Intra-operative tissue sampling and microbiological analyses during minor lower limb amputations in patients with diabetes are poorly reported and difficult to interpret

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Abstract

Diabetic foot disease (DFD) is a leading cause of acute sepsis and has long-term consequences for patients. It poses a strain on health resources in both the developed and developing world, with a significant impact on patient quality of life due to the associated complications of DFD and the often multiple interventions required to control infection and preserve limb tissue. Although there is evidence in the literature regarding early detection and prompt management of this debilitating condition, there is little structured evidence on how to gain accurate tissue sampling with processing to allow targeted antimicrobial therapy from minor amputations where bone cultures have been sent.

Methods: A literature review was conducted to establish the publications on intra-operative bone sampling and processing taken during diabetic foot minor amputations and the pathways described for processing sample acquisition.

Findings: Thirty papers were identified which highlighted some of the processes involved in the procurement of intra-operative tissue samples. No published paper reported a complete pathway for the ascertainment of samples, transfer and processing of these specimens.

Conclusion: There is no published consistent pathway published for procurement of intra-operative diabetic foot specimens, for their storage, transportation and processing. Without documented, reproducible processes, it is difficult to interpret published results. This makes planning for targeted antibiotic therapy more difficult.

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Key words: processing, intra-operative, diabetic foot, tissue

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Introduction

It is estimated that 10% of patients with diabetes will have a foot ulcer during their lifetime.[1] This can lead to serious consequences such as major lower limb amputation. People with diabetes, when compared to the general population, are twice as likely to have amputations later in their lives.[1] Mortality following diabetic foot amputation is 70% within five years and 50% following diabetic foot ulceration.[1] In the UK in 2015, it was estimated that more than 135 minor and major diabetic foot amputations were carried out per week, more than 6,677 per annum.[2] DFD is associated with substantial cost implications. Annual spending on diabetic foot care and amputations is estimated at between £837 million and £962 million.[3]

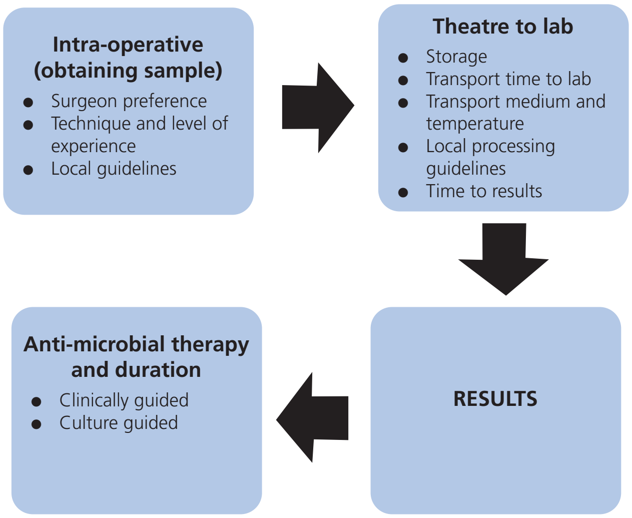
Digital amputation is a commonly performed procedure for foot salvage in the case of diabetic forefoot sepsis to drain infection. Recommendations exist on the type of specimen (bone or tissue) to take but guidance on the procurement of those specimens, transportation and processing is less well documented.[1,4] Difficulties in understanding the sampling and processing pathway could have a negative impact on treatment optimisation in the pre-, peri- and post-operative period. This may result in delayed wound healing, poor antibiotic stewardship, increased rates of re-admissions or further surgery, leading to possible sub-optimal care to this cohort of patients.[5]

The aim of this literature review is to document the reporting of the techniques deployed in bone sampling of patients who require diabetic foot minor amputations. The review looks at the process of sample acquisition, sample storage, transportation and processing. This not only highlights the literature that has been published but also portrays the ease of data interpretation to optimise patient care. Fundamental steps in sample attainment and processing, along with confounding factors, are highlighted in Figure 1.

