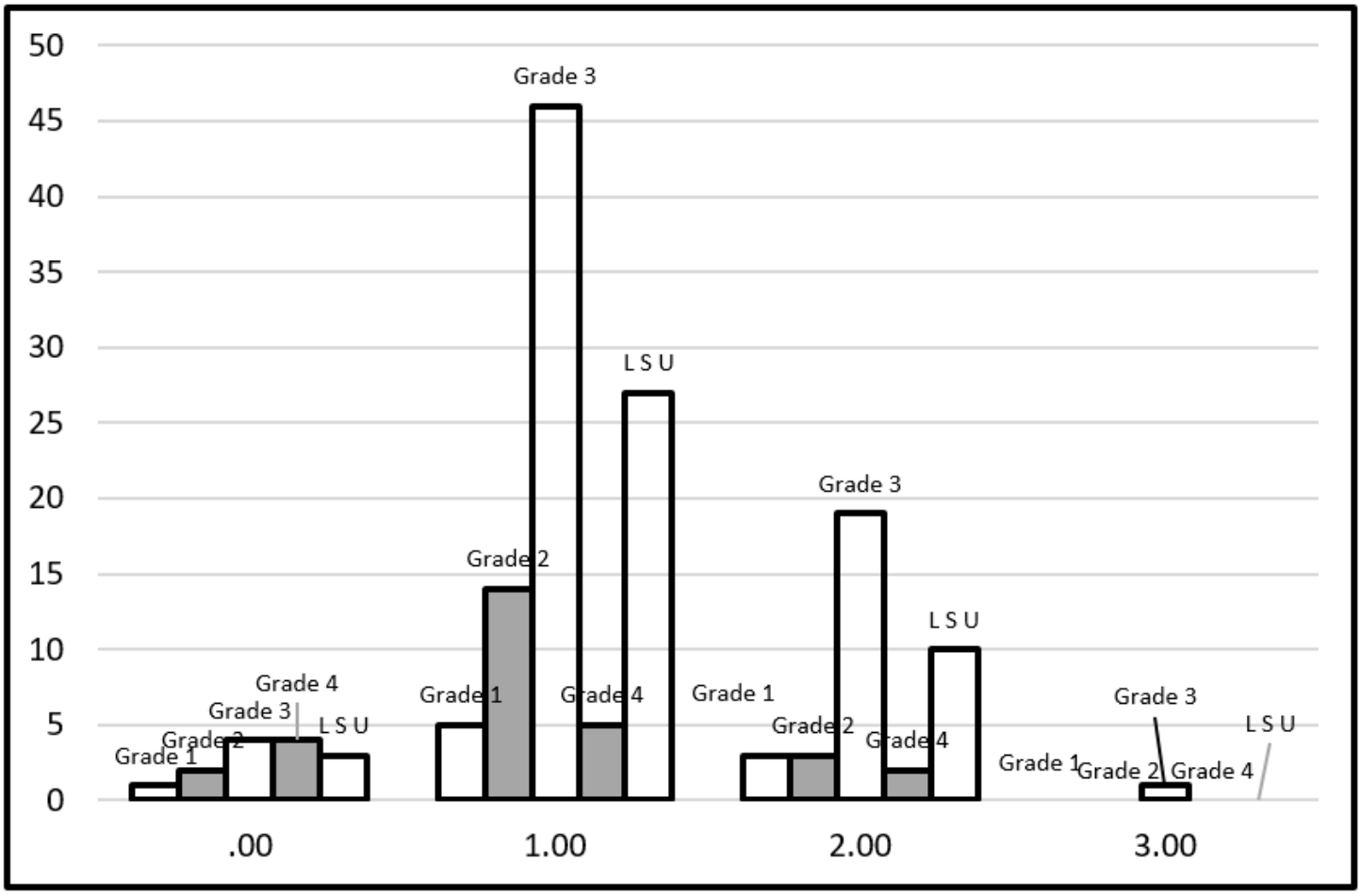

Figure 2

The relation between Wound grade and number of bacterial species isolated

