International Journal of Applied Research 2016; 2(5): 521-525

ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 5.2
IJAR 2016; 2(5): 521-525
www.allresearchjournal.com
Received: 12-03-2016
Accepted: 15-04-2016

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Surveillance of *Pseudomonas aeruginosa* in delayed wound healing on diabetic patients at tertiary care teaching hospital

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Abstract
Foundation: Foot contaminations are an incessant complexity of patients with diabetes mellitus, representing up to 20% of diabetes-related clinic confirmations. Irresistible specialists are related with the most noticeably bad results, which may at last lead to removal of the tainted foot except if brief treatment methodologies are followed. The present investigation looked to uncover the bacterial etiology of diabetic foot ulcers in Bhubaneswar, the diabetic capital of India.
Techniques: A 10-month-long distinct examination was done to break down the oxygen consuming and anaerobic bacterial confines of all patients conceded with diabetic foot contaminations giving Wagner grade 2– 5 ulcers. Bacteriological determination and anti-toxin affectability profiles were done and investigated utilizing standard methodology.
Results: Diabetic polyneuropathy was observed to be normal (56.8%) and gram-negative microbes (57.6%) were disconnected more regularly than gram-positive ones (42.3%) in the patients screened. The most continuous bacterial disconnects were *Pseudomonas aeruginosa*, Staphylococcus aureus, coagulase-negative staphylococci (CONS), and *Enterobacteriaceae*. Forty-nine societies (68%) demonstrated polymicrobial association. About 44% of *P. aeruginosa* were multi-sedate safe, and MRSA was recuperated on eight events (10.3%). *Bacteroides* spp. Furthermore, *Peptostreptococcus* spp. were the major anaerobic confines.
Ends: Our investigation bolsters the perspective set forth by past South Indian creators that the dissemination of gram-negative microscopic organisms (57.6%) is more typical than that of gram-positive ones (42.3%) and it is in opposition to the perspective that diabetic foot diseases are much of the time monomicrobial. Moreover, recuperation of multi-medicate safe *P. aeruginosa* detaches is of genuine worry, as nobody has revealed the equivalent from the Bhuaneswar.

Keywords: Diabetes; foot contaminations; Wagner grade

Introduction
Perpetual damage pollution is an essential clinical issue that prompts high ghastliness, mortality, and cost. While there is no understanding significance of unending wounds, it is regularly agreed that a human damage is assigned interminable if it remains open for longer than 6– 8 weeks [1, 2]. The wounds are depicted by bacterial weight, perpetual exacerbation, and an uneven cell protect mechanism [2]. Multiple kinds of microorganisms, including Staphylococcus aureus, *Pseudomonas aeruginosa*, *Bacteroides* spp., *Peptostreptococcus*, Enterococcus spp., and Streptococcus pyogenes, have been segregated from steady wounds, in spite of the way that the damage may not allude to any clinical kept sickness. Various other bacterial species have been recognized by using advanced sub-nuclear techniques [3]. Recent clinical data have seemed microbial biofilms are likely going to be accessible in unending wounds, and it has been recommended that biofilms are a significant supporter of the improvement and upkeep of chronicity of wounds [4]. Biofilms are a composed system of microorganisms encased in a self-diminished structure that is devotee to both an inert or living surface [5]. The sicknesses achieved by biofilms are depicted by surface-related sullying microorganism, microorganism clusters encased in an extracellular cross section (ECM), kept neighborhood pollution, and security from against microbial treatment [6]. Current topical and key enemy of microbials are irrelevantly effective in the treatment of