Methods

A review of electronic databases (PubMed, MEDLINE and EMBASE)

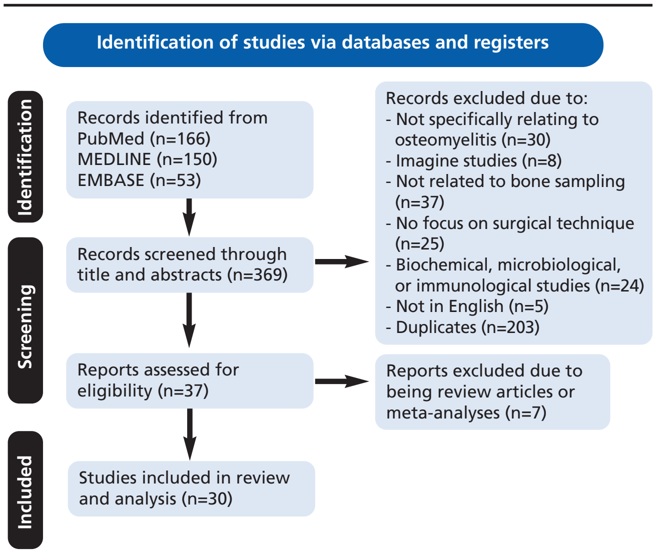
Figure 1. Steps in obtaining samples intra-operatively following minor amputations and processing pathway



was conducted with the aim of identifying papers which included bone sampling in patients who had minor/digital or forefoot amputations due to complications of diabetes. The search included papers up until 20th February 2021. Relevant search terms were used to identify associated papers. The search criteria used were: "(bone biopsy OR bone sample OR bone culture) AND diabetic AND amputation AND technique NOT percutaneous "

The results of these searches are summarised in Figure 2. The titles and abstracts were screened for relevance to our purposes and two of the authors (RS and HT) further examined the publications and assessed the relevant articles based on the inclusion and exclusion criteria.

Figure 2. Flow diagram showing selection of included studies



All studies published which signposted how bone sampling was performed in patients who underwent minor amputations (forefoot, hallux, single/multiple digits) were included. Publications were excluded if sampling was performed percutaneously, if there was no mention of surgical intervention or documented surgical technique and if abstracts were not available in English. All papers were then reviewed by two of the remaining authors (MG and MW) independently to ensure adequacy of the included studies.

Results

Results from our search criteria yielded a total of 37 papers (Appendix 1 online www.bjd-abcd.com). However, 30 studies were included in our analysis as there were six review articles and one systematic review requiring extraction. These are highlighted accordingly. The majority of these papers (21, 70%) were cohort series. All studies emphasised the importance of accurate bone sampling techniques for robust antimicrobial treatment in diabetic foot sepsis.

Three (10%) studies describe preparation techniques prior to sampling taking place, whilst seven (23.3%) papers described their intra-procedural sterility techniques (e.g. change of gloves, change of instruments). Twenty-five (83.3%) studies declared that a form of tissue specimen (bone, soft tissue or both) was taken intra-operatively but only 27 (90%) of the studies reported the type of analysis performed to identify the causative organism and rate of residual disease. Six (20%) studies did not specify whether any tissue sample was taken intra-operatively. These studies did not clarify whether treatment was therefore based on standard antimicrobial guidelines for diabetic foot disease. Twelve (40%) studies mentioned that a form of bone was sampled but did not specify the type or whether the site was infected or clean. All results are shown in Tables 1 and 2.

Eight (26.6%) of studies included type of incubation media use and six (20%) studies identified the control temperature and stor-

Table 1 Number of studies detailing each step of specimen processing and analysis

	Type of specimen analysis			Type of culture medium	Process time and temperature
	Microbiology and histology	Microbiology alone	Histology alone		
No. of Studies (N=30)	14 (46.6%)	9 (30%)	4 (13.3%)	8 (26.6%)	6 (20%)

Table 2 Number of studies detailing the site of origin of specimen

Type of specimen taken	Bone				Deep tissue or swab	Not specified
	Clean Only	Infected Only	Clean and infected	Not specified		
No. of Studies (N=30)	5 (16.6%)	4 (13.3%)	1 (3.3%)	12 (40%)	3 (10%)	6 (20%)

age of samples prior to processing; however, none of the studies specify the exact transportation methods, timing or medium used once the specimens had been retrieved intra-operatively.

