American Journal of Clinical and Experimental Medicine
2015; 3(1): 6-23
Published online January 23, 2015 (http://www.sciencepublishinggroup.com/j/ajcem)
doi: 10.11648/j.ajcem.20150301.12
ISSN: 2330-8125 (Print); ISSN: 2330-8133 (Online)

Microbiology of diabetic foot ulcer with special reference to ESBL infections

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To cite this article:
Mohammad Zubair, Abida Malik, Jamal Ahmad. Microbiology of Diabetic Foot Ulcer with Special Reference to ESBL Infections. *American Journal of Clinical and Experimental Medicine.* Vol. 3, No. 1, 2015, pp. 6-23. doi: 10.11648/j.ajcem.20150301.12

Abstract: Chronic foot wounds represent an increasing burden to healthcare systems as the age of the population increases. The deep dermal tissues of all chronic wounds harbor microorganisms, however, the precise interaction between microbes in the wounds and impaired healing is unknown. With regard to antibiotic therapy, there is a lack of evidence concerning its effectiveness, optimal regimens or clinical indications for treatment. Despite this lack of evidence, antibiotics are frequently a feature of the management of chronic wounds and these patients receive significantly more antibiotic prescriptions (both systemic and topical) than age and sex-matched patients. Current guidelines for antibiotic prescribing for such diabetic foot wounds are often based on expert opinion rather than scientific fact and may present difficulties in interpretation and implementation to the clinician. Although the increasing prevalence of antibiotic resistance is widely recognized, the relationships between antibiotic resistance, chronic wound microbiology and rationales for antibiotic therapy have yet to be determined. This review discusses the role of microbes in chronic diabetic foot wounds from a clinical perspective with particular focus on the occurrence of type bacteria and their impact on such wounds and resistance pattern, Extended Spectrum Beta Lactamases [ESBL] studies with special reference to Indian studies. The evidence and role of antibiotics in the treatment of such wounds are outlined and current practice of antibiotic usage for chronic wounds in the primary care setting described. The implications of antibiotic usage with regard to antibiotic resistance are also considered.

Keywords: Diabetic Foot, India, ESBL

1. Introduction

A diabetic foot infection is most simply defined as any infra-malleolar infection in a person with diabetes mellitus [1]. Spectrum of infection includes paronychia, cellulitis, myositis, abscesses, necrotizing fasciitis, septic arthritis, tendonitis, and osteomyelitis. The wound may progress to become actively infected, and, by contiguous extension, the infection can involve deeper tissues. This sequence of events can be rapid (occurring over days or even hours), especially in an ischemic limb. Various poorly characterized immunologic disturbances, especially those that involve polymorphonuclear leukocytes, may affect some diabetic patients, and these likely increase the risk and severity of foot infections [2,3,4,].

Selection of the antibiotic regimen involves decisions about the route of therapy, the spectrum of microorganisms to be covered, and the specific drugs to be administered and involves choosing the definitive regimen and the duration of treatment. Initial therapy is usually empirical and is based on the severity of the infection and on any available microbiological data, such as recent culture and antimicrobial sensitivity. On the basis of the available studies, no single drug or combination of agents appears to be superior to others [5], although the available data do not allow us to recommend any specific antibiotic regimen for diabetic foot infections.

The β-lactam antibiotics are among the most widely prescribed antibiotics and are important components of empirical therapy. Because of its extensive use, resistance to drugs has become a major problem especially after the introduction of newer broad-spectrum cephalosporins,

β-lactamase inhibitor / β-lactam antibiotics, monobactams and carbapenems [6]. However, there is paucity of data on the current aspect from Indian hospitals on the isolates of diabetic foot origin. There are only few published reports covering this aspect. No molecular study regarding the occurrence and detection of this class of β-lactamase is published from our country in DFU infections. It is really worrying that we, from India, do not stand on the platform of international debate on these emerging bugs because of the paucity of Indian data, while whole of the world is actively involved in the scientific studies unraveling the burden and implementing the strategies to combat the problem.

2. Microbial Consideration

The skin of person is coated with bacteria that are harmless and many of these organisms are present permanently, but some are transient bacteria that are more often isolated from skin and soft tissue infection, such as *S. aureus* and *beta haemolytic streptococci* which are rarely present on skin. It may be present transiently on the skin and wounds. When the bacteria multiply in the tissue of diabetic patients, it releases toxins inciting a host response; the wound is defined as infected. Infection involves the invasion of host tissue inflammatory response (erythema, indurations, pain or tenderness, warmth, loss of function, purulent discharge)the number and type of organisms, their interaction within each other and with the wound, vascular status , and host resistance will collectively influence whether or not the wound will heal or become more infected [7]. This may be due to single organism's infection called monomicrobial and more than one organism's infection called polymicrobial infection.

2.1. Wound Culture

A culture of samples will identify the microbes present in infection, but only if the specimen(s) are collected properly and processed according to the recommended guidelines. A curettage or tissue scraping from the base of ulcer provides more accurate result [9]. Specimen should be send to microbiology laboratory for aerobic and anaerobic culture and sensitivity report. Mild infections in patients who not receive antibiotic therapy may have 1 or 2 bacterial infection in their foot ulcer, whereas serious infection usually caused by polymicrobial infection (aerobic alone or with anaerobic bacteria) [10]. Anaerobes are rarely the sole pathogen but most often participate in mixed infection [11,12].

3. Diagnosis and Assessing Severity

3.1. Diagnosing Infection

Infection should be accessed first by appearance of a local foot problem (e.g. pain, swelling, ulceration, sinus tract, or crepitation) a systemic infection (e.g. rigor, vomiting, tachycardia, confusion, malaise, and fever) or a metabolic disorder (severe hyperglycemia, ketosis, azotemia). It should be considered when the local signs are less severe than expected [13]. Proper evaluation of diabetic foot infection requires critical and methodological approaches which are listed in table 1.

Table 1. *Recommended evaluation of diabetic patients with foot ulcer.*

Describe the lesion (cellulites, ulcer, etc) and any drainage (serous, purulent).
Enumerate the presence or absence and degree of various signs of inflammation.
Define the status of infection and determine the probable cause.
Examine the soft tissue for evidence of crepitus, abcesses, sinus tract, foreign particle.
Probe any skin break with sterile probe to see whether bone is exposed or not
Measure the size of wound, take photograph
Palpate and record pedel pulse, use Doppler instrument if necessary
Evaluate neurological status
Cleaned and debride the wound
Culture the cleaned wound
Order the plain radiograph

3.2. Severity of Infection

Various classification systems were already proposed for diabetic foot infection but none were universally accepted. Assessing the severity is essential in selecting the appropriate antibiotics. This will influence the route of drug administration and need for hospitalization. It will also help in assessing the potential necessary and timing of surgery. A simple clinical classification of infection has been presented in table 2.

Table 2. *Simple Clinical classification of severity of Diabetic Foot Ulcer[17].*

	Superficial ulcer or cellulitis present	**Deep tissue or bone involve**	**Tissue necrosis or gangrene present**	**Systemic toxicity or metabolic instability present**
Mild	√	-	±	-
Moderate	√	± (no gas or fasciitis)	± (minimal)	-
Severe	√	±	±	√

√=present; ± = may or may not; - = not present

The clinical features that help to define the severity of an infection were represented in table 3.

Table 3. *Clinical characteristics that help to define the severity of an infection [17].*

Features	Mild Infection	Serious infection
Presentation	Slowely progressive	Acute or rapidly progressive
Ulceration	Involves skin only	Penetration to subcutaneous tissue
Tissue involved	Epidermal and dermal	Fascia, muscle, tendon, joint, bone.
Cellulitis	Minimal (<2cm ring)	Extensive, or distant from ulceration
Local signs	Slight inflammation	Severe inflammation, crepitus, bullae.
Systemic signs	None or minimal	Fever, chills, hypotension, confusion, volume depletion, leukocytosis
Metabolic control	Mildly abnormal	Severe hyperglycemia, acidosis, azotemia
Foot vasculature	Minimal impaired (reduce pulse)	Absent pulse, reduced ankle or toe blood pressure
Complicating features	None or minimal (callus, ulcer)	Gangrene, Escher, foreign body, abscess, marked edema, osteomyelitis.

3.3. Criteria of Infection

A critical bacterial load, synergic relationship between bacterial species and the presence of specific pathogens have been proposed as predictors of infection. The critical microbial load might directly affect the healing of both acute and chronic wounds as first reported by Bendy *et al*, [14]. Guidelines of The British Association of Dermatologists and the Royal College of Physicians recommend that infection should be considered if one of the following is present along with the wound: increased pain, increasing erythema of surrounding skin, lymphangitis or rapid increase in ulcer size and pyrexia [15]. The Consensus Development Conference on Diabetic Foot Wound Care [16], agreed that a DFU should be considered infected when there are purulent secretions or there is presence of two or more signs of inflammation (erythema, warmth, tenderness, heat, induration). Chronic wounds by their very nature may not always display the classic symptoms of infection (pain, erythema, oedema, heat and purulence) and it has been suggested that an expanded list, including signs specific to secondary wounds (such as serous exudate plus concurrent inflammation, delayed healing, discolouration of granulation tissue, friable granulation tissue, foul odour and wound breakdown) be employed to identify infection [17].

