Review of microbiological sampling in diabetic foot disease

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Abstract

Introduction: Diabetes mellitus is a significant cause of morbidity and mortality. Foot-related complications affect 2–2.5% of people with diabetes. There is significant variation in outcomes for patients with diabetic foot disease within the UK. The multidisciplinary approach to diabetic foot disease is well publicised and protocols, guidance and consensus approaches exist for most components of the management of diabetic foot disease. Antimicrobial therapy to treat diabetic foot infections based on microbiological sampling and culture is well documented, but no consensus exists on how these samples should be obtained, processed and reported.

Methods: A literature review was undertaken to establish the reporting of techniques used in obtaining and processing microbiological samples in diabetic foot disease to establish if consensus exists in the methodologies used with a view to develop best practice guidelines.

Results: Six out of 102 papers reported all processes in obtaining and processing microbiological samples.

Conclusion: No gold standard consensus exists for microbiological sampling of diabetic foot infections, preventing optimisation of this aspect of management of diabetic foot disease and ultimately potentially adversely affecting the outcomes of this growing patient cohort.

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Key words: diabetic foot; microbiology sampling; osteomyelitis

Introduction

Diabetes mellitus is a significant cause of morbidity and mortality.[1] Foot-related complications affect 2–2.5% of people with diabetes, equating to a point prevalence of approximately 58,000 people in England alone.[2]

There is significant regional variation in outcomes for patients with diabetic foot disease within the UK.[3] The National Diabetes Foot Care Audit aims to quantify these variations at an organisational level so that markers of an effective service can be identified. However, low levels of participation have so far made it difficult to draw any consensus on this.[4]

The management of diabetic foot disease is complex, involving input from a multidisciplinary team of professionals.[5] The mainstays of treatment in these challenging cases are off-loading of pressure areas and appropriate footwear, surgical debridement of infected and necrotic tissue, revascularisation if required, appropriate wound care and dressings, and antimicrobial therapy. Healthcare institutions managing diabetic foot disease should have clear pathways and guidance for management of these patients with alignment of services and processes to ensure the best patient outcomes and reduce major limb amputation rates and the associated morbidity and mortality.[4]

Each facet of the management of diabetic foot disease has been subject to review in the medical literature with consensus documents produced advising on the best practice for the treatment. The use of antimicrobial therapy and prolonged courses to treat osteomyelitis is well documented[6] and should be based on tissue or bone sampling, culture and appropriate sensitivity testing cultures.[5] However, how these samples should be obtained, processed and reported is poorly documented, making alignment of services difficult. Targeted antimicrobial therapy relies on certain steps to be completed, as demonstrated in Figure 1. Each of the steps shown has the potential to affect the subsequent accuracy of results and must be clearly described so accurate comparison can be made between techniques and results.

A literature review was undertaken to clarify the practice for reporting of tissue sampling techniques in the diabetic foot population and to determine if consensus exists in the literature for sampling techniques and processing, with the aim of developing best practice guidelines particularly in relation to the intraoperative bone sampling techniques used.

Methods

The NICE Healthcare Databases Advanced Search (https://hdas.nice.org.uk) was used to search EMBASE and Medline databases in September 2020. The search strategy is detailed in Appendix 1. Studies were restricted to human subjects, in the English language, published between 2010 and 2020 with an abstract available.

A total of 707 papers were identified. Duplicates, case reports and conference abstracts were removed and abstracts were screened by HT and JD for relevance and any conflicts were resolved by the senior author (MW). One hundred and forty-nine full-text articles were deemed relevant for review and 102 were included in the analysis. Figure 2 shows the PRISMA flow diagram.

