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Review Article

Current trend in wound infections: Microbial profiling and techniques

Ravichandran B, Thyagarajan Ravinder, Radhika Katrakadda, K. V. Leela

Govt. Kilpauk Medical College, Chennai, Tamilnadu, Dr. MGR Medical University, Chennai, Tamilnadu, India

Abstract

Wound occurs on destruction of the first line of defense, the skin; and thus disturbs the normal microflora of the body. This, in addition to the exposure of an optimal environment for both the normal flora and the pathogens to colonise, establish and infect results in wound infections. Depending on the type of wound, location of the wound, microbial load, microbial diversity and the patient history wound infections are categorised as surgical wound infections, acute soft tissue infections, cellulitis, chronic wounds and diabetic foot ulcer infections. Wound microbial profiling for understanding the role of microbes in wound infections will require detailed microbiological studies unlike the screening of prime etiological agents for scrutinizing the antibiotic regime for treatment. Despite the duration required for microbiological reports will take more than two days, the need for such tests are mandatory with the advent of resistant strains like ESBL Enterobacteriaceae that requires screening for effective antibiotics. The development of rapid microbiological techniques will thus aid in reducing the prevalence of wound infections.

Keywords: Wound infections, Wound microbial profiling, Enterobacteriaceae

***Corresponding author: Ravichandran,** Govt. Kilpauk Medical College, Chennai, Tamilnadu, Dr. MGR Medical University, Chennai, Tamilnadu, India. E- mail: drrashy1974@yahoo.co.in

1. Introduction

Skin is the largest organ in human body and plays a crucial role in regulation of water and electrolyte balance, thermoregulation, besides being a barrier to external noxious agents including microorganisms. The disruption of epithelial integrity of the skin results in a wound.[1] The resultant exposure of subcutaneous tissue provides an optimal moist, warm and nutritive environment for microbial colonization and proliferation.[2] Wound infections are one of the most common hospital acquired infections and are an important cause of morbidity that account to 70-80% mortality [3,4].

They can be classified into two major categories [5]:

Exogenous wound infections on traumatic injury or decubitus pressure ulcer, animal or human bites, burns or foreign bodies in skin or mucous membrane; and endogenous wound infections and abscess like appendicitis, cholecystitis, cellulitis, dental infection, septic arthritis, osteomyelitis, empyema, sinusitis. While exogenous infections are contracted after invasive procedures, surgical manipulation or placement of prosthesis, while others are derived from hematogenous spread from primary site of infection [6].

The potential wound pathogens are Gram positive cocci (*Staphylococcus aureus*, *Streptococcus* species, coagulase-negative *Staphylococcus* species, *Enterococcus* species), Gram negative bacilli (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus* species, *Enterobacter* species), and anaerobes (Bacteroids, Clostridial species) [7]. Wound infections by nosocomial pathogens, on the other hand exhibits varying diversity between countries and at local

regional levels [8] (Figure 1 depicts the wound infection microbial profile in the year 2009 – 2010), thereby being the main cause for postoperative morbidity [9].

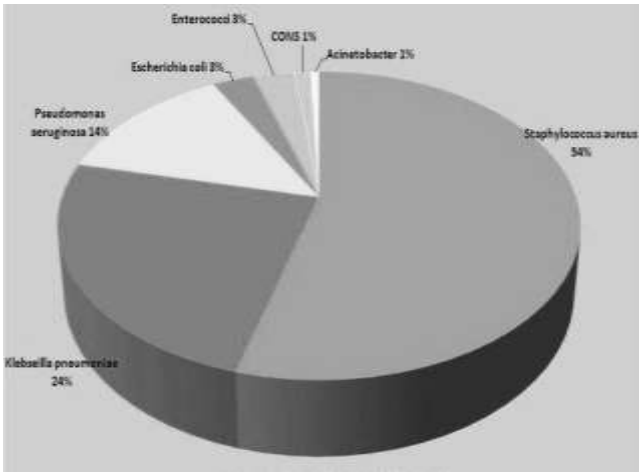

Figure 1: Distribution of micro-organisms from wound infections.

Furthermore, what aggravates wound infection as worldwide problem [8] is infection by antibiotic resistant bacteria [10]. This poses a serious threat in developing countries owing to irrational prescription of antimicrobial agents [11]. Some of the common measures to curb the this problem will include development of novel antimicrobials, better infection prevention and control program and efficient microbial profiling techniques for appropriate use of existing antimicrobial agents [12,13,14]. Many researchers made different recommendations on the susceptibility of microorganisms to drugs [15]. This review paper contemplates on the techniques used to profile wound microbes for efficient use of existing antimicrobials.

1. Microbiology of wound infections:

2.1 Pathogenesis of Wound Infections:

2.1.1 Infection and Colonization:

Exposed subcutaneous tissue provide a favourable substratum for a wide variety of microorganism to contaminate and colonize because the tissue is devitalized (eg: ischemic, hypoxic or necrotic) and the host immune response is compromised making growth conditions optimal for microbial growth.

Wound contaminants are likely to originate from three main sources:

1. The environment (exogenous) microorganism in the air or those introduced by traumatic injury;

2. The surrounding skin (involving members of the normal skin microflora like *Staphylococcus epidermidis*, *Micrococci* sp., skin diptheroids and Propionibacteria); and

3. Endogenous sources are mucous membranes (primarily the gastrointestinal, oropharyngeal and genitourinary mucosa) [16] hosting an array of normal microflora in the gut, oral cavity and vagina that can colonize wounds.