4. Antibiotic Therapy

4.1. Route of Therapy

The antibiotic therapy usually is given intravenously for systemic ill patients, with severe infection and those who are unable to tolerate oral agents. After a patient significantly responds to the antibiotic treatment in 3-5 days, most of the patients are shifted to oral antibiotics [18]. Oral antibiotic therapy is less expensive and more convenient. For mildly infected patients, tropical therapy is the better option of treatment. This treatment has several advantages, including high local drug levels, avoidance of systemin adverse effect [19]. The patients with PVD, therapautic antibiotic concentrations with many agents are often not achieved in tissue even while the serum concentrations are adequate [20,21,22]. In one procedure, called retrograde venous perfusion, antibiotic solution are injected under pressure into a foot vein while sphygmomanometer is inflated on the thigh. Recently calcium sulphate beads were used in the surgical sites and open wound [23].

4.2. Choice of Antibiotic Therapy and Duration

Table 4(a). *For* Enterobacteriaceae *[96]*

Enterobacteriaceae				
Molecule	**Dosage/ 24h**	**Route of administration**	**Dose interval**	**Comment**
Cefotaxime ofloxacin or ciprofloxacin	200 mg/ kg per day	IV	4–6 h	Oral route as soon as possible
	600 mg per day	IV/ Oral	8 h	
	800–1200 mg per day	IV	8 h	
	or	or	or	
	1000–1500 mg per day	Oral	12 h	
OR				
ofloxacin or ciprofloxacin	600 mg per day	IV/ Oral	8 h	Oral route as soon as possible
	800–1200 mg per day	IV	8 h	
	or	or	or	
	1000–1500 mg per day	Oral	12 h	

Table 4(b). *For Streptococcus infection [96]*

Streptococcus spp				
Molecule	**Dosage/24h**	**Route of administration**	**Dose interval**	**Comment**
Amoxicilin + rifampicin	150–200 mg/kg per day 20–30 mg/kg per day	IV IV/ Oral	4–6 h 8 or 12 h	Change to oral route as soon as possible IV for first 24–48 hours, then oral route at the physician's discretion
OR				
Clindamycin[b] + rifampicin	1800 mg per day 20–30 mg/kg per day	IV/ Oral IV/ Oral	4–6 h 8–12 h	oral route as soon as possible
OR				
Vancomycin + rifampicin	1 g (loading dose) then 30 mg/kg 20-30 mg/kg per day	IV IV infusion IV/ Oral	Loading dose (1h) IV infusion or/ 12h 8–12h	Adjust to serum assays[a]
OR				
Teicoplanin + rifampicin	24 mg/ kg per day then 12 mg/ kg per day 20–30 mg/kg per day	IV/ subcutaneous Subcutaneous IV/ Oral	12 h loading dose 24 h 8 or 12 h	For 48 h, then Every 24 h[a]

[a] Adjust the dosages to obtain tough concentrations (discontinuous IV) or plateau concentrations (continuous IV) of 30 mg/l for vancomycin, or a tough concentration of 30-40 mg/l by HPLC for teicoplanin

[b] Only if susceptible to erythromycin

Table 4(c). *For MRSA & MSSA [96]*

Methicilin – resistant *S. Aureus*				
Molecule	**Dosage/24h**	**Route of administration**	**Dose interval**	**Comment**
Vancomycin ± gentamicin OR + rifampicin OR + fosfomycin	1 g (loading dose) then 30 mg/ kg 4 mg/ kg per day 20–30 mg/ kg per day 200 mg/kg per day	IV IV infusion IV IV/ Oral IV	Loading dose (1h) IV infusion or/ 12h 24 h 8 or 12 h 8 h	Adjust according to serum assays[a] For 48 h IV for first 24–48 hours, then oral route as soon as possible Infusion over 1–2 h
OR				
Rifampicin + fusidic acid	20 – 30 mg/ kg per day 1500 mg per day	IV/ Oral IV/ Oral	8 or 12 h 8 h	IV for first 24–48 hours, then oral route as soon as possible
OR				
[Trimethoprim + Sulfamethoxazole] + rifampicin	640/3200 mg 20–30 mg/ kg per day	IV/ Oral IV/ Oral	(equivalent to 2 tab/12h of [Trimethoprim + Sulfamethoxazole] 8 or 12 h	IV for first 24–48 hours, then oral route as soon as possible
OR				
Teicoplanin + rifampicin	24 mg/ kg per day 12 mg/kg per day 20–30 mg/ kg per day	IV/ Subcutaneous Subcutaneous IV/ Oral	12 h loading dose 24 h 8 or 12 h	for 48 h, then every 24 h[a]

[a] Adjust the dosage to obtain trough concentrations (discontinuous IV) or plateau concentrations (continuous IV) of 30 mg/l for vancomycin, or a trough concentration of 30–40 mg/l by HPLC for teicoplanin

Methicillin – susceptible *S. Aureus*				
Molecule	**Dosage/24h**	**Route of administration**	**Dose interval**	**Comment**
Oxacillin or cloxacillin = gentamicin	100-15 mg/ kg per day 4 mg/ kg per day	IV IV	4 or 6 h 24 h	Until reception of specimens 4 mg/ kg per day
OR				
Ofloxacin or perfloxacin[b] + rifampicin	600 mg per day 800 mg per day 20–30 mg per day	IV/ Oral IV/ Oral IV/ Oral	8 h 12 h 8 or 12 h	Oral route as soon as possible
OR				
Ofloxacin or perfloxacin[b] + fusidic acid	600 mg per day 800 mg per day 1500 mg per day	IV/ Oral IV/ Oral IV/ Oral	8 h 12 h 8 h	Oral route as soon as possible
OR				
Rifampicin + fusidic acid	20–30 mg per day 1500 mg per day	IV/ Oral IV/ Oral	8 or 12 h 8 h	Oral route as soon as possible
OR				
Clindamycin[a] + rifampicin	1800 mg per day 20–30 mg per day	IV/ Oral IV/ Oral	8 h 8 or 12 h	Oral route as soon as possible
OR				

Methicillin – susceptible *S. Aureus*				
Molecule	**Dosage/24h**	**Route of administration**	**Dose interval**	**Comment**
[Trimethoprim + sulfamethoxazole]	640/ 3200 mg	IV/ Oral	12 h (equivalent to 2 tab/ 12 h of [Trimethoprim + sulfamethoxazole])	Oral route as soon as possible
+ rifampicin	20–30 mg per day	IV/ Oral	8–12 h	

[a] Only if susceptible to erythromycin
[b] Caution in subjects > 60 years (1/2 dose)

Table 4(d). *First line antibiotics for Diabetic foot ulcer (excluding osteomyelitis [96]).*

First–line antibiotics in diabetes foot infections (excluding osteomyelitis)		
Type of infection	**Suspected pathogens**	**Antibiotic therapy**
Recent infection of a superficial wound (< 1 month)	MSSA[b] *S.pyogenes* MRSA[c]	Cloxacillin or cephalexin or [amoxicillin + clavulanate] or clindamycin Pristinamycin or linezolide or vancomycin or teicoplanin
Extensive cellulitis	MSSA[b] *S.pyogenes* MRSA[c]	Oxacillin AG[a] Vancomycin or teicoplanin or linezolide
Deep and/ or chronic lesion with or without sepsis	MSSA[b] *S. pyogenes*, GNB[d], anaerobes MRSA[c] MSSA[b] *S. pyogenes*, GNB[d], anaerobes MRSA[c], GNB[d], anaerobes	[Amoxicillin + clavulanate] AG[a] + vancomycin or teicoplanin or linezolide [Piperacillin + tazobactam] or [ticarcillin + clavulanate] + AG[a] Imipenem or ertapenem + [vancomycin or teicoplanin or linezolide] + AG[a]

Shaded zone: oral outpatient treatment; for the other cases, treatment is initially parenteral, followed by oral therapy when possible, depending on the course and susceptibility profile of the bacteria isolated.
[a] AG: aminoglycosides (gentamicin or netilmicin)
[b] MSSA: methicillin- susceptible *Staphylococcus aureus*
[c] MRSA: methicillin- resistant *Staphylococcus aureus*
[d] GNB: Gram-negative bacilli

Many patients will begin therapy, with pending the results of would culture. The narrow spectrum antibiotics may be used in mild infected ulcers until the report of culture & sensitivity are received to modify the treatment accordingly, selecting antibiotic agents that empirically active against *Staphylococci* and other *Streptococci* also. The wounds with foul smell and necrotic and gangrenous usually be treated with anti-anaerobic antibiotics and later on the treatment will be modified according to the reports. On the other hand if the infection is not significantly responding to treatment, the treatment should be changed to cover all the isolated organisms. The antimicrobial spectrum, are shown in table 4 (a, b, c, d). The necessary duration of antibiotics therapy has not been well studied. For mild to moderate infections, 1-2 week course are found to be effective [24], and for severe, it was 2-4 weeks time. The antibiotic treatment should be discontinued when the clinical sign and symptoms of infection have resolved, even the wound has not completely healed.

Table 4. Selected antibiotics that may be used for diabetic foot infections (Adopted from Clinical Practice Guideline-2007. Médecine et maladies infectieuses 37 (2007) 14–25.)

4.3. Outcome of Antibiotic Therapy

The clinical response to mild and moderate infection can be expected to be 80-90 % and this rate of treatment output are further reported to decrease to 50-60%, the infections are of deep or more extensive type, patients usually require surgical intervention in the form of minor or major. Approximately 2/3 of these patients require amputations in their feet or one or more bone resection [25] and long term outcome are reported to be achieved in 80 % of patients [1,26]. In many patients, above ankle amputations are avoided and the uses of aggressive antibiotic therapy with minor and major surgical intervention are required [27]. The factors which can predict the ulcer healing includes (i) absence of exposed bone, (ii) palpable popliteal pulse, (iii) toe pressure of >45 mmHg and for ankle >80mmHg, and (iv) WBC count <12000 m^3 [28].