these microbial systems. Moreover, the host's searing response is deficient in doing combating the closeness of biofilm [7]. An imperative inconvenience in characterizing new medicines for endless wounds is the nonattendance of an attractive animal presentation in which steady wounds can be productively inspected. Investigators have endeavored a couple of strategies to prompt relentless injuries, for instance, the ischemic rabbit ear model [8], radiation impaired rats [9], and diabetic mice [10, 11]. Studies, to date, have exhibited that it is less requesting to hurt the tissue than to impel a reproducible model in which the unending damage recovering response is initiated [12]. Wound retouching in these models is for the most part delayed just by a brief time period or advances so mightily that the pollution winds up key and results in high mortality [13, 14]. The db/db mouse has been comprehensively used for concentrates related with diabetes. The db/db mouse has an unconstrained inherited change of the leptin receptor (LEPR) in the operational hub and loses control of satiation. The mouse revels and at last takes after human sort 2 diabetesmellitus, with heaviness, hyperglycemia, periphery neuropathy, and conceded wound repairing. Despite the sort 2 diabetes phenotype, db/db mice take around twice as long to patch wounds as nondiabetic heterozygous littermates under explicit conditions. Turned end in db/db mice is essentially by epidermal movement rather than wound contraction [15]. Much of the force ask about on curved repairing in diabetic mice has focused on the effects of such factors as flammable cytokines, improvement factors, neuropeptides, arrange metalloproteinases, tissue inhibitor of metalloproteinases, low oxygen, and cell senescence [16, 17]. Targeting biofilms that are accessible outwardly of unlimited wounds may be an incredible strategy to propel patching of perpetual wounds [1]. Our goal was to develop a diabetic mouse appear in which we could inspect the activity of biofilm in relentless wounds. In this examination, we developed a biofilm-tried damage show in the db/db mouse by inoculating the damage with P. aeruginosa biofilm and keeping up unhealed damage for 28 days. This model gives a reproducible mouse twisted with bound cutaneous sickness while keeping up a vital separation from basic defilement. The model may enable the examination of unequivocal frameworks by which biofilms debilitate recovering, and support the screening of rising antibiofilm methodology on wound repairing. Relentless damage sullying is an essential clinical issue that prompts high depressingness, mortality, and cost. While there is no agreement importance of interminable wounds, it is generally agreed that a human damage is assigned unending if it remains open for longer than 6– 8 weeks [1, 2]. The wounds are depicted by bacterial weight, ceaseless aggravation, and an unequal cell protect mechanism [2]. Multiple sorts of microorganisms, including Staphylococcus aureus, *Pseudomonas aeruginosa*, *Bacteroides* spp., *Peptostreptococcus*, Enterococcus spp., and Streptococcus pyogenes, have been detached from consistent wounds, in spite of the way that the damage may not indicate any clinical confined tainting. Various other bacterial species have been perceived by using advanced nuclear techniques [3]. Recent clinical data have seemed microbial biofilms are most likely going to be accessible in endless wounds, and it has been prescribed that biofilms are an essential supporter of the improvement and upkeep of chronicity of wounds [4]. Biofilms are a composed system of microorganisms encased in a self-diminished matrix that is devotee to both a latent or living surface [5]. The defilements achieved by biofilms are depicted by surface-related polluting microorganism, microorganism bundles encased in an extracellular cross section (ECM), confined adjacent malady, and security from against disease treatment [6]. Current topical and principal counter agents poisons are irrelevantly convincing in the treatment of these microbial systems. Similarly, the host's red hot response is lacking in battling the proximity of biofilm [7]. A significant inconvenience in enumerating new medications for wearisome wounds is the nonappearance of a classy animal show in which unending wounds can be methodicallly thought about. Pros have endeavored a couple of systems to affect interminable injuries, for instance, the ischemic rabbit ear model,8 radiationimpaired rats [9], and diabetic mice [10, 11]. Studies, to date, have shown that it is less requesting to hurt the tissue than to incite a reproducible model in which the unending damage retouching response is initiated [12]. Wound recovering in these models is ordinarily conceded just by a brief time allotment or advances so mightily that the malady winds up primary and results in high mortality [13, 14]. The db/db mouse has been comprehensively used for concentrates related with diabetes. The db/db mouse has an unconstrained genetic difference in the leptin receptor (LEPR) in the operational hub and loses control of satiation. The mouse gorges and definitely takes after mankind 2 diabetesmellitus, with weight, hyperglycemia, periphery neuropathy, and conceded wound repairing. Despite the sort 2 diabetes phenotype, db/db mice take generally twice as long to repair wounds as nondiabetic heterozygous littermates under explicit conditions. Curved end in db/db mice is on a very basic level by epidermal movement instead of wound contraction [15]. Much of the rhythmic movement look at on contorted recovering in diabetic mice has focused on the effects of such factors as red hot cytokines, improvement factors, neuropeptides, matrix metalloproteinases, tissue inhibitor of metalloproteinases, low oxygen, and cell senescence [16, 17]. In this investigation, we assessed deferred twisted mending in diabetic patients with *Pseudomonas aeruginosa* biofilm at IMS and Sum medical clinic, Bhubaneswar.