Discussion

Diabetic foot disease is a growing pandemic which requires good data to treat effectively. Vascular surgeons, diabetologists, diabetic podiatrists, microbiologists, radiologists and other members of the MDT all have a crucial role to play in the management of this complex issue.[1] Minor amputation techniques have been poorly taught and poorly understood for some time.[6] The authors have concerns that the process of sampling is being overlooked when it comes to gaining accurate and useful information. No core outcome set could be identified for diabetic foot disease. Recently published work by the authors would suggest that DFD sampling reporting in the medical literature is heterogeneous and leads to findings that cannot be interpreted or reproduced with ease.[7] The fact that minor amputation for foot sepsis in the presence of diabetes with or without the presence of osteomyelitis is performed by junior surgeons, often out of hours, in the UK remains of concern. This stimulated the authors to look at the published literature on the techniques reported for bone sampling as recommended by NICE (NG19) in the population undergoing minor amputations.[1,6]

The published literature on minor amputation with sampling for diabetic foot disease is based around small series of patients in single centres.[4,5] These studies fail to report the process of sampling adequately and make interpretation of results difficult, as described previously. Authors who have undertaken systematic reviews on similar subjects report the concerns with sampling techniques and therefore accuracy of results that we have raised through this publication.[5,8] Diabetic foot amputation is often performed in septic patients bearing tissues with gross tissue destruction whereas most published studies are based around chronic diabetic foot ulceration, which is clearly a different situation.[5,9,13] Understanding the differences between the success and process of sampling between the two situations will help benefit planning and delivery of services in the future.

Atway *et al* and colleagues in 2012 and Schmidt *et al* in 2020 emphasized that the presence of residual osteomyelitis or positive bone margins is associated with poor outcomes, with higher rates of residual infection leading to morbidity.[4,9] Their studies emphasised the importance of debridement technique and robust proximal bone sampling to guide optimal antimicrobial therapy and achieve better outcomes. This sampling can often be supported by effective imaging, such as recommended by Cohen *et al*, and by the use of MRI.[10] Accurate sampling is vital for targeted antibiotic therapy. Authors sporadically report the use of culture mediums to support sample transport, suggesting more accurate yields from their specimens to improve the accuracy for targeted antibiotic therapy, but there are only a few small-scale studies in the literature.[11] This concerns the authors, as the growing pandemic of diabetic foot disease will require a concerted effort backed by reproducible data and robust guidelines to maximise good outcomes for patients.

Key messages

- Accurate tissue sampling techniques from minor lower limb amputations intra-operatively provides an optimal medium for robust microbiological analyses.
- Tissue sampling techniques are non-standardised and unstructured within the published literature.
- Clinicians should aim and work towards publishing standardised pathways for sampling to ensure gold standard treatment strategies are employed within daily medical practice.

It is clear that the literature supports the use of intra-operative bone specimens in diabetic foot treatments to guide accurate diagnosis,[5,7] but as can be seen from this study the techniques deployed to gain these samples are poorly reported, with no standard of care present in the literature or in the NICE NG19 guidelines. Moreover, standardised methods of immediate intra-operative culture storage and transportation are not specified at all, which leaves uncertainty in a crucial part of treating this complex disease. Public Health England in 2015 published a standard operating procedure to guide specimen storage and processing time. The recommendation was for transfer of specimen collection to the laboratory within two hours. We have been unable to validate from the published literature whether this guidance is being followed. In practical terms, this can be difficult to deliver in the theatre or clinic environment due to logistical difficulties such as out-of-hours working.[6,12]

At present, there is a lack of evidence to show that rapid transfer times to the laboratory or short turnaround times have any long-term benefits on the outcomes after minor amputation where bone sampling has been performed. Further investigation into the process of sampling technique and specimen handling should be considered to gain accurate yields from bone sampling in minor amputation. Future cohort studies are required to see if this leads to beneficial outcomes to patients. Appropriate sampling guidelines backed by appropriate education for clinicians should be developed to guide future sampling, transfer and processing techniques to maximise benefit from therapies.[6,12,13]

Conclusion

This literature review concludes that there is no standardised method for sampling, transportation or processing of bone biopsy specimens taken for those DFD patients who require minor amputation. Considering the high prevalence of this condition and requirements for surgical interventions, a robust pathway and standardised technique must be ascertained and described routinely in the medical literature to allow for the optimal utilisation of antibiotic therapy in this disease.