5. Wound Healing in Diabetic Foot Ulcer

The healing of diabetic wound is complex mechanism which involves an intricately regulated sequence of celluler and biochemical events orchestrated to restore tissue integrity after injury (Figure 01).

The wound healing process involves the three distinct but overlapping phases (Figure 02):

(i) Hemostasis and inflammation,
(ii) Proliferation &
(iii) Maturation and Remodelling.

Each of these phases is controlled by biologically active agents called growth factors [29]. Growth factors are hormone like polypeptides, present in very small amounts in the body that control the growth, differentiation and

metabolism of cells [30].

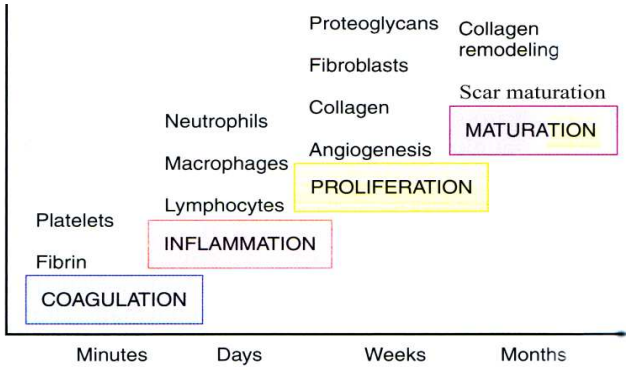

Figure 1. *Normal wound healing is an intricately regulated sequence of cellular and biochemical events orchestrated to restore tissue integrity after injury.*

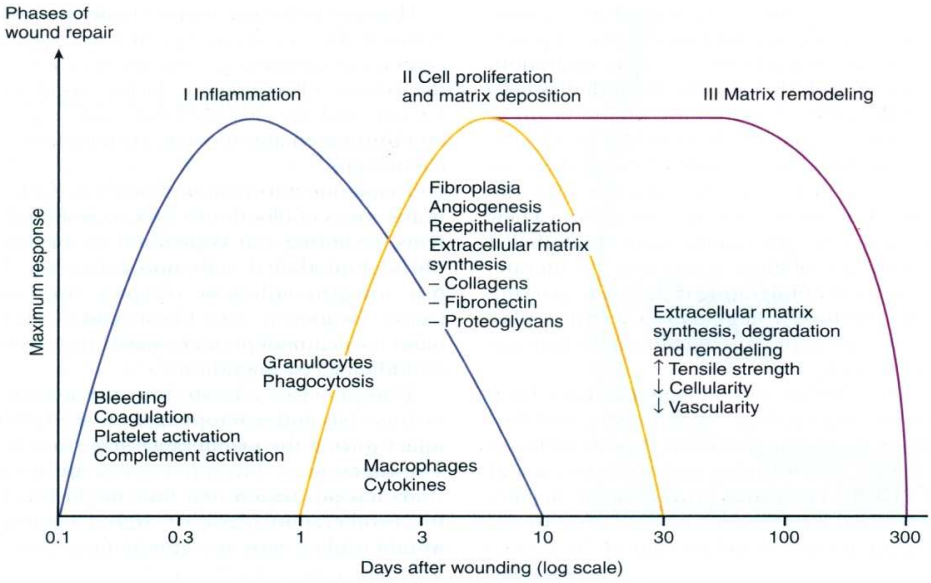

Figure 2. *Time sequence of normal wound healing*

5.1. Hemostasis and Inflammation

Hemostasis follows the traumatic rupture of blood vessels exposed to sub-endothelial collagen and platelets resulting the activation of intrinsic part of coagulation cascade [31]. The inflammation is characterized by increased in vascular permeability, poly-morphonuclear cells (chemotaxis) from circulation into wound and it activates the migrating cells. The neutrofills are the first to arrive at site of wound and its role are primary phagocytic and wound debridement and it is a source of proinflammatory cytokines which serve to activation of the local fibroblast and keratinocytes [32,33]. It decreases the infection in wound, as they are not essential because their role in phagocytosis and antimicrobial defence are taken up by macrophages. The macrophage will migrate to into wound 48-98 hrs after injury and participate in inflammatory process. Their antimicrobial functions will include phagocytosis and generation of reactive free radicals, such as nitric oxide, oxygen, and peroxide. It develops functional complement receptors and undertake similar operations to the neurophils [34].

5.2. Proliferation

The new tissue matrix are essential for physical support for new blood vessel, arising from the intact vasculature in the surrounding dermis, and angiogenesis is essential to provide the basic nutrient and oxygen which help in synthesis of collagen, hence collagen and capillary proliferation occurs in co-dependent manner. During this phase, fibroblast and endothelial cells are primary cells that multiply in this process. Fibroblast responsible for replacing the fibrin matrix called clot and also produce and release proteoglycans and glycosaminoglycans, an extra-component for tissue granulation. The fibroblast production will stop when sufficient collagen were produced. During healing and repair, angiogenesis a fundamental biological mechanism is activated [35], which is characterized by invasion, migration and proliferation of endothelial cells and those which are close to side walls of vessel start migration because of angiogenesis. The cytoplasmic pseudopods starts sprouting in wound and forms perivascular space and the endothelial cells also proliferate to cell migration and produce degradation

enzyme [36]. Several biological factors like, platelets, macrophages, lymphocyte, keratinocyte production (fibroblast growth factor α & β, transforming growth factor α & β, epidermal growth factor, hepatocyte growth factor and IL-1) are reported to be potent stimuli for new vessel formation [37]. Hypoxia (create oxygen granulation), lactate and extracellular matrix also mediate cell growth [38]. Several adhesive protein like Willebrand factor, fibrinogen, fibronectin up regulate the α & β integrin adhesive receptors. These integrin factors initiate the calcium dependent signalling pathways leading to cell migration. Once the wound is filled with new granulation tissue, angiogenesis stops and many vessels disintegrate as a result of apoptosis.

5.3. Maturation and Wound Healing

The main feature in the maturation phase is collagen deposition in wound space. The rate of maturation, quality and total amount of matrix deposition determine the strength of scar. To support the future matrix deposition and remodelling, glycosaminoglycans and proteoglycans are synthesized. The collegen is a predominant scar protein and extracellular matrix components are remodelled by matrix metaloproteinases. The source of collagenases in the wound is the inflammatory cells and endothelial cells as well as fibroblastand keratinocyte [31].

6. Pathogenesis and Impact of Microbes on DFU

Diabetic peripheral sensorimotor neuropathy is the key factor in the majority of cases. As a result of damage to sensory nerves, minor trauma can go unnoticed. Neuropathy can also deform the architecture of the foot to such an extent that joints and digits are placed in mechanically unfavourable positions, making them highly vulnerable to injury. Once the skin is breached, continued mobilization on a broken area impairs the healing process. Inevitably, direct contiguous spread of microbes on the skin follows on, with colonization and infection of superficial and then deeper tissues is, likely if the process is allowed to proceed unchecked. Both the healing process and the response to infection are further compromised by vascular insufficiency, which is commonly present in patients burdened with complications of diabetes [39]. The impact of microbes on wounds has been extensively studied and reviewed using different approaches to elicit their possible role in healing process of diabetic foot ulcers. The infection in diabetic foot is mainly by aerobic bacteria [6, 40- 47]. Anaerobic bacterial infection also plays a significant role in the infection of DFU but this has not been studied since the strict anaerobic culture techniques are not available at all the clinical laboratories. The impact of anaerobes has been reported first by Louie *et al*., [48] and subsequently studies by many [10,41,42,49]. There are only few reports available on the incidence of fungal pathogens in diabetic foot infections [50-55]. DFU infection is usually polymicrobial in nature and this was first reported by Louie *et al*., [48], and subsequently by Anandi *et al*., [56]; Gadepalli *et al*., [41]; Ramakant *et al*., [6]; Zubair et al.,[44-47].

7. Etiology and Prevalence of Bacterial Infection in DFU

7.1. Global Scenario

The first report of polymicrobial etiology of DFU infection was from Boston, USA in 1976. On an average, 5.2 organisms infection per patient were reported; which included 3.2 for aerobic and 2.6 for anaerobic organisms respectively. Majority of isolates were *Bacteroides sp* (14.6%), followed by *Peptococci* (13.7%), *Proteus* sp (9.4%), *Enterococcus* sp (7.7%), *S. aureus* (7.7%), *Clostridium* sp (7.7%) and *E coli* (7.0%) [48].

Sapico *et al*., [50] did the qualitative assessment of 13 DFU patients in California, USA and found that 84.6% patients had infection in their foot. A total of 4.7 strains per patient were isolated (range 3-8). They have collected different samples (ulcer swab pre/post amputed, curettage, saline aspirates and deep tissue biopsy) from the same patient. Almost equal number of aerobic (51.7%) and anaerobic (43.1%) bacteria were isolated and only 5.1% were fungal pathogens. Among the aerobic bacteria, majority (53.3%) of infection in their foot were caused by Gram negative bacilli, Gram negative cocci (3.3%) Gram positive cocci (36.6%) and Gram positive bacilli (6.6%). Among the anaerobic bacterial infections, majority (52%) were caused by gram positive bacilli followed by Gram negative bacilli (44%) and gram positive cocci (4%). Later in year 1984, [10] the polymicrobial nature in their DFU patients. On an average, 4.8 organisms per patient were isolated. The high prevalence of *Bacteroides* sp, *Clostridium* sp, gram negative enteric bacilli, anaerobic bacteria and *Enterococci* were recovered in their study patients.