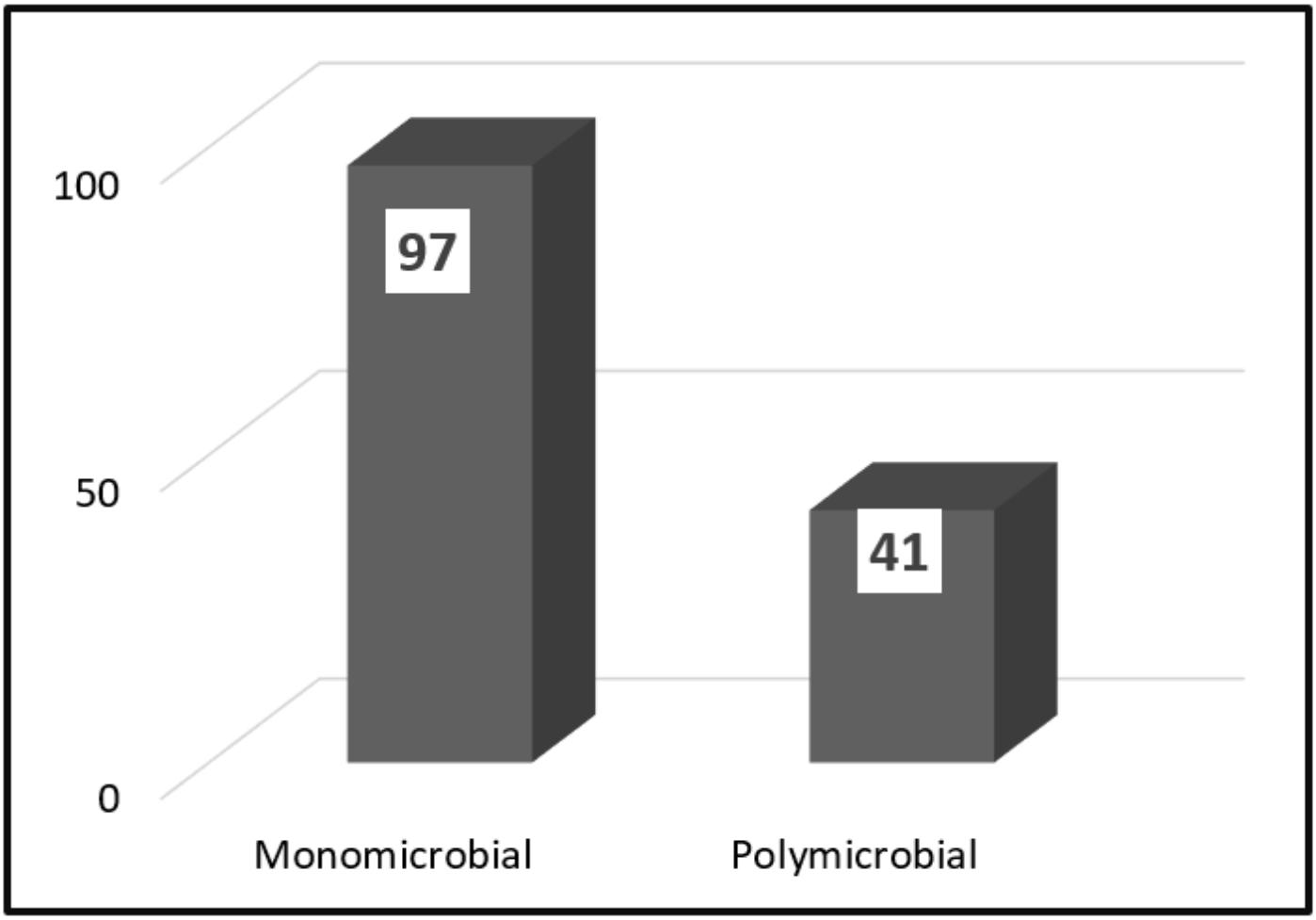

Figure 3

Types of microbial infection of diabetic wounds