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Appendix 1. Intra-operative tissue sampling in minor lower limb amputations is poorly reported.
(All papers yielded in our qualitative review)

Year	Author	Title	Study Type	Study Size	Preparation	Technique (Margins/ Debridement)	Technique for sampling	Specimen analysis	Collection container, medium	Process Technique
2010	Aragon-Sanchez [1]	Treatment of Diabetic Foot Osteomyelitis: A Surgical Critique	Review Article	NA	Not Mentioned	Various techniques	Not Mentioned	Micro & Histology	Not Mentioned	Not Mentioned
2012	Aragon-Sanchez et al[2]	Limb salvage for spreading midfoot osteomyelitis following diabetic foot surgery	Case report	2	Not Mentioned	MT head resection to affected OM bones	Not Mentioned	Micro & Histology	Not Mentioned	Not Mentioned
2012	Atway et al[3]	Rate of Residual Osteomyelitis after Partial Foot Amputation in Diabetic Patients: A Standardized Method for Evaluating Bone Margins with Intraoperative Culture	Retrospective observational study	27	Not Mentioned	Healthy proximal bone margins	3 litres of saline, gloves changed	Micro only	Not Mentioned	Not Mentioned
2014	Boffeli et al[4]	In-office distal Symes lesser toe amputation: a safe, reliable, and cost-effective treatment of diabetes-related tip of toe ulcers complicated by osteomyelitis	Case series	48	Standard prep and drape till mid- calf	Plantar flap - distal phalanx and distal third to half of middle phalanx	Irrigated, new instrument	Micro (distal phalanx) and histology of healthy proximal bone	Not Mentioned	Not Mentioned
2007	Chantelau et al[5]	Bone Histomorphology May Be Unremarkable in Diabetes Mellitus	Case series	45	Not Mentioned	Bone from infected site and clean bone from distant site (same foot)	Not Mentioned	Histology	Not Mentioned	Not Mentioned
2018	Cohen et al[6]	Added value of MRI to X-ray in guiding the extent of surgical resection in diabetic forefoot osteomyelitis: a review of pathologically proven, surgically treated cases	Case series	32	NA	Removal of bone at level of disease based on MRI	Not Mentioned	Micro only	Not mentioned	Not mentioned
2019	Couturier et al[7]	Comparison of microbiological results obtained from per-wound bone biopsies versus transcutaneous bone biopsies in diabetic foot osteomyelitis: a prospective cohort study	Cohort study	43	Taken at bedside, sterile gloves, gown.	Healthy skin was sterilised with iodine and washed with sterile saline. Per wound biopsies taken using scalpel/curette before the bone sample was taken using metal forceps.	Not Mentioned	Micro only	Sterile tube with a few drops within 2 hours of sampling.	Bacteria identified by using VITEK MS system

Appendix 1.

1997	Craig et al[8]	Osteomyelitis of the Diabetic Foot: MR Imaging-Pathologic Correlation' Index	Case series	13	Not Mentioned	Bone from affected areas based on MR findings	Not Mentioned	Histology	Not Mentioned	Not Mentioned
2016	Dalla Paola et al[9]	Extension and grading of osteomyelitis are not related to limb salvage in Charcot neuropathic osteoarthropathy: A cohort prospective study	Cohort study	33	Not Mentioned	Multiple infected bone taken	Not Mentioned	Micro & Histology	Not Mentioned	Not Mentioned
2006	Ertugrul et al[10]	The diagnosis of osteomyelitis of the foot in diabetes microbiological examination vs. magnetic resonance imaging and labelled leucocyte scanning	Case series	31	Not Mentioned	Bone samples	Aseptic technique using debridemen t and bone sampling	Micro & Histology	Anerobic-Schaedler agar, aerobic 5% sheep blood agar, MacConkey and Sabourd agar	24-48 hour at 35 degrees
2008	Ertugrul et al[11]	Pathogens Isolated From Deep Soft Tissue and Bone in Patients With Diabetic Foot Infections	Case series	45	Not Mentioned	Deep soft tissue and bone sample	Aseptic technique for sampling	Micro & Histology	Anerobic-Schaedler agar, aerobic 5% sheep blood agar	24-48 hour at 35 degrees
2016	Fujii[12]	Surgical treatment strategy for diabetic forefoot osteomyelitis	Cohort study	28	Not Mentioned	5micrometer sections of formalin fixed and paraffin embedded bones stained with H+E. Sent for histopathology	Not Mentioned	Histology	Not Mentioned	Not Mentioned
2013	Game [13]	Osteomyelitis in the Diabetic Foot	Review Article	NA	Not Mentioned	Bone sample	Not Mentioned	Not Mentioned	Not Mentioned	Not Mentioned
2012	Garcia-Morales et al[14]	Surgical complications associated with primary closure in patients with diabetic foot osteomyelitis	Cohort study	46	Not Mentioned	Bone sample-debridement/ osteotomy/ arthrodesis of IP joint	Not Mentioned	Micro & Histology	Not Mentioned	Not Mentioned
1994	Grayson et al[15]	Use of Ampicillin/Sulbactam versus Imipenem/Cilastat in in the Treatment of Limb-Threatening Foot Infections in Diabetic Patients	Double-blinded RCT	96	Not Mentioned	Deep wound swab and tissue debrided	Not Mentioned	Micro & Histology	anaerobic-yeast, 5% sheep blood agar	Standard
2019	Johnson et al[16]	Outcomes of Limb-Sparing Surgery for Osteomyelitis in the Diabetic Foot: Importance of the	Cohort study	66	Not Mentioned	Bone tissue	Not Mentioned	Histology of margins	Not Mentioned	Not Mentioned