In the year 1996, a study by Tan *et al*., [27] in Ohio, a total of 112 DFU patients were studied and ulcer infection was found in 74.1% cases. Single isolates were found in 21.1%, and multiple organisms in 75.2%. *Staphylococcus* sp were the most common single organisms isolated. *Corynebacterium* isolates were usually found as one of multiple organisms.

Lipsky *et al*., [19] studied the microbiology of DFU in Washington USA. 108 patients were enrolled at 12 centres across the United States. Culture specimens were obtained by swabbing in 72% of cases, by needle aspiration in 9%, and by tissue sampling in 19%. The mean number of pathogens isolated was 1.6 (range, 0-7). 92% of the pathogens were aerobic organisms among them 67% were gram-positive cocci.

A study by Abdulrazak *et al*., [53] on 86 DFU patients from Kuwait showed an average of 1.6 organisms per infection. 82.5% of the pathogens were aerobic organisms, among them 73.6% were aerobic gram-positive cocci and 26.4% were gram-negative aerobes. Anaerobic infection

were present in 10.5% Among anaerobic infection (10.5%), the most frequently isolated bacteria were *Bacteriodes fragilis* and the fungal infection was represented by 7%.

Another study conducted in Mahaboudha, Kathmandu by Sharma *et al*., [56] reported polymicrobial nature in 62.7% patients while 37.2% had monomicrobial infection. *S. aureus* was the most predominant (38.4%) isolate followed by *P. aeruginosa* (17.5%) and *Proteus mirabilis* (14%).

Ako Nai *et al*., [57] characterized the bacteria isolated from DFU infection in Nigeria, 50.6% were from superficial swabs and 49.3% from deep tissue biopsies. Altogether, 90.7% were aerobes whereas 9.2% were strict anaerobes. 47.6% were *S. aureus*, coagulase negative *staphylococci* (33%) and *Streptococcus* spp. (19.0%). *Proteus species* 32.9%, *E. coli* 26.1%, *P. aeruginosa* 12.5%, *Klebsiella sp*. 11.3%, *Enterobacter cloaca* 5.6%, *Citrobacter freundii* (6.8%), while *Serratia sp* and *Alcaligenes* spp, contributed 3.4% and 1.1%, respectively. 9.2% were strict anaerobes of which *Corynebacterium* spp. accounted for 16.0% of Gram-positive isolates.

Citron *et al*., [42], in a multicentric trial (the SIDESTEP trial) conducted in United States of America on 433 moderate to severely infected DFU patients isolated a total of 1,607 organisms from 454 specimens. Infections were present in 93.7% patients in which 83.8% had polymicrobial infection and 16.2% had monomicrobial infection. Gram-positive comprised 80.3% of the aerobic organisms. The predominant aerobic species were *S. aureus* (76.6%). *Enterococci* were found in 35.7% of the patients. Among Gram-negative rods, 19.7% were aerobic organisms. *P. aeruginosa* was the predominant species followed by *Proteus mirabilis* and *Klebsiella* sp.

In France, Sotto *et al*., [59] studied 173 DFU patients. Polymicrobial infection was found in 73.9% patients with 24.8% cases having monomicrobial infection. Aerobic Gram-positive cocci were predominant (59.7%) with *S. aureus* (38.1%). Gram-negative aerobic bacilli (31.7%) of them *Enterobacteriaceae* were the most frequent pathogens. Of the anaerobes, *Peptostreptococcus sp*. and *Bacteroides sp*. were the most commonly isolated species (7.3% of all isolates).

Alavi SM *et al*., [60] studied found polymicrobial infection was found in 50% of the cases and monomicrobial infection in 31.2% cases. Aerobic Gram-positive bacteria accounted for 42.9 % and 54.8% were Gram-negative rods. *S. aureus* (26.2%) was the most frequent microorganism, followed by *E. coli* (23.8%), *P. mirabilis* (9.5%), *P. aeruginosa*, *Enterobacter* spp and *Morganella* spp (4.76% each). No anaerobes were isolated in their study by using standard anaerobic culture methods.

Raja [54] recovered 287 microbes on an average of 1.47 organisms per patients. Polymicrobial growth was found in 42.7% patients while 57.2% patients had pure growth. 52% were aerobic gram negative bacteria while 45% showed gram positive aerobic bacterial infection. The organisms isolated were *S aureus* (17%) followed by *Proteus* sp (15%), *P aeruginosa* (13%), Group B *Streptococcus* (11%) and *Bacteroides* sp (1%). 1.7% anaerobes were isolated; *Peptotreptococcus spp*, *Bacteroides* spp and *Clostridium* sp. *Candida* sp (0.69%) were also isolated.

Lily SY *et al*., [49] in their study comprised of tissue (65.7%), swabs (28.9%) and bone (5.2%). The most common anaerobic isolates were *Peptostreptococcus* sp (47%) and *Bacteroides fragilis* group (19%), *Prevotella sp* (3%), *Clostridium sp* (2%), *Fusobacterium sp* (2%), *Eggerthella sp and Acidominococcus sp* (1% each respectively).

Tascini *et al*., [60], studied a total of 4332 samples were collected from 1295 patients with an average of 3.3 samples per patient, over three years. Specimens collected by aspiration yielded a positive culture more frequently than swabs and tissue specimens (72.4% vs 59.7% and 50.3%). About 40% of the positive samples were polymicrobial. The Gram positive bacteria were more frequently isolated (52.6%) and *S. aureus* was the most commonly isolated organism (29.9%). *Enterococcus* spp. was isolated in around 10% of samples, mainly *E. faecalis*. *Streptococci* were only 4.6% of isolates. The Gram negative rods were isolated from 40.6% of cases, consisting of Enterobacteriaceae (23.5%) and *P. aeruginosa* (10.3%). Anaerobes were isolated in only 0.3% of cases. *Candida* sp was isolated from 6% of cases.

8. Indian Scenario

Anandi *et al*., [40] studied the bacterial etiology of 107 diabetic foot ulcer patients from India. Polymicrobial etiology was observed in 76.6% patients and only 23.3% had monomicrobial infection. Aerobic bacteria were isolated from 79.2% while 20.2% showed anaerobic infection. The most common aerobic bacteria was *E. coli* (27.7%) followed by *Proteus sp* (16.9%), *S. aureus* and *Klebsiella sp* (13.6% each). The commonest among anaerobes was *Clostridium* spp (60%), followed by *Bacteroides fragilis* (20%), *Prevotella* sp (13.3%) and *Peptostreptococcus* sp (6.7%).

Gadepalli *et al*., [41] in North India on 80 cases having infection in their foot. Isolated a total of 183 organisms on an average of 2.3 species per patients. Polymicrobial infection was found in 82.5% patients. The majority (65.0%) were infected with aerobes & anaerobic infection alone was found in 1.2% patients. Both aerobic and anaerobic organisms' infection was found in 33.8% patients. Gram-positive organisms were found in 13.8% patients, and 28.7% patients had gram-negative organisms. The remaining 57.5% had both Gram-positive and Gram-negative organisms.

Bansal *et al*., [55] from Chandigarh, India studied 103 DFU patients with 37.5% having polymicrobial infection. Total of 157 organisms (91.2% aerobic, 8.9% fungi) were isolated, averaging of 1.52 organisms per patient. In 85.6% specimens, only bacteria were isolated and, mixed infection of bacteria and fungi in 8.4% specimens. *P aeruginosa* (21.6%) were the most common organisms isolated followed by *S aureus* (18.8%), *E coli* (18.1%) and *K pneumonia* (16.7%). Fungal isolates comprises of 9% of total samples. *Candida tropicalis* (29%), *C albicans* (14%), and *C guililermondi* (7%); followed by *Aspergillus flavis* (21%), *A*

niger (14%) and *Fusarium* (14%).

Zubair et al., [43], total of 75 bacterial isolates were isolated, averaging 1.2 species per patient. 56.6% had monomicrobial infection and polymicrobial etiology was observed in 33%. Among the bacterial isolates, gram-positive cocci comprised of 44% and gram-negative bacilli accounted for 56%. *Staphylococcus aureus* was the most common isolate, accounting for 28%; followed by *Escherichia coli* 26.6%, *Pseudomonas aeruginosa* 10.6%, beta haemolytic *Streptococcus spp* 6.6%, *K. oxytoca 5.3%, Enterococcus faecalis 4%, Acinetobacterspp 4%, Coryneformspp 2%, CONS 2%* and *Proteus vulgaris 2%.*

Ramakant *et al*., (6) studied the changing microbiological profile of pathogenic bacteria isolated from DFU in SGPGI, Lucknow, India over a period of 8 years. 1632 cultures were isolated from 434 DFU patients, showing polymicrobial infection in 66% & 23% had monomicrobial infection. They reported that Gram-negative bacterial infection was increasing from 50.6% to 66%. The most common isolate was *P. aeruginosa* (16.9%) followed by *E. coli* (16.1%) and *Proteus* sp. (8.8%). Other Gram negative aerobes recovered were *Citrobacter* sp., *Enterobacter* sp. and *Acinetobacter* sp.