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Figure 1. The journey of the bone/deep tissue specimen

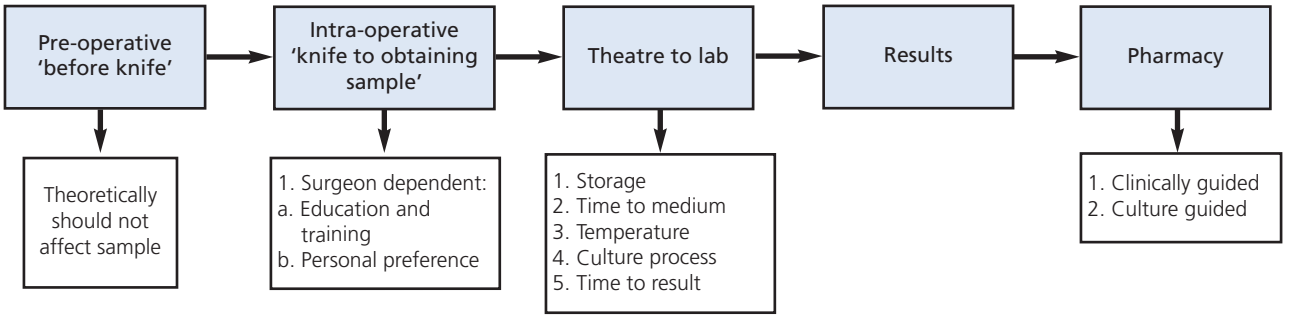
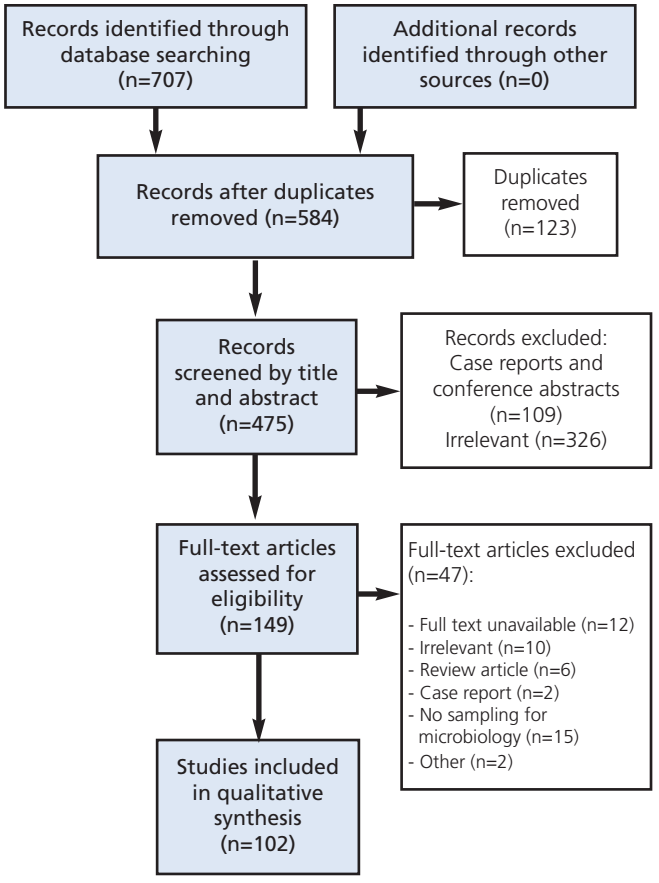


Figure 2. Prisma Flow Diagram



Studies were reviewed by the authors and basic information was collected on the study type and population. The papers were reviewed for the following aspects of their methodology with a view to whether the study would be reproducible: what was sampled, how it was sampled, whether the wound was cleaned prior to sampling and how, how the specimen was transported for processing and what processing occurred. This information was compiled and analysed using Microsoft Excel (Windows 10).

Results

Of the 707 papers identified through database searching, 123 duplicates and 109 case reports and conference abstracts were removed; 475 were screened by title and abstract and 326 were deemed irrelevant and excluded. Of the 149 full-text articles assessed for eligibility, a further 47 were excluded (reasons detailed in Figure 2). One hundred and two papers were included in qualitative analysis (see Appendix 2), of which 45 were prospective studies, 25 were retrospective studies and in 32 the time frame was unclear. There were 16 observational studies, 1 case series, 3 case–control studies, 55 cohort studies, 22 cross-sectional studies, 4 randomised controlled trials and 1 pilot study.

Eighty (78%) studies described the sampling technique used, 58 (57%) described how the wound was cleaned prior to sampling, 50 (49%) described how the specimen was kept prior to processing and 80 (78%) described the processing techniques used.

Samples taken

Wound or ulcer swabs only were performed in 26 of the papers and pus cultures in five. Bone sampling alone was used in 17 papers, tissue including skin in 17 and other samples in one paper. Thirty-one papers described more than one specimen type being taken.