Materials and Methods

Study subjects

Patients (n =77) admitted to a particular diabetes care center in at IMS and Sum medical clinic, Bhubaneswar, from May 2016 to March 2015 were penniless down to choose the etiology of their illnesses and their reality on the Wagner scale. Age, sex, metabolic control, cardiovascular peril factors, wound sort and its imprisonment and Wagner survey were chronicled. The related co-dreary conditions in all of the 77 patients were in like manner recorded. Survey 1 was described as cellulitis or a shallow ulcer, audit 2 as subcutaneous damage, fascitis, or tendonitis without osteomyelitis, audit 3 as osteomyelitis, survey 4 as a kept gangrene, and grade 5 as vast gangrene. Wagner survey 1 patients were not considered for the examination as these bruises regularly harbor skin contaminants that as often as possible lead to false-positive results.

Methods

Culture materials from every one of the injuries were gathered, either by washing the injury with 100 cm3 of clean physiological saline and afterward making a cut desire

at the base of the injury or by applying a sterile cotton swab (Himedia, Mumbai) to the injury. The examples were transported to the preparing lab on Cary and Blair media and Robertson's cooked meat media in a 0° smaller than normal cooler (Tarson Labs, Mumbai) and were refined under standard microbiological conditions. Distinguishing proof was made according to strategies proposed CLSI 24.

Results

An entirety of 77 patients (46 folks and 31 females) with diabetes mellitus (5 type 1 and 72 type 2) and diabetic foot ulcers were admitted to the medicinal facility. Their mean age was 63 quite a while and their mean Hb A1C was 6.3%. The related comorbid conditions recorded from the 77 patients are shown in Table 1. The confinement of wounds was for the most part on the toes, with 40% on the colossal toe and 31% on exchange toes, 27% on the underside, and 2% on various pieces of the foot. Twenty-three percent of the patients (n =18) were named Wagner sort out 2, 38.9% (n =30) as stage 3, 20.7% (n =16) as stage 4, and 6.4% (n =5) as stage 5. The component of evacuation was settled with the help of Doppler ultrasound contemplates. The sorts of watchful evacuation performed were according to the accompanying: (I) underneath the knee, n =1; (ii) Symes task (through the lower leg), n =1; (iii) through the knee, n =2; (iv) trans metatarsal, n =1; and exceptional toe, n =3. The center facility stay was 8 days. Bacterial limits experienced in the examination are showed up.

Table 1: One hundred and eighteen bacterial disconnects were Table 1 Bacteriology of 77 different Wagner grade diabetic foot ulcers

Micro-organisms	**W2**	**W3**	**W4**	**W5**	**Total**	**%**
Gram-negative aerobes	39	21	5		65	84.4
Pseudomonas aeruginosa	14	6	3		23	29.8
Escherichia coli	12	4	1		17	22.0
Klebsiella pneumoniae	5	4			9	11.6
Proteus mirabilis	5	2	1		8	10.3
Proteus vulgaris		1			1	1.2
Citrobacter koseri	1	1			2	1.2
Citrobacter freundi		1			1	1.2
Klebsiella oxytoca		1				1.2
Edwardsiella tarda	1				1	1.2
Klebsiella ozaenae	1				1	1.2
Enterobacter aerogenes		1			1	1.2
Gram-positive aerobes	28	9	6	**5**	48	62.3
Methicillin-sensitive *Staphylococcus aureus*	6	2	1	**2**	11	14.2
Methicillin-resistant *Staphylococcus aureus*	2		3	**3**	8	10.3
Coagulase-negative staphylococci (CONS)	16	3	1		20	25.9
Enterococcus faecalis		2	1		3	3.8
Corynebacterium jeikeium	2	1			3	3.8
Bacillus subtilis	2	1			3	3.8
Gram-negative anaerobes		3			3	3.8
Bacteroides fragilis		3			3	3.8
Gram-positive anaerobes		1	1		2	2.5
Peptostreptococcus spp.		1	1		2	2.5
Sterile culture	1	1	1	**2**	5	6.4
Polymicrobial etiology					49	68.0

In the table refers to different Wagner grade diabetic foot ulcers.