Umadevi *et al*., [61] conducted a prospective study and found a total of 171 bacteria from 105 DFU patients. Infection was present in 89.7% case, in which 44.8% had monomicrobial infection and 52.4% had polymicrobial infection. Gram positive organisms infection was found in 8.6% patients, 52.4% had Gram negative organisms infection while remaining 39.0% had mixed gram positive and gram negative. The most commonly found bacteria was *K. pneumonia* (20.5%) followed by *P. aeruginosa* and *S. aureus* (17.0% each), *E coli* (14.6%), *CONS* (7.0%), *Proteus mirabilis* (5.8%), *Enterococcus* sp (5.3%), *Citrobacter* sp (4.1%), *Proteus vulgaris* (3.5%) *Acinetobacter* sp (3.5%), *Pseudomonas* sp (1.2%) and *Providencia* sp (0.6%).

Zubair et al., [46], 152 aerobic bacteria were isolated, averaging of 1.49 species per patient. 38% patients had monomicrobial infection and polymicrobial etiology was observed in 62%. *E. coli* was the most common isolate (42.2%) followed by S. *aureus* 24.3%, *Pseudomonas aeruginosa* 23.7%, *K. oxytoca* 11.3% and *K. pneumoniae* 9.2%. Both aerobic and anaerobic organisms were isolated in the remaining patients (96.2%). Among the anaerobic bacteria isolated, gram positive comprised of 88.2% and gram negative for 11.7%

Tiwari et al., [62] conducted a prospective study from BHU, Varanasi, India, on 62 cases, 43.5% had mono-microbial infection, 35.5% had poly-microbial infections, and 21% had sterile culture. A total of 82 bacteria were isolated, 68% were Gram negative and 32% were Gram positive. *E. coli* was the most common pathogen isolated followed by *S. aureus*. Other commonly isolated bacteria were *P. aeruginosa*, *Streptococci*, *P. mirabilis*, *Citrobacter* sp., *P. vulgaris*, *K. pneumoniae*, *Bacillus* sp., *Morganella* sp., *Acinetobacter* sp., *E. faecalis*, *K. oxytoca*, *E. aerogenes*, Coagulase –ve Staph, *Pneumococcus*, *Enterococci*. Co-infection with *Candida* spp. was also found in one case with Gram-negative infection (*E. coli*).

Recently Zubair et al., [47] total, 272 (aerobic + anaerobic) bacteria were isolated, averaging 1.67 (1.57 aerobic, 0.10 anaerobic) species per patient, the Gram-positive to Gram-negative ratio was 1:1.8. E. coli was the most common isolate, accounting for 27.8%, followed by *S. aureus* (23.5%), *P. aeruginosa* (15.6%), and *K. oxytoca* (7%). Among the anaerobic bacteria isolated, Gram positive cocci made up 58.8% of infections comprises of *Peptostreptococcus anaerobius* (23.5%), *Propionibacterium sp*. (17.6%), *Bacteroides ureolyticus* (11.7%), *Clostridium perfringens* (5.8%), and *Eggerthella lenta* (5.8%)

9. Antibiotic Resistance in Diabetic Foot Ulcer

The infection of DFU and the drug resistance in this group of patients is an important and major health issue which needs to be addressed. Different populations of DFU patients show wide variation in the level of antibiotic resistance encountered. A prospective study of uninfected chronic venous leg ulcer from 66 patients who had received no antibiotics in the previous month showed a very low level of antibiotic resistance, only two patients were found to have MRSA. Day & Armstrong [63] reviewed the limited evidence on risk factors for the carriage of resistance in diabetic foot wounds. While they found no studies that had directly addressed this issue, suggested risks include cross-contamination of wounds from the patients themselves, inanimate objects or health care personnel, long-term use of antibiotics, prior hospitalization and severity of illness. High prevalence of antibiotic resistance, especially MRSA, affects treatment decisions concerning wounds and raises the question of whether the empirical regimens could cover these resistant organisms [1]. Whilst the additional impact of antibiotic-resistant organisms on wound healing is not known, overall, the morbidity, mortality and cost associated with infections in hospitalised patients caused by antibiotic-resistant organisms has been shown to be 1.3- to 2-fold higher than those infections caused by antibiotic-sensitive organisms [64].

9.1. Global Scenario

Abdulrazak *et al*., [53] showed that imipenem, meropenem, and cefepime were between 80-100%. Metronidazole was found effective for anaerobes. Candida isolates were susceptible to Amphotericin B, nystatin, econazole and fluconazole. They suggested that the spectrum of causative organisms, their resistant patterns, efficacy, and safety should be taken into account before choosing antimicrobials for the treatment.

Ako-Nai *et al*., [57] shows the resistance to erythromycin was 67.1% and tetracycline 61.4%. Similarly, 48.6% of the isolates were resistant to cotrimoxazole while resistance to chloramphenicol was 45.7%. Resistance to a relatively new beta-lactamase-resistant antibiotic, augmentin was seen in

38.6% of the isolates. Only 7.4% of these isolates were resistant to ofloxacin. Among the anaerobes screened, 55% were resistant to gentamicin while 42.9% were resistant to cotrimoxazole.

Alavi *et al*., [59] revealed that the gram negative isolates were resistant to cloxacillin, amoxycillin, clindamycin and vancomycin except that 50% of isolates of *Proteus mirabilis* were susceptible to these antibiotics. *Pseudomonas aeruginosa* was the second most resistant isolate with resistance to all antibiotics used and have 100% sensitivity to Ciprofloxacin and 50% to Ceftriaxone.

Raja *et al*., [54] reported the resistance to methicillin in *S aureus* isolates was 16%, while sensitive to vancomycin and rifampin. Resistance to fusidic acid was found in 7% cases. Sensitivity to penicillin, ampicillin, vancomycin, imipenem, cefuroxime and clindamycin by group B streptococci was found as 100%. *Enterococci sp were* detected resistant to imipenem (8%), ampicillin (17%) and co-trimoxazoles (25%). Metronidazole, imipenem and clindamycin had good activity against all anaerobes. Imipenem was effective against gram negative and gram positive isolates equally in their study.

Citron *et al*., [42] showed all aerobic gram-positive organisms were susceptible to vancomycin, daptomycin, and linezolid. Piperacillin-tazobactam and amoxicillin-clavulanate were the next most active drugs against the gram-positive aerobes. Ciprofloxacin was the least sensitive throughout of the quinolones, especially against all species of Streptococci, however, moxifloxacin was the most active quinolone. *P aeruginosa* strains and the *Enterobacteriaceae* group were largely susceptible to imipenem, piperacillin-tazobactam, ceftazidime, aminoglycosides, and ciprofloxacin. Piperacillin tazobactam and the quinolones were active against more than 90% of the gram-negative organisms, while amoxicillin-clavulanate, doxycycline, and cephalexin were the least active of the drugs tested. Among the anaerobes, all isolates were susceptible to ertapenem except two strains, one B. fragilis strain and one *Bacteroides vulgates.* Overall, 18% of the anaerobes were resistant to clindamycin and 24% were resistant to moxifloxacin.

Lily SY *et al*., [49] reported that 81% were susceptible to clindamycin, 98% to imipenem and 99% to metronidazole. Clindamycin resistance was predominantly present in the *Bacteroides fragilis* group and *Peptostreptococci*. The results of this study showed that metronidazole and imipenem resistance remained low.

Tascini C *et al*., [60] shows all *S. aureus* isolates were susceptible to vancomycin and teicoplanin and 22% were found MRSA positive. 85% were susceptible to Co-trimoxazole, doxycicline and rifampin. Piperacillin tazobactam, cefepime, ceftazidime and meropenem were active against 80% of *P aeruginosa*. Imipenem was active against 74% of strains. Quinolones susceptibility was around 60% with ciprofloxacin, more active than levofloxacin. Levofloxacin had better clinical activity as compared to other quinolones. All the 70 *Candida* isolates were susceptible to fluconazole except one strain of *Candida ciferrii.*

9.2. Indian Scenario

Anandi *et al*., [40] shows average sensitivity of *E coli* was 97% followed by *Klebsiella* sp (94%), *Proteus* spp (92%), *Enterobacter* sp (90%) and *Pseudomonas* sp (84%) for ciprofloxacin, ofloxacin and pefloxacin. All the aerobic isolates were sensitive to amikacin and gentamycin and, cefotaxime in their study. They concluded that the results of sensitivity testing played an important role in the treatment of infection in patients especially with cellulitis and gangrene.

In a study conducted by Sharma *et al*., [56], 82.2% of *S. aureus* were resistant to ampicillin. Cloxacillin and Ciprofloxacin were second most sensitive drug, having a resistance of 11.6% for *S aureus*. Amikacin had a slightly better sensitivity for *E coli*. Amikacin remained the best antibiotic for *Proteus* and *Pseudomonas* also.

Gadepalli *et al*., [41] exhibited high levels of resistance to erythromycin, tetracycline, and ciprofloxacin (40% each) were found in *Enterococcus* species. All the isolates were uniformly susceptible to vancomycin and linezolid. All the anaerobes were susceptible to metronidazole and amoxicillin clavulanate.

Bansal *et al*., [55] shows that 55.5% was MRSA and almost all the isolates were sensitive to ceftriaxone and imipenem. Amikacin and ciprofloxacin shows good sensitivity. *E fecalis* showed 75% resistance to erythromycin and 42% to amoxycillin. All the isolates were 85% sensitive to cefoparazone sulbactam, 71% for amoxycillin clavulanic acid, 62% for ciprofloxacin, and 66% for gentamycin. *P aeruginosa* were sensitive to cefoparazone sulbactam, ceftazidime and imipenem. *E coli* showed 100% sensitivity to imipenem.