Sampling technique

The percentage of papers reporting the use of different techniques for obtaining samples in the systematic review is shown in Table 1.

Wound cleaning

The percentage of papers reporting how the wound was cleaned prior to microbiology sampling is shown in Table 2.

Specimen transport

All three variables (time, medium, temperature) of transportation of specimens were reported in 6.9% of papers, 50% of papers detailed no information about how the specimen was kept or transported prior to processing, 22.5% of papers reported only one of the three transport variables (medium 15.7%, time frame 4.9%, temperature 2.0%) and 19% reported on two of the

Table 1. Percentage of papers reporting the use of different techniques for obtaining samples in the systematic review

Sample Type	Technique	Percentage of papers reporting
Wound swab	Levine's	8.8%
	Other	5.9%
	Insufficient description/ no comment	22.5%
Tissue	Described	6.9%
	Insufficient description/ no comment	14.7%
Bone	Described	6.9%
	Insufficient description/ no comment	9.8%
Multiple sample types	Technique specified	1.0%
	Samples taken using "established method" referencing another paper	2.9%
	Insufficient description/ no comment	13.7%
Other samples (pus/ fluid/ulcer)	Insufficient description/ no comment	6.9%

three variables. One paper stated that the specimens were transported by "conventional methods".

Specimen processing

Detailed processing methods were described in 23.5% of papers, 30.4% stated "conventional methods" or "culture and sensitivity" were used, 18.6% made no comment about the processing techniques, 11.8% were sent for aerobic and anaerobic culture and 3.9% for aerobic culture only, and 11.8% of papers described molecular microbiological techniques.

Complete sampling protocols

Thirty-five papers (34%) described all four stages of microbiological sampling and processing and six papers (6%) sampled bone and described all four stages. These papers were all studies in patients with diabetic foot disease. The techniques described in these six papers are summarised in Appendix 3.

Discussion

Diabetic foot disease is an international pandemic with a large socioeconomic burden on people and healthcare systems worldwide. Attempts to improve the treatment of diabetic foot disease have been ongoing throughout the medical community with identification of trends in microbiology and the best sampling techniques. Duration of antimicrobial therapy is guided by the culture and sensitivity of samples taken from active diabetic foot infections. Positive bone cultures attract a prolonged (6-week) course of antimicrobial therapy.[7,8] Inappropriate use of antimicrobials is not without its morbidity and therefore accurate culture and sensitivity is imperative to optimise management.

Table 2. Percentage of papers reporting how the wound was cleaned prior to microbiology sampling

Method of cleaning	Percentage of papers reporting
No comment	42.2%
"Asepsis/ Conventional methods"	5.9%
Cleaning/ Irrigation - solution specified	22.5%
Cleaned/ Irrigation - solution not specified	5.9%
"Cleaned (solution specified) and debrided"	7.8%
"Cleaned (solution not specified) and debrided"	3.9%
Debridement	6.9%
Multiple steps, well described	4.9%

The management of diabetic foot infection requires a multidisciplinary approach and it is the links between specialities that improve patient care. The authors, as surgeons, were concerned that the process by which specimens are sampled and transported to the laboratory for microbiological processing may well be impacting upon the reliability of results. Having standard operating procedures and protocols is well documented in healthcare to improve outcomes; however, there is no gold standard for microbiology sampling and processing to guide antimicrobial therapy in the management of diabetic foot disease. A standardised approach to the sampling process will reduce variation in technique and may help avoid inaccurate results, therefore leading to greater reliability and reproducibility.

There are some limitations to this study. It is a qualitative literature review rather than a systematic review due to the fact that the authors are examining methodology and reporting rather than study results. Non-English language studies were excluded and 12 studies were not available as full-text articles. This may have led to exemplary studies being excluded from this literature review but, if they are not readily available to clinicians treating diabetic foot disease internationally, it is difficult for their results to influence practice.