Among 77 social orders of which 5 were sterile. Otherworldly vegetation was *Pseudomonas aeruginosa* (29.8%), trailed by Staphylococcus aureus (24.6%), coagulase-negative staphylococci (CONS) (25.9%), Escherichia coli (22%) and distinctive *Enterobacteriaceae*. In 49 positive social orders (68%), polymicrobial etiology was experienced. There were three occasions on which Corynebacterium jeikeium was separated. Five anaerobic isolates were gotten viz. *Bacteroides* fragilis (n =3) from Wagner audit 3 ulcers, however *Peptostreptococcus* spp. (n =2) had a spot with one each from assessments 3 and 4. An antibiogram of the oxygen devouring gram-negative disconnects revealed that ciprofloxacin, amikacin and, somewhat, cefotaxime and gentamicin were fruitful, while an extensive part of the gram-positives were sensitive to gentamicin. Additionally, among the 19 detaches of S. aureus, 8 (10.3%) were methicillin-safe that were sensitive to vancomycin (30 Ag/circle). Ten isolates of P. aeruginosa (43.5%) were multidrug-safe with something like two of the customary antipseudomonal hostile to contamination specialists like amikacin, gentamicin, cefotaxime or ciprofloxacin.

Discussion

With an extending diabetic masses worldwide and with essential centrality to the world's diabetic capital, there is a basic rising in the prevalence of foot defilements moreover in Bhubaneswar, India, the Indian diabetic capital. Eastern India has the greatest number of diabetic individuals and their money related conditions are poor. Subsequently, an examination of the pieces of vivacious and anaerobic tiny life forms and their antibiogram profile acknowledge mind blowing centrality. Essentially as with past data showed up by Gin *et al.* [18] the present South Indian examination recorded the intensity of diabetic polyneuropathy (56.8%) among most of the other related co-inauspicious conditions of diabetes mellitus. It is represented to be the prime figure incorporated the initiation of a sullying in the more significant tissues of the diabetic foot after starting bacterial presentation [19]. Unfortunately our examination revealed an obvious, overwhelming commitment of gram-negative detaches (84.4% aerobes and 3.8% anaerobes) appeared