Ramakant *et al*., [6] showed antibiotic sensitivity patterns change before and after 1999: piperacillin–tazobactam 74% vs 66%, imipenem 77% vs 85%, cefoperazone–sulbactam 47% vs 44%, amikacin 62% vs 78%, ceftriaxone 41% vs 36%, amoxicillin–clavulanate 51% vs 43% and clindamycin 43% vs 36% respectively. They suggested that the treatment modes can be modified based on the severity of infection and on any available microbiological data such as recent culture results or current Gram-stained smear findings.

Zubair et al., [45] isolated 152 aerobic and 17 anaerobic organisms shows higher percentage of resistance (73.5%) among the Penicillin group, followed by cephalosporin group (54%), quinolones and fluroquinolones (52.8%), aminoglycosides group (38.5%), beta lactam inhibitors (32.2%) and carbapenems (18.4%). All the anaerobes were susceptible to metronidazole, amoxicillin + clavulanate and imepenem.

Umadevi *et al*., [61] demonstrated that 65.5% of *S aureus* were MRSA positive. 56% of *Enterobacteriaceae* member were ESBL producers, in which 62.5% of *Proteus* spp were ESBL positive followed by *Klebsiella pneumoniae* (60%) and *Escherichia coli* (56%). Amikacin, piperacillin tazobactam, imipenem were sensitive against gram-negative bacilli, while vancomycin was sensitive against gram-positive bacteria.

Murugan *et al*., [65] shows that *E. coli* exhibited 100% susceptibility to imipenem and meropenem and resistant to cephalexin, erythromycin, gentamycin and norfloxacin. ESBL positive isolates showed 52.9% resistance to amikacin, 64.7% to gentamicin, 70.6% to cloxacin, 76.5% to co-trimoxazole and 82.4% to cephalexin.

Recently, Zubair et al., [47] shows higher percentage of antibiotic resistance (67.1%) by coagulase-negative staphylococcal spp., followed by *P. aeruginosa* (63.7%), *P. mirabilis* (57.5%), *M. morganii* (57.5%), *E. faecalis* (55.2%), *Acinetobacter sp*. (51.9%), *P. vulgaris* (50.3%), b-hemolytic *Streptococcus* (47.6%), *E. coli* (45.9%), *K. pneumoniae* (44.8%), *S. aureus* (44.3%), *K. oxytoca* (42.9%), and *Corynieform sp*. (37.1%). All the anaerobes were susceptible to metronidazole, amoxicillin + clavulanate, and imepenem.

It is clear from the literature that antibiotics have an important role to play in the treatment of clinically infected diabetic foot. However, there are no conclusive scientific studies to support antibiotic use that might definitively guide antibiotic choice, dose and duration. The use of antibiotics is not risk-free for the individual with both the immediate risk associated with anaphylactic reactions [66] and the longer term prospect of antibiotic use making co-morbidities more difficult to treat. In addition, antibiotic resistance in the general population is a continuing and growing concern. The contribution made to the development, maintenance and dissemination of resistance by those antibiotics issued for DFUs is not yet known, although there is reason to believe that the DFU patient population may be of importance due to the high levels of antibiotic prescribing to these patients, the degree of microbial load associated with their lesions and the potential they provide for dissemination of resistant organisms to others. The resistant organisms have been isolated from both infected and colonized chronic wounds, however, the true prevalence and impact on the wider community are, again, not known. Research needs to be undertaken to elicit the interactions between microbes, antibiotics and antibiotic resistance in chronic wounds for the benefit of both chronic wound patients and the population in general.

10. Extended Spectrum Beta Lactamases (ESBL)

ESBLs are a group of enzymes that break down antibiotics belonging to the penicillin and cephalosporin groups and render them ineffective. ESBL has traditionally been defined as transmissible beta-lactamases that can be inhibited by clavulanic acid, tazobactam or sulbactam, and which are encoded by genes that can be exchanged between bacteria. There is no consensus of the precise definition of ESBLs. A commonly used working definition is that the ESBLs are beta lactamases capable of conferring bacterial resistance to the penicillins, first, second, and third-generation cephalosporins, and aztreonam (but not the cephamycins or carbapenems) by hydrolysis of these antibiotics, and which are inhibited by betalactamase inhibitors such as clavulanic acid.

10.1. History, Evolution and Dissemination of β-Lactamases

The increase in antibiotic resistance among Gram-negative bacteria is a notable example of how bacteria can procure, maintain, and express new genetic information that can confer resistance to one or several antibiotics. This genetic plasticity can occur both inter- and intragenerically. Gram-negative bacterial resistance possibly now equals to gram-positive bacterial resistance and has prompted calls for similar infection control measures to curb their dissemination. Reports of resistance vary, but a general consensus appears to prevail that quinolone and broad-spectrum β-lactam resistance is increasing in members of the family *Enterobacteriaceae* and *Acinetobacter spp*. and that treatment regimens for the eradication of *Pseudomonas aeruginosa* infections are becoming increasingly limited.

The first report of plasmid-encoded beta-lactamases capable of hydrolyzing the extended-spectrum cephalosporins was published in 1983 [67]. The gene encoding the beta-lactamase showed a mutation of a single nucelotide compared to the gene encoding SHV-1. Other beta-lactamases were soon discovered which were closely related to TEM-1 and TEM-2, but had the ability to confer resistance to the extended-spectrum cephalosporins [68, 69]. Hence these new beta-lactamases were coined extended-spectrum beta-lactamases (ESBLs). In the first substantial review of ESBLs in 1989, it was noted by Philippon, Labia, and Jacoby that the ESBLs represented the first example in which beta lactamase-mediated resistance to beta-lactam antibiotics resulted from fundamental changes in the substrate spectra of the enzymes [70].

Gram-negative bacteria have at their disposal a plethora of resistance mechanisms that they can sequester and/or evince, eluding the actions of carbapenems and other β-lactams. The common form of resistance is either through lack of drug penetration (i.e., outer membrane protein [OMP] mutations and efflux pumps), hyper production of an AmpC-type β-lactamase, and/or carbapenem-hydrolyzing β-Iactamases. Based on molecular studies, two types of carbapenem-hydrolyzing enzymes have been described: serine enzymes possessing a serine moiety at the active site, and metallo β-lactamases (MBLs), requiring divalent cations, usually zinc, as metal cofactors for enzyme activity [71].

The series Carbapenems are invariably derivatives of class A or class D enzymes and usually mediate Carbapenem resistance in *Enterobacteriaceae* or *Acinetobacter* spa The enzymes characterized from *Enterobacteriaceae* include NmcA, Smel-3, IMI-1, KPC1-3, and. GES-2, Despite the avidity of these enzymes for carbapenems, they do not always mediate high-level resistance and not all are inhibited by clavulanic acid. In contrast, the oxacillinases have been characterized from *Acinetobacter brumannii* only and include OXA 23 to 27, OXA-40, and OXA-48. These enzymes hydrolyze carbapenems poorly but are able to confer

resistance and are only partially inhibited by clavulanic acid. The class A and class D carbapenemases are encoded by genes that have been procured by the bacterium and can be chromosomally encoded (sometime associated with integrons) or carried on plasmids.

Extended-spectrum p-lactamases (ESBLs) and AmpC β-iactamases are of increasing clinical concern. ESBLs are most commonly produced by *Klebsiella* spp. and *E. coli* but may also occur in other gram-negative bacteria. They are typically plasmid mediated, ciavulanate susceptible enzymes that hydroiyze penicillins, expanded-spectrurn cephalosporins (cefotaxime, ceftriaxone, ceftazidime, cefepirne and others) and aztreonam. AmpC class p-lactamases are cephalosporinases that are poorly inhibited by clavulanic acid. They can be differentiated from other ESBLs by their ability to hydrolyze cephamycins as well a3 other extended-spectrum cephalosporins. AmpC p-lactamases, demonstrated or presumed to be chromosomaily o;- plasmid mediated, have been described in pathogens e.g., *K. pneunioniae, E. coli, Salmonella spp., Proteus mirabilis, Citrobacter freundii, Acinetobacter, Enterobacter spp and. P. aeruginosa*. Although reported with increasing frequency, the true rate of occurrence of AmpC β-iactamases in different organisms, including members of *Enterobacteriaceae*, remains unknown.

In 2001, the ESBLs were reviewed by Patricia Bradford [72]. The body of knowledge pertaining to ESBLs has grown rapidly since that time. A Pub-Med search using the key-words extended-spectrum β-lactamase reveals more than 1,300 relevant articles, with more than 600 published since the time Bradford's review was written.

The total number of ESBLs now characterized exceeds to 200. These are detailed on the website of the nomenclature of ESBLs hosted by George Jacoby and Karen Bush (http://www.lahey.org/studies/webt.htm). Published research on ESBLs has now originated from more than 30 different countries, reflecting the truly worldwide distribution of ESBL producing organisms. However, primarily due to genomic sequencing, increasingly more chromosomally mediated genes are being discovered but are often found in obscure nonclinical bacteria.

10.2. Mechanism of Action of β Lactamases.

β-Lactamase enzymes destroy the β -lactam ring by two major mechanisms of action. Firstly, most common β -lactamase have a serine based mechanism of action. They are divided in to three major classes (A, C and D) on the basis of the ammo acid sequences. They contain an active site consisting of a narrow longitudinal groove, with a cavity on its floor (the oxyanion pocket), which is loosely constructed in order to have conformational flexibility in terms of substrate binding. Close to this lies, the serine residue that irreversibly reacts with the carbonyl carbon of the β -lactam ring, resulting- in an open ring (inactive β -lactam) and regenerating the β-lactamase. These enzymes are active against many penicillins, cephalosporins and monobactam. Secondly a less commonly encountered group of β -lactamases is the metallo β-lactamases or class B β-lactamases. These use a divalent transition metal ion most often zinc, linked to a histidine or cysteine residue or both, to react with the carbonyl group of the amide bond of most penicillins, cephalosporins and carbapcnems but not monobactams [71].