This literature review clearly demonstrates that there is no standardised methodology for reporting of specimen type, sampling method or processing methods for microbiological culture for the diagnosis and treatment of diabetic foot infection in the medical literature. This heterogeneous reporting means that it is difficult for readers and practitioners to draw accurate conclusions from the published literature in order to improve their own practice or to train the future generation of the multidisciplinary team managing this disease. A recent survey conducted by the author showed a lack of consistency in the sampling techniques in the trainee surgical community.[9] It also demonstrated a lack of understanding of the processing techniques, procedural reporting and a lack of ongoing training in the surgical debridement of diabetic foot disease, specifically toe amputations.

The authors feel that a consensus must be sought for the sam-

Key messages

- Gold standard consensus in microbiology sampling techniques and reporting in diabetic foot management is lacking
- Optimal sampling techniques need to be established to increase specimen yield and allow targeted antimicrobial therapy
- Optimisation and standardisation of all aspects of management is key to reduce morbidity and mortality of diabetic foot disease

pling and processing of diabetic foot samples. The publication of papers in relation to microbiology sampling in diabetic foot disease must clearly delineate the steps in sampling, transportation and processing, making the studies transparent and reproducible. This will allow the reader to interpret the results and optimise all aspects of management of diabetic foot disease, allow for further studies into techniques, allow rationalisation of antimicrobial therapy and ultimately reduce the long-term sequelae, morbidity and mortality of diabetic foot disease.

Conflict of interest All authors have non to declare.
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Appendix 1. Search strategy

Search	Search Term
1	exp DIABETES MELLITUS/
2	(Diabet*).ti,ab
3	1 or 2
4	FOOT DISEASES/
5	ULCER/
6	GANGRENE/
7	OSTEOMYELITIS/
9	"SOFT TISSUE INFECTION"/ OR "wound infections/"
10	((foot* OR feet* OR toe* OR tissue* OR wound*) ADJ4 (infect* OR disease*)).ti,ab
11	(4 OR 5 OR 6 OR 7 OR 9 OR 10)
12	(3 AND 11)
13	(diabetic foot).ti,ab
14	(diabet* ADJ4 (foot* OR feet* OR toe* OR ulcer* OR gangrene* OR osteomyelit*)).ti,ab
15	(12 OR 13 OR 14)
16	(micro*).ti,ab
17	(culture).ti,ab
18	(organis*).ti,ab
19	(sampl*).ti,ab
20	(16 OR 17 OR 18)
21	(20 ADJ4 samp*).ti,ab
22	(19 AND 20)
23	(21 OR 22)
24	(15 AND 23)
25	(15 AND 23) [English language] [Humans]