differently in relation to gram-positive minuscule life forms (62.3% aerobes and 2.5% anaerobes). Besides, unlike mulls over by others [20, 21], we found a 29.8% (n =23) recovery rate of P. aeruginosa in the cases screened. In 65.21% of the cases (15 of the 23 isolates), P. aeruginosa exhibited a mixed commitment with various living creatures, for instance, E. coli, Enterococci spp., S. aureus, CONS, and Proteus spp. A progressing clinical examination by Dhanasekaran *et al*. [22] revealed that 84% of diabetic foot ulcers are as regularly as conceivable monomicrobial, instead of our disclosures. Thinks by Viswanathan *et al*. [23] from another center in South India, uncovered 35% gram-positive pathogens segregated and 65% gram-negative ones. These disclosures resemble our own, underlining the high inescapability of gram-negative pathogens in Eastern India. Three broad diabetes examine centers have gotten on a very basic level equivalent to results. Besides, 43.5% (n =10) of the P. aeruginosa isolates were multi-sedate impenetrable to no less than two of the customary antipseudomonal hostile to disease specialists, for instance, amikacin, gentamicin, cefotaxime, or ciprofloxacin. Wheat *et al*. have prescribed that P. aeruginosa may cause outrageous tissue hurt in diabetics and should never be insulted as immaterial in diabetic foot ulcers [24]. They should never be consider as contaminants or conventional vegetation aside from in the event that they are unquestionably isolated as pathogens. The result of considering these microorganisms as contaminants or commensals may result in sepsis and evacuation [25]. Drawn out or sweeping reach against microbial treatment may slant patients to defilements with hostile to contamination safe living things like MRSA or vancomycin-safe enterococci (VRE) [26], yet our examination was not capable give data identifying with past counter-agent poison treatment of the examination cases. In any case, data presented on the polymicrobial consideration may, somewhat, illuminate a possible prior treatment history of the patients inspected, as Lipsky *et al*. [20] and others [27] have definite that polymicrobial etiology in diabetic foot ulcers may as often as possible be a direct result of past treatment history. MRSA has ended up being dynamically transcendent in diabetic foot wounds [28]. Among the 19 disengages of S. aureus, 8 (10.3%) were methicillin-safe. Regardless, most of the isolates were delicate to vancomycin (30 Ag/plate), which expect criticalness as vancomycin-safe S. aureus (VRSA) is all things considered dynamically declared by others [29]. Our unit of C. jeikeium (diphtheroid) from 3 patients (3.8%) suggests that they may hold some pathogenicity in the diabetic host as there reliably is apparently a connection between C. jeikeium and diabetes mellitus. C. jeikeium is seen as a deft pathogen in diabetic patients 30 that gets segment into necrotic fragile tissue or bone and anticipate a pathogenic activity, paying little personality to its being a for the most part low-ruinous microorganisms. In a past report, Corynebacterium spp. was represented in 12% of diabetic patients with positive social orders [30]. Bessmann *et al*. have revealed that B. fragilis and *Peptostreptococcus* spp. can be recovered in high numbers from extraordinary pollutions. Our imprisonment of B. fragilis with E. faecalis on two occasions confirms its synergistic activity, as supported by Bessmann *et al*. [31] Reports on polymicrobial etiology were practically identical, while a couple of makers have declared a larger amount of anaerobic limits [32]. This qualification could be related to the model gathering and transport structure, which never fused a specific conditions for anaerobic greenery, in spite of the way that Cary and Blair and RCM media support the eagerness of anaerobic microorganisms [31]. We need to consider progressively trustworthy methods, like curettage of the base of the ulcers and significant tissue social orders [33]. Gram-negative infinitesimal life forms that are seen as normal vegetation of the skin, as Citrobacter spp. also, P. aeruginosa, may cause extraordinary tissue hurt in diabetics and should never be normally disregarded as immaterial in diabetic foot ulcers [28]. The Wagner grades with the most separates were 2 and 3, as in past examinations [34]. We couldn't develop any connection between's damage significance and microbiological disclosures, though most of the anaerobes were recovered from audit 3 and 4 wounds. In once-over, our examination supports the point of view put forward by past South Indian makers that the movement of gram-negative microorganisms (57.6%) is more average than that of gram-positive ones (42.3%), anyway it doesn't reinforce the see that diabetic foot infections are a great part of the time monomicrobial. Furthermore, recovery of multi-cure safe P. aeruginosa separates is an authentic stress, as no one has nitty gritty the proportional from the South Indian milieu. Future undertakings must be engaged at understanding the activity of bacterial pathogens in diabetic foot ulcers, and beginning treatment should be composed at both oxygen devouring and anaerobic tiny living beings. The endorsement of a fundamental, cost-effective computation for the assurance of diabetic foot sicknesses will be of chief use. In any case, as proposed by Lipsky *et al*. [18], area of neuropathy before its ensnarements make is the best way to deal with prevent diabetic foot illnesses.

References

1. Fonder MA, Lazarus GS, Cowan DA, Aronson-Cook B, Kohli AR, Mamelak AJ. Treating the chronic wound: a practical approach to the care of nonhealing wounds and wound care dressings. J Am Acad Dermatol. 2008; 58:185-206.
2. Tomic-Canic M, Agren MS, Alvares OM. Epidermal repair and chronic wounds. In: Rovie DT, Maibach H, editors. The epidermis in wound healing. New York: CRS Press, 2004, 25-57.
3. Dowd SE, Sun Y, Secor PR, Rhoads DD, Wolcott BM, James GA *et al*. Survey of bacterial diversity in chronic wounds using pyro sequencing, DGGE, and full ribosome shotgun sequencing. BMC Microbiol. 2008; 8:43.
4. James GA, Swogger E, Wolcott R, Pulcini E, Secor P, Sestrich J *et al*. Biofilms in chronic wounds. Wound Repair Regen. 2008; 16:37-44.
5. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. Science. 1999; 284:1318-22.
6. Parsek MR, Singh PK. Bacterial biofilms: an emerging link to disease pathogenesis. Annu Rev Microbiol. 2003; 57:677-701.
7. Kelly NM, Kluftinger JL, Pasloske BL, Paranchych W, Hancock RE. *Pseudomonas aeruginosa* pili as ligands for nonopsonic phagocytosis by fibronectin-stimulated macrophages. Infect Immun. 1989; 57:3841-5.
8. Ahn ST, Mustoe TA. Effects of ischemia on ulcer wound healing: a new model in the rabbit ear. Ann Plast Surg. 1990; 24:17-23.