10.3. Classification of β – Lactamases

Classifications involve two major approaches, first and older one based on the biochemical and functional characteristics of the enzyme and the second approach is based on molecular structure of enzyme.

Functional classification schemes were started by describing penicillinases and cephalosporinases by using the response to antisera -as an additional discriminator. Their classification was based on hydrolytic spectrum, substrate profile and whether they are encoded by chromosome or by plasmids. Bush [71] expanded the substrate profile, added the reaction with EDTA, and correlated between function and molecular classification.

Molecular structure classifications were first proposed by Ambler [73] when only four ammo acid sequences of β -lactamases were known. At that time a single class of serine enzyme was designated, the class A β-lactamases that included the *Staphylococcus aureus* PCI penillinase, in contrast to the class B metallo β-lactamases from *Bacillus cereus*. The class C cephalosporinases and class D oxacillinases were segregated from other serine p-lactamases in late 1980s. However, the latest classification of β -Iactamases based on biochemical properties, molecular structure and amino acid sequence [71]. They suggested classification in to four groups (1-4) on the basis of the spectrum of activity and other functional characteristics.

10.4. Schematic Outline of Functional Classification β-Lactamases. [71]

GROUP 1: Cephalosporinase, Molecular Class C *(not inhibited by clavulanic acid)*. They are cephalosporinases not inhibited by clavulanic acid, belonging to the molecular class C.

GROUP 2: Penicillinases, Cephalosporinases, or both inhibited by clavulanic acid, corresponding to the molecular classes A and D reflecting the original TEM and SHV genes. However, because of the increasing number of TEM- and SHV-derived {beta}-lactamases, they were divided into two subclasses, 2a and 2b.

Group 2a: Penicillinase, Molecular Class A

The *2a subgroup* contains just penicillinases.

Group 2b Broad-Spectrum, Molecular Class A

2b Opposite to 2a, 2b are broad-spectrum {beta}-lactamases, meaning that they are capable of inactivating penicillins and cephalosporins at the same rate. Furthermore, new subgroups were segregated from subgroup 2b.

GROUP 2be: Extended-Spectrum, Molecular Class A

Subgroup 2be, with the letter "e" for extended spectrum of

activity, represents the ESBLs, which are capable of inactivating third-generation cephalosporins (ceftazidime, cefotaxime, and cefpodoxime) as well as monobactams (aztreonam).

GROUP 2br: Inhibitor-Resistant, Molecular Class A *(diminished inhibition by clavulanic acid)*

The *2br* enzymes, with the letter "r" denoting reduced binding to clavulanic acid and sulbactam, are also called inhibitor-resistant TEM-derivative enzymes; nevertheless, they are commonly still susceptible to tazobactam, except where an amino acid replacement exists at position at 69.

GROUP 2c: Carbenicillinase, Molecular Class A

Later *subgroup 2c* was segregated from group 2 because these enzymes inactivate carbenicillin more than benzylpenicillin, with some effect on cloxacillin.

GROUP 2d: Cloxacilanase, Molecular Class D or A

Subgroup 2d enzymes inactivate cloxacillin more than benzylpenicillin, with some activity against carbenicillin; these enzymes are poorly inhibited by clavulanic acid, and some of them are ESBLs. The correct term is "OXACILLINASE". These enzymes are able to inactivate the oxazolylpenicillins like oxacilli, cloxacilli, dicloxacillin. The enzymes belong to the molecular class D not molecular class A.

GROUP 2e: Cephalosporinase, Molecular Class A

Subgroup 2e enzymes are cephalosporinases that can also hydrolyse monobactams, and they are inhibited by clavulanic acid.

GROUP 2f: Carbapenamase, Molecular Class A

Subgroup 2f was added because these are serine-based carbapenemases, in contrast to the zinc-based carbapenemases included in group 3.

GROUP 3: Metalloenzyme, Molecular Class B *(not inhibited by clavulanic acid).*

Group 3 are the zinc-based or metallo {beta}-lactamases, corresponding to the molecular class B, which are the only enzymes acting by the metal ion zinc, as discussed above. Metallo B-lactamases are able to hydrolyse penicillins, cephalosporins, and carbapenems. Thus, carbapenems are inhibited by both group 2f (serine-based mechanism) and group 3 (zinc-based mechanism)

GROUP 4: Penicillinase, No Molecular Class *(not inhibited by clavulanic acid)*

Group 4 are penicillinases that are not inhibited by clavulanic acid, and they do not yet have a corresponding molecular class.

11. Molecular Classification of ESBL

The majority of ESBLs contains a serine at the active site and belongs to Ambler's molecular class A. Class A enzymes are characterized by an active-site serine, a molecular mass of approximately 29,000 Da, and the preferential hydrolysis of penicillins Class A β -lactamases include such enzymes as TEM-1, SHV-1, and the penicillinase found in S. aureus. The molecular classification scheme is still used to characterize β -lactamases; however, it does not sufficiently differentiate the many different types of class A enzymes. The classification scheme was based on the substrate profile and the location of the gene encoding the p-lactamase. This classification scheme was developed before ESBLs arose, and it did not allow for the differentiation between the original TEM and SHV enzymes and their ESBL derivatives. More recently, uses the biochemical properties of the enzyme plus the molecular structure and nucleotide sequence of the genes to place β-lactamases into functional groups. Using this scheme, ESBLs are defined as β -lactamases capable of hydroiyzing oximino-cephalosporins that are inhibited by clavuianic acid and are placed into functional group 2be [71].

11.1. Diversity of ESBL Types

11.1.1. SHV beta-Lactamases

The SHV-type ESBLs was frequently found in clinical isolates than any other type of ESBLs [71]. SHV refers to sulfhydryl *v*ariable. This designation was made because it was thought that the inhibition of SHV activity by *p*-chloromercuribenzoate was substrate related, and was variable according to the substrate used for the assay [75]. (This activity was never confirmed in later studies with purified enzyme.) In 1983, a *Klebsiella ozaenae* isolate from Germany was discovered which possessed a beta-lactamase which efficiently hydrolyzed cefotaxime, and to a lesser extent ceftazidime [67]. Sequencing showed that the betalactamase differed from SHV-1, by replacement of glycine by serine at the 238 position. This mutation alone accounts for the extended-spectrum properties of this beta lactamase, designated SHV-2. Within 15 years of the discovery of this enzyme, organisms harbouring SHV-2 were found in every inhabited continent [76], implying that selection pressure from third-generation cephalosporin in the first decade of their use was responsible. SHV-type ESBLs have been detected in a wide range of *Enterobacteriaceae* [77].

11.1.2. TEMbeta-Lactamases

The TEM-type ESBLs are derivatives of TEM-1 and TEM-2. TEM-1 was first reported in 1965 from an *Escherichia coli* isolate from a patient in Athens, Greece, named Temoneira (hence the designation TEM) [78]. TEM-1 is able to hydrolyze ampicillin at a greater rate than carbenicillin, oxacillin, or cephalothin, and has negligible activity against extended-spectrum cephalosporins. It is inhibited by clavulanic acid. TEM-2 has the same hydrolytic profile as TEM-1, but differs from TEM-1 by having a more active native promoter and by a difference in isoelectric point (5.6 compared to 5.4). TEM-13 also has a similar hydrolytic profile to TEM-1 and TEM-2 [79]. TEM-1, TEM-2, and TEM-13 are not ESBLs. However, in 1987 *Klebsiella pneumoniae* isolates detected in France as early as 1984 were found to harbor a novel plasmid-mediated beta lactamase coined CTX-1 [68,71]. The enzyme was originally named CTX-1 because of its enhanced activity against cefotaxime. The enzyme, now termed TEM-3, differed from TEM-2 by two amino acid substitutions [80].

In retrospect, TEM-3 may not have been the first TEM-type ESBL. *K. oxytoca*, harboring a plasmid carrying a gene encoding ceftazidime resistance, was first isolated in Liverpool, England, in 1982 [81]. The responsible beta lactamase was what is now called TEM-12. Interestingly, the strain came from a neonatal unit which had been stricken by an outbreak of *K. oxytoca* producing TEM-1. Ceftazidime was used to treat infected patients, but subsequent isolates of *K. oxytoca* from the same unit harboured the TEM-type ESBL [81]. This is a good example of the emergence of ESBLs as a response to the selective pressure induced by extended-spectrum cephalosporins. Well over 100 TEM-type beta lactamases have been described, of which the majority are ESBLs. Their isoelectric points range from 5.2 to 6.5. The amino acid changes in comparison with TEM-1 or TEM-2 are documented at http://www.lahey.org/ studies/ temtable.htm.

11.1.3. CTX-M Beta-Lactamases

The name CTX reflects the potent hydrolytic activity of these beta lactamases against cefotaxime. Organisms producing CTX-M-type beta lactamases typically have cefotaxime MICs in the resistant range (>64 μg/ml), while ceftazidime MICs are usually in the apparently susceptible range (2 to 8 μg/ml). However, some CTX-M-type ESBLs may actually hydrolyse ceftazidime and confer resistance to this cephalosporin (MICs as high as 256 μg/ml) [82]. Tazobactam exhibits an almost 10-fold greater inhibitory activity than clavulanic acid against CTX-M-type beta lactamases [83]. It should be noted that the same organism may harbor both CTX-M-type and SHV-type ESBLs or CTX-M-type ESBLs and AmpC-type beta lactamases, which may alter the antibiotic resistance phenotype [84]. The number of CTX-M-type ESBLs is rapidly expanding.