Appendix 2. All papers included in the qualitative review

Year	Authors	Title
2020	Macdonald KE et al	A retrospective analysis of the microbiology of diabetic foot infections at a Scottish tertiary hospital
2010	Nagoba BS et al	A simple and effective approach for the treatment of diabetic foot ulcers with different Wagner grades
2019	Thanganadar AS et al	A Study on isolation, characterization, and exploration of multiantibiotic-resistant bacteria in the wound site of diabetic foot ulcer patients
2019	Niazi NS et al	Adjuvant antibiotic loaded bio composite in the management of diabetic foot osteomyelitis - a multicentre study
2020	Manas AB et al	Admission time deep swab specimens compared with surgical bone sampling in hospitalized individuals with diabetic foot osteomyelitis and soft tissue infection
2011	Landsman A et al	An open-label, three-arm pilot study of the safety and efficacy of topical Microcyn Rx wound care versus oral levofloxacin versus combined therapy for mild diabetic foot infections
2019	Malone M et al	Analysis of proximal bone margins in diabetic foot osteomyelitis by conventional culture, DNA sequencing and microscopy
2016	Wolcott RD et al	Analysis of the chronic wound microbiota of 2,963 patients by 16S rDNA pyrosequencing
2020	Monami M et al	Antimicrobial photodynamic therapy in infected diabetic foot ulcers: a multicenter preliminary experience
2018	Pugazhendhi S and Dorairaj AP	Appraisal of biofilm formation in diabetic foot infections by comparing phenotypic methods with the ultrastructural analysis
2019	Lavery LA et al	Are we misdiagnosing diabetic foot osteomyelitis? Is the gold standard gold?
2020	Min KR et al	Association between baseline abundance of Peptoniphilus, a Gram-positive anaerobic coccus, and wound healing outcomes of DFUs
2018	Vatan A et al	Association between biofilm and multi/extensive drug resistance in diabetic foot infection
2016	Karmaker M et al	Association of bacteria in diabetic and non-diabetic foot infection - an investigation in patients from Bangladesh
2017	Sanchez-Sanchez M et al	Bacterial prevalence and antibiotic resistance in clinical isolates of diabetic foot ulcers in the Northeast of Tamaulipas, Mexico
2020	Ullah I et al	Bacteriological profile and antibiotic susceptibility patterns In diabetic foot infections at Lady Reading Hospital, Peshawar
2017	Amjad SS et al	Bacteriology of diabetic foot in tertiary care hospital; frequency, antibiotic susceptibility and risk factors
2018	Yasin M et al	Baseline characteristics of infected foot ulcers in patients with diabetes at a tertiary care hospital in Pakistan
2010	Sotto A et al	Beneficial effects of implementing guidelines on microbiology and costs of infected diabetic foot ulcers
2015	Lipsky BA et al	Ceftaroline fosamil for treatment of diabetic foot infections: the CAPTURE study experience.
2014	Murali TS et al	Characteristics of microbial drug resistance and its correlates in chronic diabetic foot ulcer infections.
2020	Goh TC et al	Clinical and bacteriological profile of diabetic foot infections in a tertiary care
2012	Mendes JJ et al	Clinical and bacteriological survey of diabetic foot infections in Lisbon
2018	Kim PJ et al	Clinic-based debridement of chronic ulcers has minimal impact on bacteria
2011	Zubair M et al	Clinico-microbiological study and antimicrobial drug resistance profile of diabetic foot infections in North India
2018	Nelson A et al	CODIFI (Concordance in Diabetic Foot Ulcer Infection): a cross-sectional study of wound swab versus tissue sampling in infected diabetic foot ulcers in England
2016	Nelson EA et al	Concordance in diabetic foot ulceration: A cross-sectional study of agreement between wound swabbing and tissue sampling in infected ulcers
2019	Bellazreg F et al	Correlation between superficial and intra-operative specimens in diabetic foot infections: Results of a cross-sectional Tunisian study
2011	Lesens O et al	Culture of per-wound bone specimens: A simplified approach for the medical management of diabetic foot osteomyelitis
2013	Aslangul E et al	Diagnosing diabetic foot osteomyelitis in patients without signs of soft tissue infection by coupling hybrid 67Ga SPECT/CT with bedside percutaneous bone puncture.
2012	Sotto A et al	Distinguishing colonization from infection with Staphylococcus aureus in diabetic foot ulcers with miniaturized oligonucleotide arrays: a French multicenter study
2018	Wu M et al	Distribution of microbes and drug susceptibility in patients with diabetic foot infections in Southwest China
2017	Malone M et al	Effect of Cadexomer iodine on the microbial load and diversity of chronic non-healing diabetic foot ulcers complicated by biofilm in vivo
2019	Malone M et al	Effect on total microbial load and community composition with two vs six-week topical Cadexomer iodine for treating chronic biofilm infections in diabetic foot ulcers
2018	Saseedharan S et al	Epidemiology of diabetic foot infections in a reference tertiary hospital in India
2016	Reveles KR et al	Epidemiology of methicillin-resistant Staphylococcus aureus diabetic foot infections in a large academic hospital: implications for antimicrobial stewardship
2019	MacDonald A et al	Evidence of differential microbiomes in healing versus non-healing diabetic foot ulcers prior to and following foot salvage therapy
2019	Couturier A et al	Comparison of microbiological results obtained from per-wound bone biopsies versus transcutaneous bone biopsies in diabetic foot osteomyelitis: a prospective cohort study