Appendix 1.

		Histopathologic Margin								
1995	Lavery et al[17]	Microbiology of Osteomyelitis in Diabetic Foot Infections	Case series	36	Not Mentioned	Deep tissue and bone specimen	Not Mentioned	Micro only	Not Mentioned	Not Mentioned
2009	Lavery et al[18]	Risk factors for developing osteomyelitis in patients with diabetic foot wounds	Cohort study	1666	Not Mentioned	Percutaenous bone biopsy or open surgical bone cultures	Not Mentioned	Micro only	Not Mentioned	Not Mentioned
2019	Lavery et al[19]	The Infected Diabetic Foot: Re-Evaluating the IDSA Diabetic Foot Infection Classification	Cohort study	294	Not Mentioned	Bone sample	Not Mentioned	Micro & Histology	Not Mentioned	Not Mentioned
2019	Lederma nn et al[20]	A novel intraoperative technique seeding morselized bone tissue into paediatric blood culture bottles improves microbiological diagnosis in patients with foot and ankle osteomyelitis	Cohort study	107	Not Mentioned	Paired bone samples from affected region	Not Mentioned	Paired samples- UCM and PCB	UCM- plastic contained at room temp, PCB- morselized into sterile solution and seeded in Bact/Alert PF plus bottles	both 72 hours
2010	Malizos et al[21]	Ankle and foot osteomyelitis: Treatment protocol and clinical results	Cohort study	84	Not mentioned	Not specified	Not specified	Micro & Histology	Not Mentioned	Not Mentioned
2013	Malone et al[22]	Deep Wound Cultures and Bone Biopsy in Diabetic Foot Osteomyelitis.	Cohort study	66	Not mentioned	Bone biopsies were obtained during surgical removal of infected bone	Sent in standard transport system	Micro only	Used blood agar and gram stained	Not Mentioned
2018	Mijuskovi c et al[23]	Culture of Bone Biopsy Specimens Overestimates Rate of Residual Osteomyelitis After Toe or Forefoot Amputation	Cohort study	51	Wrapping toe for amputation in sterile gauze and removing it before obtaining specimens	In exarticulation, two bone cylinders were obtained using Jamshidi needle. In transmetatars al, a 3-5mm of corticocancell ous metatarsal bone was removed using an oscillating saw. Half was sent for histology and half for microbiology	Using oscillating saw or Jamshidi needle	Micro & histology	Sterile closable transport tubes send immediately after surgery	Micro- firm samples placed in thioglycolat e broth and soft samples crushed and plated onto agar media. Columbia blood again with 5% sheep blood agar were incubated in 5% CO2, brucella agar were incubated in anaerobic workstation, and

Appendix 1.