12. Epidemiology of ESBLs in DFU

Currently there is paucity of data on the epidemiology of ESBL producing organisms isolated from DFU patients in the World and also from India. The extensive studies on infection with ESBL producing organisms in DFU patients in India are scarce. Mathur et al., from Chandigarh, India [85] have reported 68.5% of their DFU isolates were ESBL producers. Babypadmini and Appalaraju [86] have shown 40% of *K. pneumoniae* isolates and 41% of *E. coli* isolates were ESBL producers in their study from Vellore, India. The prevalence of ESBL was 6% in *E. coli* isolated from DFU patients from Brazil [1]. Gadepalli et al [41] have reported that 54.5% *E. coli* isolates were ESBL producers in diabetic foot infections in Delhi, India. In a study carried out at Shobha et al [87] have reported 27.3% *K. pneumonia*, 25.2% *E. coli*, 21.42% *Pseudomonas* spp, 25% *Enterobacter* spp and 17% *Acinetobacter* spp were ESBL producer. In my study in 2011[45] a total of 68.5% isolates were ESBL producers isolated from diabetic foot ulcer infection in a North Indian tertiary care hospital and later on in year 2012 [47], on average, 74.2% of Gram-negative DFU isolates were positive in the screening of ESBL by the disc diffusion method: 142 (85.0%) isolates were positive using cefotaxime, followed by 127 (76.0%) for cefpodoxime, 117 (70.0%) for aztreonam, and 116 (69.4%) for ceftriaxone and ceftazidime each. In the confirmatory ESBL test, 67.8% were found to be positive by the disc potential method: 132 (79%) using cefoperazone/ cefoperazone + sulbactam, followed by 126 (75.4%) by piperacillin/piperacillin + tazobactum, and 114 (68.2%) by cefotaxime/cefotaxime + clavulanic, whereas ceftazidime/ceftazidime + clavulanic acid showed only 81 (48.5%) ESBL producer.

There is also paucity of data on molecular studies regarding the occurrence of ESBL bla genes (CTX-M, TEM, SHV) from India on diabetic foot ulcer infection. In India, the first report on the molecular detection of ESBL from clinical isolates was from Aligarh, India the study on the prevalence of bla Genes (CTX-M, SHV, TEM) [47]. On average, 89.3% cefotaxime-resistant isolates were found positive for bla genes, of which CTX-M was found to be the most prevalent ESBL noted in 54 (81.8%), followed by TEM in 33 (50%) isolates and SHV b-lactamases in 31 (46.9%) isolates. Twenty-three (37.2%) strains had all three genes (CTX-M +TEM+ SHV), nine (15.2%) strains had CTXM+SHV, four (6.7%) strains had CTX-M + TEM, two (3.3%) strains had TEM+ SHV, 19 (32.2%) strains had CTX-M only, one (1.6%) strain had SHV only, and two (3.3%) strains had TEM only.

13. Importance of ESBL Detection in DFU Isolates

The empiric management of polymicrobial infections in DFU involves the use of a combination of antibiotics that are effective against specific bacterial species, for example, a combination of aminoglycosides and anti-anaerobic agents, such as metronidazole or clindamycin. However, to overcome the incompatibility of certain antimicrobial agents and the resultant toxicity, separate administration is sometimes necessary. The avoidance of potentially nephrotoxic agents is particularly important in diabetic patients because of the likely presence of renal impairment. In addition to this, monitoring of pharmacokinetics is essential in order to ensure that adequate therapy is given. Cost of drugs (acquisition, preparation, and administration) also tends to be higher if combination regimens are given. A more practical approach is to use a single parenterally administered antimicbial agent due to the seriousness of such infections [88]. The second-generation cephalosporins also possess anti-anaerobic activity (notably cefoxitin and cefotetan) provide one therapeutic option [89]. However, both these agents can only be administered parenterally; if subsequent oral treatment is required, an alternate agent must be prescribed. Another disadvantage of the second-generation cephalosporins is that they are relatively poor in vitro activity against enterococci [90]. The use of a

beta lactam: beta-lactamase inhibitor combination is another option. These agents possess a broad spectrum activity and provide coverage against all potential pathogens, including anaerobes. The β-lactam: β-lactamase inhibitor combinations ticarcillin–clavulanic acid and piperacillin–tazobactam antibiotics have similar activity to that of sulbactam–ampicillin, which were administered parenterally. The treatment requires that adequate tissue levels of antibiotic are achieved to ensure eradication of the likely pathogens. Pharmacokinetic studies have shown that concentrations of sulbactam–ampicillin in colonic tissue exceed the MIC90 of *Bacteroides fragilis* [91], and levels in peritoneal fluid are high enough to inhibit most susceptible β-lactamase-producing pathogens encountered in intra-abdominal sepsis [92]. Similarly, sulbactam–ampicillin penetration of the myometrium is sufficient to provide effective therapy for pelvic infections [93]. Penetration of infected tissue is particularly crucial in diabetic patients because of peripheral vascular insufficiency. Seabrook *et al*. [94] reported that, after a single therapeutic dose of sulbactam–ampicillin, 42.8% of patients studied showed level of drug higher than 10 mg/day in diseased soft tissue and this combination is significantly superior to those achieved with either gentamicin or clindamycin. The microbiological and pharmacokinetic properties of sulbactam–ampicillin suggest its suitability for the treatment of mixed infections. Extensive clinical evaluation has been conducted to confirm both the clinical and bacteriological efficacy of sulbactam–ampicillin, and to demonstrate the cost-effectiveness of the combination. The findings of these studies are summarized below. Infections in the feet of patients with diabetes mellitus are responsible for 20% of admissions in this patient group [95] and are a major cause of limb amputation [96]. Sulbactam–ampicillin is an appropriate choice of treatment because of its efficacy and relative lack of renal toxicity.

Three randomized trials were done to assess the efficacy of drugs in diabetic patients with infected foot ulcer. Two of these trials addressed non-limb threatening infections, third one addressed non-limb threatening but treatment resistant infection and the fourth one on invasive infection.

a) FIRST TRIAL: The first randomized trial to access the efficacy of beta lactam drugs in the treatment of DFUs was done in 1990 by Lipsky *et al[24]*. He randomized 56 patients with infected lesions regardless of type or duration to oral Clindamycin or oral cephalexin in an outpatient setting. After 2 weeks, no statistical significant difference were found between treatment, either for response to infection or wound healing, the latter occurring in 40 % of patients receiving clindamycin and 33% receiving cephalexin.
b) SECOND TRIAL: Grayson *et al*., [96] in the year 1994 randomized 93 patients to intravenous imipenem/cilastatin or ampicillin/sulbactam. Patients had severe infections of the lower extremities, threatening to the lower limb and identified by the presence of cellulites, with or without ulceration or purulent discharge. Osteomyelitis was diagnosed in 59(63%) patients. After 5 days, cure had been affected in 60% of the ampicillin/ sulbactam group and 58% in imipenem/cilastatin group.
c) THIRD TRIAL: Lipsky *et al*., [19] randomized 88 patients to intravenous Ofloxacin followed by oral Ofloxacin or intravenous ampicillin sulbactum followed by oral amoxicillin clavulanate in patients who were hospitalized for soft tissue infections that had not responded to outpatient management but which were not limb threatening. At 28 days there were no statistically significant differences in the efficacy of the two therapies. Cure occurred in 49% of the Ofloxacin group and 56% of the amino-penicillin group.

14. Conclusion

It is clear from the literature that expert opinion suggests that antibiotics have an important role to play in the treatment of clinically infected chronic wounds. However, there are no conclusive scientific studies to support antibiotic use, let alone those that might definitively guide antibiotic choice, dose and duration. The use of antibiotics is not risk-free for the individual with both the immediate risk associated with anaphylactic reactions and the longer term prospect of antibiotic use making co-morbidities more difficult to treat. In addition, antibiotic resistance in the general population is a continuing and growing concern. The contribution made to the development, maintenance and dissemination of resistance by those antibiotics issued for chronic wounds is not yet known, although there is reason to believe that the chronic wound patient population may be of importance due to the high levels of antibiotic prescribing to these patients, the degree of microbial load associated with their lesions and the potential they provide for dissemination of resistant organisms to others. MRSA and other resistant organisms have been isolated from both infected and colonized chronic wounds, however, the true prevalence and impact on the wider community are, again, not known. Whereas, ESBLs have evolved greatly over the last 20 years. Their presence, plus the potential for plasmid-mediated quinolone and carbapanem resistance, will be sure to create significant therapeutic problems in the future. It is unlikely that many new antibiotic options will be available in the next 5 to 10 years to tackle such multiresistant infections. Enhanced infection control, coupled with antibiotic stewardship programs, therefore plays an important role in limiting the spread of ESBL-producing organisms. As previously stated, there is no doubt that the ESBLs will become increasingly complex and diverse in the future. This will create increasing challenges for those creating guidelines for detection of ESBLs in the clinical microbiology laboratory. Alteration of antibiotic susceptibility breakpoints may become necessary but need to be carefully considered in combination with pharmacokinetic, pharmacodynamic, and clinical data.Research needs to be undertaken to elicit the interactions between microbes, antibiotics and antibiotic resistance in

chronic wounds for the benefit of both chronic wound patients and the population in general.

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