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Year	Authors	Title
2018	Elmarsafi T et al	Concordance between bone pathology and bone culture for the diagnosis of osteomyelitis in the presence of Charcot neuro-osteoarthropathy
2017	Esposito S et al	Deep tissue biopsy vs. superficial swab culture, including microbial loading determination, in the microbiological assessment of skin and soft tissue infections (SSTIs)
2013	Malone M et al	Deep wound cultures correlate well with bone biopsy culture in diabetic foot osteomyelitis
2011	Tascini C et al	Microbiology at first visit of moderate-to-severe diabetic foot infection with antimicrobial activity and a survey of quinolone monotherapy
2018	Noor S et al	Molecular and culture based assessment of bacterial pathogens in subjects with diabetic foot ulcer
2013	Djahmi N et al	Molecular epidemiology of staphylococcus aureus strains isolated from inpatients with infected diabetic foot ulcers in an Algerian University Hospital
2017	Oli AN et al	Multi-antibiotic resistant extended-spectrum beta-lactamase producing bacteria pose a challenge to the effective treatment of wound and skin infections
2016	Smith K et al	One step closer to understanding the role of bacteria in diabetic foot ulcers: Characterising the microbiome of ulcers
2014	Mannucci E et al	Photodynamic topical antimicrobial therapy for infected foot ulcers in patients with diabetes: A randomized, double-blind, placebo-controlled study - The D.A.N.T.E (Diabetic ulcer Antimicrobial New Topical treatment Evaluation) study
2010	Saltoglu N et al	Piperacillin/tazobactam versus imipenem/cilastatin for severe diabetic foot infections: A prospective, randomized clinical trial in a university hospital
2015	DaCosta RS et al	Point-of-care autofluorescence imaging for real-time sampling and treatment guidance of bioburden in chronic wounds: first-in-human results
2014	Dunyach-Remy C et al	Polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE): A promising tool to diagnose bacterial infections in diabetic foot ulcers
2011	Bernard L et al	Predicting the pathogen of diabetic toe osteomyelitis by two consecutive ulcer cultures with bone contact
2017	Chisman R et al	Prescribing antibiotics in diabetic foot infection: what is the role of initial microscopy and culture of tissue samples?
2019	Jaju K et al	Profile and antibiotic susceptibility of bacterial pathogens associated with diabetic foot ulcers from a rural area
2014	Merlet A et al	Prognostic factors of calcaneal osteomyelitis
2013	Redel H et al	Quantitation and composition of cutaneous microbiota in diabetic and nondiabetic men
2012	Atway S et al	Rate of residual osteomyelitis after partial foot amputation in diabetic patients: a standardized method for evaluating bone margins with intraoperative culture.
2011	Elamurugan TP et al	Role of bone biopsy specimen culture in the management of diabetic foot osteomyelitis
2019	Sloan TJ et al	Examining diabetic heel ulcers through an ecological lens: Microbial community dynamics associated with healing and infection
2018	Jneid J et al	Exploring the microbiota of diabetic foot infections with culturomics
2019	Beroukhim G et al	Factors predicting positive culture in CT-guided bone biopsy performed for suspected osteomyelitis
2020	Kosmopoulou OA et al	Feasibility of percutaneous bone biopsy as part of the management of diabetic foot osteomyelitis in a 100% neuropathic, grade 3 IDSA/IWGDF population on an outpatient basis
2013	Aragon-Sanchez J et al	Gram-negative diabetic foot osteomyelitis: Risk factors and clinical presentation
2011	Weiner RD et al	Histology versus microbiology for accuracy in identification of osteomyelitis in the diabetic foot
2016	Kumar D et al	Identification, antifungal resistance profile, in vitro biofilm formation and ultrastructural characteristics of Candida species isolated from diabetic foot patients in Northern India
2017	Ottolino-Perry K et al	Improved detection of clinically relevant wound bacteria using autofluorescence image-guided sampling in diabetic foot ulcers
2013	Ray GT et al	Incidence, microbiology, and patient characteristics of skin and soft-tissue infections in a U.S. population: a retrospective population-based study.
2013	Turhan V et al	Increasing incidence of Gram-negative organisms in bacterial agents isolated from diabetic foot ulcers
2015	Cervantes-García E et al	Infections of diabetic foot ulcers with methicillin-resistant Staphylococcus aureus
2017	Noor S et al	Inflammatory markers as risk factors for infection with multidrug-resistant microbes in diabetic foot subjects
2019	Park J et al	Influence of microbiota on diabetic foot wound in comparison with adjacent normal skin based on the clinical features
2018	Saltoglu N et al	Influence of multidrug resistant organisms on the outcome of diabetic foot infection
2014	Boffeli TJ et al	In-office distal Symes lesser toe amputation: a safe, reliable, and cost-effective treatment of diabetes-related tip of toe ulcers complicated by osteomyelitis
2018	Makki D et al	Is it necessary to change instruments between sampling sites when taking multiple tissue specimens in musculoskeletal infections?
2011	Vinodkumar CS et al	Isolation of bacteriophages to multi-drug resistant Enterococci obtained from diabetic foot: a novel antimicrobial agent waiting in the shelf?
2018	Meyr AJ et al	Level of agreement with a multi-test approach to the diagnosis of diabetic foot osteomyelitis
2017	Dunyach-Remy C et al	Link between nasal carriage of Staphylococcus aureus and infected diabetic foot ulcers