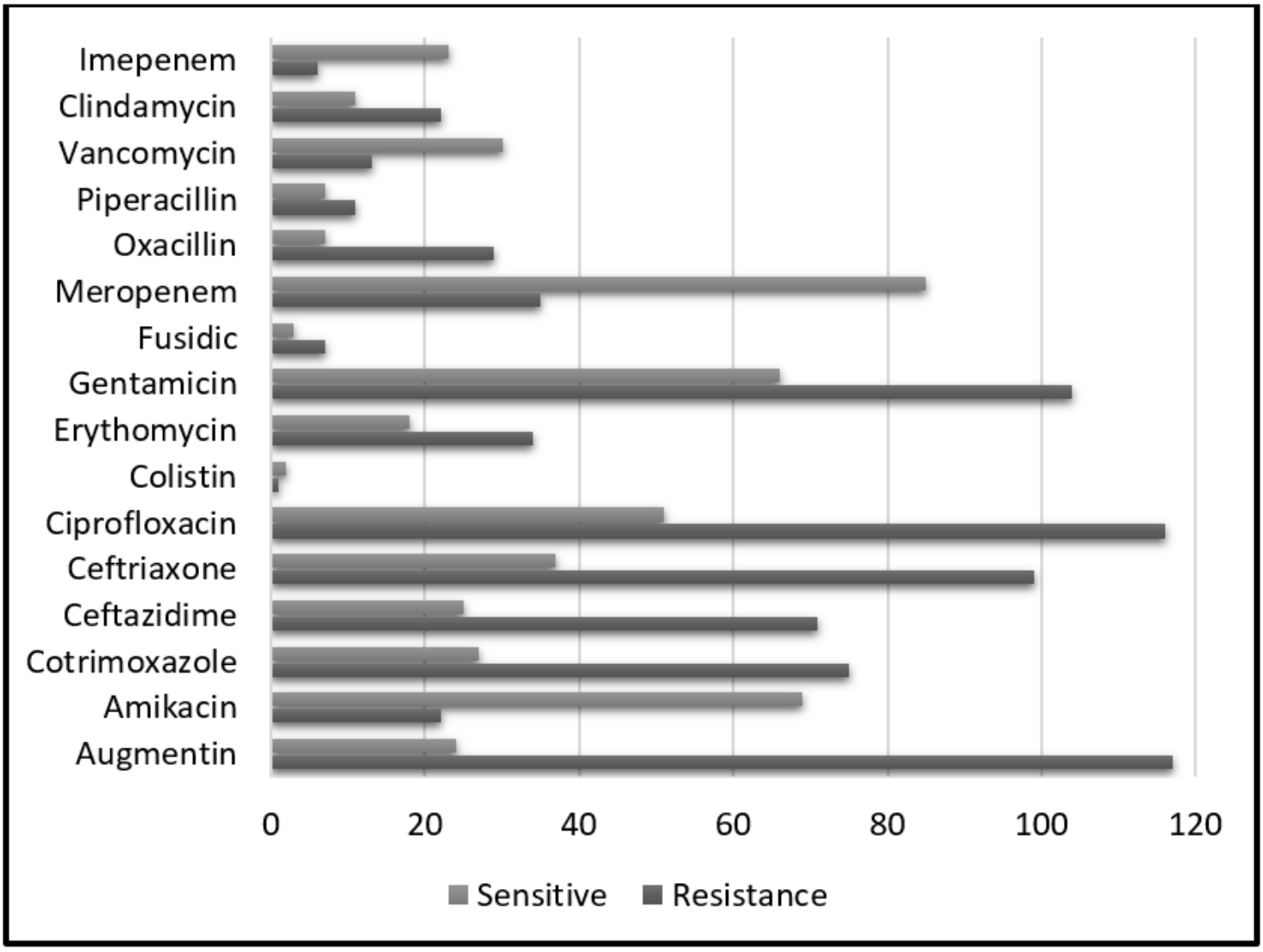

Figure 4

Frequency distribution of sensitivity of antibiotics among all patients