9. Gu Q, Wang D, Gao Y, Zhou J, Peng R, Cui Y *et al*. Expression of MMP1 in surgical and radiation-impaired wound healing and its effects on the healing process. J Environ Pathol Toxicol Oncol. 2002; 21:71-810.
10. Greenhalgh DG, Sprugel KH, Murray MJ, Ross R. PDGF and FGF stimulate wound healing in the genetically diabetic mouse. Am J Pathol. 1990; 136:1235-46.
11. Gibran NS, Jang YC, Isik FF, Greenhalgh DG, Muffley LA, Underwood RA *et al*. Diminished neuropeptide levels contribute to the impaired cutaneous healing response associated with diabetes mellitus. J Surg Res. 2002; 108:122-8.
12. Davidson JM. Animal models for wound repair. Arch Dermatol Res. 1998; 290:S1.
13. Davis SC, Ricotti C, Cazzaniga A, Welsh E, Eaglstein WH, Mertz PM. Microscopic and physiologic evidence for biofilm- associated wound colonization *in vivo*. Wound Repair Regen. 2008; 16:23-9.
14. Brown RL, Greenhalgh DG. Mouse models to study wound closure and topical treatment of infected wounds in hearing-impaired and normal healing hosts. Wound Repair Regen. 1997; 5:198-204.
15. Sullivan SR, Underwood RA, Gibran NS, Sigle RO, Usui ML, Carter WG *et al*. Validation of a model for the study of multiple wounds in the diabetic mouse (db/db). Plast Reconstr Surg. 2004; 113:953-60.
16. Brem H, Tomic-Canic M. Cellular and molecular basis of wound healing in diabetes. J Clin Invest. 2007; 117:1219-22.
17. Terasaki K, Kanzaki T, Aoki T, Iwata K, Saiki I. Effects of recombinant human tissue inhibitor of metalloproteinases-2 (rh-TIMP-2) on migration of epidermal keratinocytes *in vitro* and wound healing *in vivo*. J Dermatol. 2003; 30:165-72.
18. Gin H. Infection and diabetes. Rev Med Interne. 1993; 14:32-8.
19. Caputo GM, Cavanagh PR, Ulbrecht JS, Gibbons GW, Karchmer AW. Assessment and management of foot disease in patients with diabetes. N Engl J Med. 1994; 331:854-60.
20. Wheat LJ, Allen SD, Henry M, Kernek CB, Siders JA, Kuebler T *et al*. Diabetic foot infections. Arch Intern Med. 1986; 146:1935-40.
21. Mike E, Ali F. The use of antibiotics in the diabetic foot. Am J Surg. 2004; 187:25S-8S.
22. Hartemann-Heurtier A, Robert J, Jacqueminet S, Ha Van G, Golmard JL, Jarlier V *et al*. Diabetic foot ulcer and multidrug-resistant organisms: risk factors and impact. Diabet Med. 2004; 21:710-5.
23. Ge Y, MacDonald D, Hait H, Lipsky B, Zasloff M, Holroyd K. Microbiological profile of infected diabetic foot ulcers. Diabet Med. 2002; 19:1032-5.
24. Dang CN, Prasad YDM, Boulton AJM, Jude EB. Methicillin-resistant *Staphylococcus aureus* in the in the diabetic foot clinic: a worsening problem. Diabet Med. 2003; 20:159-61.
25. Eady EA, Cove JH. Staphylococcal resistance revisited: community acquired methicillin-resistant *Staphylococcus aureus*-an emerging problem for the management of skin and soft tissue infections. Curr Opin Infect Dis. 2003; 16:103-24.
26. Bessmann AN, Sapico FL, Tabatabai M, Montgomerie JZ. Persistence of polymicrobial abscesses in the poorly controlled diabetic host. Diabetes. 1986; 35:448.
27. Smith AJ, Daniels T, Bohnen JMA. Soft tissue infections and the diabetic foot. Am J Surg.