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Year	Authors	Title
2018	Ramanujam CL et al	Medical imaging and laboratory analysis of diagnostic accuracy in 107 consecutive hospitalized patients with diabetic foot osteomyelitis and partial foot amputations
2018	Suryaletha K et al	Metataxonomic approach to decipher the polymicrobial burden in diabetic foot ulcer and its biofilm mode of infection
2012	Parvez N et al	Microbial profile and utility of soft tissue, pus, and bone cultures in diagnosing diabetic foot infections
2013	Islam S et al	Microbial profile of diabetic foot infections in Trinidad and Tobago
2020	Pontes DG et al	Microbiologic characteristics and antibiotic resistance rates of diabetic foot infections
2012	Tiwari S et al	Microbiological and clinical characteristics of diabetic foot infections in northern India.
2015	Parsa H et al	Microbiological features and risk factors in patients with diabetic foot ulcers
2017	Miyan Z et al	Microbiological pattern of diabetic foot infections at a tertiary care center in a developing country
2014	Sugandhi P et al	Microbiological profile of bacterial pathogens from diabetic foot infections in tertiary care hospitals, Salem
2018	Shettigar K et al	Severity of drug resistance and co-existence of Enterococcus faecalis in diabetic foot ulcer infections
2018	Drampalos E et al	Single stage treatment of diabetic calcaneal osteomyelitis with an absorbable gentamicin-loaded calcium sulphate/hydroxyapatite biocomposite: The Silo technique
2017	Kassam NA et al	Spectrum and antibiogram of bacteria isolated from patients presenting with infected wounds in a tertiary hospital, northern Tanzania.
2016	Fujii M et al	Surgical treatment strategy for diabetic forefoot osteomyelitis
2018	Chang JW et al	The appropriate management algorithm for diabetic foot: A single-center retrospective study over 12 years
2013	Malik A et al	The diabetic foot infections: Biofilms and antimicrobial resistance
2020	Crisologo PA et al	The infected diabetic foot: Can serum biomarkers predict osteomyelitis after hospital discharge for diabetic foot infections?
2017	Rastogi A et al	The microbiology of diabetic foot infections in patients recently treated with antibiotic therapy: A prospective study from India
2019	Banerjee T et al	The microflora of chronic diabetic foot ulcers based on culture and molecular examination: a descriptive study
2016	Nageen A	The most prevalent organism in diabetic foot ulcers and its drug sensitivity and resistance to different standard antibiotics
2013	Gardner SE et al	The neuropathic diabetic foot ulcer microbiome is associated with clinical factors
2012	Abbas Z et al	The utility of Gram stains and culture in the management of limb ulcers in persons with diabetes
2020	Hunter P et al	Topical oxygen therapy shifts microbiome dynamics in chronic diabetic foot ulcers
2012	Pinzur MS et al	Treatment of osteomyelitis in charcot foot with single-stage resection of infection, correction of deformity, and maintenance with ring fixation
2019	Johani K et al	Understanding the microbiome of diabetic foot osteomyelitis: insights from molecular and microscopic approaches
2016	Shettigar K et al	Virulence determinants in clinical Staphylococcus aureus from monomicrobial and polymicrobial infections of diabetic foot ulcers
2018	Haalboom M et al	Wound swab and wound biopsy yield similar culture results

Appendix 3. Summary of papers reporting all aspects of bone sampling techniques and processing in diabetic foot patients