										thioglucolate broth tubes were incubated in ambient air at 36-37 degrees. Incubated for up to 7 days. Histology- performed using EDTA decalcification of formalin fixed probes followed by paraffin embedding and staining with H+E.
1999	Nehler et al[24]	Intermediate-term outcome of primary digit amputations in patients with diabetes mellitus who have forefoot sepsis requiring hospitalization and presumed adequate circulatory status	Cohort study	92	Not Mentioned	Not mentioned	Not Mentioned	Not Mentioned	not mentioned	Not Mentioned
2016	Przybylski et al[25]	Diagnosing osteomyelitis in the diabetic foot: a pilot study to examine the sensitivity and specificity of Tc99mwhite blood cell-labelled single photon emission computed tomography/computed tomography	Cohort study	14	Not Mentioned	bone samples	Not Mentioned	Not Mentioned	Not Mentioned	Not Mentioned
2017	Ramanujam et al[26]	Medical Imaging and Laboratory Analysis of Diagnostic Accuracy in 107 Consecutive Hospitalized Patients With Diabetic Foot Osteomyelitis and Partial Foot Amputations	Cohort study	107	Not Mentioned	bone samples	Not Mentioned	Micro & Histology	Not Mentioned	Not Mentioned

Appendix 1.

2016	Reveles et al[27]	Epidemiology of Methicillin-Resistant Staphylococcus aureus Diabetic Foot Infections in a Large Academic Hospital: Implications for Antimicrobial Stewardship	Cohort study	318	Not Mentioned	deep wound cultures	Not Mentioned	Micro only	gram stain, biochemical stain and vitek 2 system	Not Mentioned
2018	Schmidt et al[28]	Prospective Analysis of Surgical Bone Margins After Partial Foot Amputation in Diabetic Patients Admitted With Moderate to Severe Foot Infections	Cohort study	72	Not Mentioned	proximal bone margins	wash with 3L of saline, gloves changed and instruments changed	Microbiology and Histology	aerobic, anaerobic, acid fast, fungal	Not Mentioned
2020	Schmidt et al[29]	Making the equivocal unequivocal: standardization of clean margins in diabetic foot osteomyelitis	Cohort study	50	Not Mentioned	Proximal clean bone margins	copious saline wash, change of gloves and instruments	Microbiology and Histology	aerobic, anaerobic, acid fast, fungal	Not Mentioned
2020	Seneville et al[30]	Surgical techniques for Bone Biopsy in Diabetic Foot Infection, and association between results and treatment duration	Review Article	NA	Not Mentioned	Bone margin biopsy using needle for ray and slices for TMT amputations	Change of gloves, instruments	Micro & Histology	Not Mentioned	Not Mentioned
2020	Seneville et al[31]	Diagnosis of infection in the foot in diabetes: a systematic review	Systematic review	NA	Not Mentioned	Bone sample or soft tissue	Not Mentioned	Not Mentioned	Not Mentioned	Not Mentioned

Appendix 1.

2006	Shank et al[32]	Osteomyelitis in the Diabetic Foot: Diagnosis and Management	Review Article	NA	Not Mentioned	bone sample and deep tissue cultures	Not Mentioned	Microbiology and Histology	Not Mentioned	Not Mentioned
2018	Shettigar et al[33]	Microbiological Profile of Deep Tissue and Bone Tissue in Diabetic Foot Osteomyelitis	Cohort study	54	Not Mentioned	No surgical technique given	Not mentioned	Micro only	Not mentioned	Transferred into brain heart infusion containing glass beads, and vortexed. Gram stained and cultured on chocolate agar, 5% sheep blood agar, MacConkeys agar and incubated at 37 degrees. Antibiotic susceptibility was done using the modified Kirby Bauer disk diffusion method.
1999	Tan et al[34]	Diagnosis and treatment of diabetic foot infections	Review Article	NA	Not Mentioned	Open culture or biopsy is superior to swabs	Not Mentioned	Micro & Histology	Not Mentioned	Not Mentioned
2018	Tardaguila-Garcia et al[35]	Complications associated with the approach to metatarsal head resection in diabetic foot osteomyelitis	Cohort study	108	Not Mentioned	Not Mentioned	Not Mentioned	Micro and histology	Not Mentioned	Not Mentioned
2013	Vaznaisiene et al[36]	Major amputation of lower extremity: Prognostic value of positive bone biopsy cultures	Cohort study	69	Not Mentioned	Two bone fragments taken. Bone fragments inoculated into Rosenows broth and the other was placed in a standard transport system	Obtained in surgical room	Aerobic and anaerobic cultures maintained for 5 days and 2 weeks respectively.	Standard transport system	Not Mentioned
2016	Wukich et al[37]	Outcomes of Osteomyelitis in Patients Hospitalized With Diabetic Foot Infections	Cohort study	229	Not Mentioned	Bone biopsy	Not Mentioned	Micro & Histology	Not Mentioned	Not Mentioned

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