Year	Authors	Title	Study type	What was sampled	How they sampled it	Was the wound cleaned prior to sampling and how	How was the specimen kept prior to processing	What processing occurred
2020	Macdonald K.E. et al	A retrospective analysis of the microbiology of diabetic foot infections at a Scottish tertiary hospital	Retrospective cohort study	Diabetic foot infections, ulcers and suspected osteomyelitis	Deep wound swab from ulcer base. If suspected osteomyelitis, bone biopsy	Yes, cleaned and debrided, no more detail given	Deep tissue swab: Amies transport medium with charcoal. Bone biopsies: sterile universal container	Cultured for aerobic and anaerobic organisms, pure cultures obtained and subjected to antibiotic sensitivity testing.
2011	Lesens O et al	Culture of per-wound bone specimens: A simplified approach for the medical management of diabetic foot osteomyelitis	retrospective cohort review	Bone	All samples taken by the same operator sterile gloves and a gown worn disposable needle holder used to harvest the fragment of infected bone.	careful debridement, wound cleaned with polyvidone iodine, then washed with sterile saline solution	Bone samples were sent to the microbiology laboratory within 2 h in a sterile tube with a few drops of sterile saline solution	Aerobic and anaerobic cultures were performed for each sample for 6 days
2019	Couturier A. et al	Comparison of microbiological results obtained from per-wound bone biopsies versus transcutaneous bone biopsies in diabetic foot osteomyelitis: a prospective cohort study	prospective cohort	Per bone biopsy and Transcutaneous bone biopsy	All samples taken at the bedside by the same operator Sterile gloves, gown, mask worn Bone biopsies: through healthy skin, performed by introducing a 13-gauge pediatric osteo-medullary biopsy trocar through a 3-mm incision made approximately 10 mm from the margins of the wound. For per-wound biopsies: bone sample was taken using metal forceps.	Debridement of the necrotic and fibrinous tissues was performed using a scalpel or curette before a bone sample was taken	Bone samples were sent to the microbiology laboratory in a sterile tube with a few drops of sterile saline solution within 2 h of sampling. All samples were transferred to, and processed by, the center's local clinical microbiology laboratory.	The laboratory identified bacteria by MALDI MS technology using a VITEK MS system (Biomérieux, La Balme, France) and determined antibiotic susceptibility using the VITEK2 system or the disk diffusion method. Susceptibility results were interpreted according to the recommendations of the Antibiogram Committee of the French Microbiology Society
2020	Kosmopoulou O.A.; Dumont I.J.	Feasibility of Percutaneous Bone Biopsy as Part of the Management of Diabetic Foot Osteomyelitis in a 100% Neuropathic, Grade 3 IDSA/IWGDF Population on an Outpatient Basis	Retrospective, Observational	Bone from foot with osteomyelitis (metatarsals or toes), away from open wound or through dorsum	Biopsy using a bone biospy needle - T shaped Jamshedi needle. No anaesthesia due to neuropathy	Cleaned with povidone iodine, sterile drape.	Sent directly to lab in 0.9% saline. If possible specimen divided into and second sample sent for histology(only 7 samples of 23)	Culture and sensitivity and histology in 7 samples.
2011	Weiner R.D. et al	Histology versus Microbiology for Accuracy in Identification of Osteomyelitis in the Diabetic Foot	Prospective	Bone from surgical field taken during other surgery	During surgical procedure, piece of suspected osteomyelitis bone was sampled and split into two	Sterile technique, no further details mentioned	Microbiology specimen transported in dry sterile container. Histology specimen transported in 10% buffered formalin	Aerobic, anaerobic and fungal cultures + histology
2012	Parvez N. et al	Microbial profile and utility of soft tissue, pus, and bone cultures in diagnosing diabetic foot infections	Prospective	Soft tissue specimens - scraping and bone specimens	During surgical debridement- with scalpel for soft tissue and bone nibbler or bone curette or ultrasound guided transcutaneous biopsies with 18 G needle	Soft tissue specimens: after washing with saline and surface debridement Bone specimen: - aseptic precautions and sterile nibbler used. Transcutaneous biopsy - detergent and antiseptic on normal skin .	Sent directly to lab in sterile saline	Aerobic and anaerobic cultures and sensitivity.