A wound commonly heals within days; however, a minor, slow-healing wound subjected to continued exposure to devitalized tissue is chronic wound and facilitates easy colonization and establishment of a wide variety of endogenous microbes. Dental plague of the gingival crevice and the contents of the colon contain approximately 10 [10] microorganism per g of tissue, of which up to 90% of the oral microflora [17] and up to 99.9% of the colonic microflora are anaerobes [18] and are potential sites for such kinds of wound infections. These wounds are thus susceptible to colonization by a wide variety of endogenous anaerobic bacteria. Ironically, until-to-date wound care practitioners are of opinion that aerobic or facultative pathogens like *Staphylococcus aureus*, *Pseudomonas aeruginosa* and beta haemolytic *Streptococci* sp. are the primary etiological agents for delayed healing and infection in both acute and chronic wounds. Recent literature, however, pointed out that the reason for dearth of information on the role of anaerobic microbes in wound infections was omission or minimal isolation of anaerobic bacteria until lately when appropriate microbiological techniques for anaerobic microbial profiling indicated the presence of a significant proportion of anaerobic microbial population in both acute and chronic wounds.

2.1.2 Factors Predisposing to Microbial Proliferation:

A study showed that surgical wounds heal rapidly if blood perfusion is maximized to deliver O_2, nutrients and cells of the immune systems to the site of injury, thus providing minimal opportunity

for micro-organism to colonize and proliferate [19]. But in chronic, non-healing wounds, besides hypoxicity due to poor blood perfusion (ischemia), host- microbial cell metabolism contributes further to the lowering of local pO_2. Thus cell death and tissue necrosis caused by tissue hypoxia or anoxia are likely to create ideal growth conditions for wound microflora, including fastidious anaerobes that will proliferate once residual O_2 is consumed by facultative bacteria. Poorly perfused wound tissue is considered to be far more susceptible to infection than wounds that are well perfused [20].

2.2 Wound Infection Types:

The progression of a wound to an infection state is likely to involve a multitude of microbial and host factors that include type, site and depth of the wound; the extent of non-viable exogenous contamination; the amount of blood perfusion to the wound; the microbial load and the virulence capacity of various microorganisms involved. Most acute and chronic wound infections involve mixed population of both aerobic and anaerobic microbes. The characteristic local responses are purulent discharge or painful spreading erythema indicative of cellulites around a wound [21]. The different kinds of wound infections are discussed in this section:

2.2.1 Surgical Wound Infections:

Definition: Clinically a surgical site is infected when there is purulent discharge from the incision site [22, 23]. According to Centre for Disease Control (CDC), the definition of surgical site infection (SSI) is diagnosed on basis of one of the following: [24]

a) Purulent discharge from an incision site drain;
b) Positive results obtained from culture of fluid obtained from a surgical site closed primarily;
c) Surgeons or attending physician's diagnosis of infection; and
d) Surgical site that require re-opening.

The bacterial flora accounting for the majority of SSI are *Staphylococcus aureus*, *Staphylococcus epidermidis* and enteric Gram negative bacteria are common in clean surgeries. When a surgery involves the gastrointestinal, respiratory or genitourinary tract, the pathogens are polymicrobial involving aerobic and anaerobic organisms.

D.C. Berridge *et al* and Bengt Gastrin *et al* [25, 26] stated in their studies on orthopaedic surgeries on the omnipresence of *Staphylococcus aureus* and *Staphylococcus epidermidis* isolates. Enterobacteriaceae, Enterococci, Streptococci, Bacteriodes and *Pseudomonas* sp. were the other isolates. Studies involving a large number of generalized wound types reported an overall infection rate of 3.4 % in 5129 operations [27], 4.7 % in 62939 operations [28] and 9.4 % in 1770 operations [29]. In the last two studies, the infection rates ranged from 1.5 % and 5.9 % following clean surgery against 40 % and 52.9 % following contaminated surgery.

Minimizing the incidence of post-operative wound infections relies on adequate sepsis and antisepsis, and preservation of the local host defences [30]. Asepsis involves utilization of effective infection control procedures (eg: air filtration, skin barrier garments, disinfection) to minimize exogenous microbial contamination during surgery; while, antisepsis involves the use of skin antiseptics on the operative site and in those cases of dirty surgical procedures- administration of prophylactic antibiotics at a time point just prior to surgery that will ensure adequate tissue levels of antibiotic during surgery.

As part of the surgical procedure, the endogenous and exogenous microbial contaminations must be minimized using good aseptic, skilled surgical techniques and reduced surgery duration concurrently optimizing the local wound conditions [31]. This primarily involves removing any devitalized tissue to re-establish blood flow to the wound area thereby maintaining adequate perfusion to enable the delivery of immune cells, oxygen and nutrients, thus reducing the microbial load.

2.2.2 Acute soft tissue infections:

Acute soft tissue infections include cutaneous abscesses, traumatic wounds and necrotizing infections. In a cataloguing bacteriological study of a large number of cutaneous abscesses (with unspecified individual predisposing causes), *Staphylococcus aureus* was the single most common aerobic facultative isolate followed by

streptococci, both groupable (A, B, C, D) and non groupable [32]. Among the anaerobic isolates, Bacteroides species (most commonly *Bacteroides fragilis*) was the most frequent followed by *Peptostreptococcus* species and *Clostridium* species. These abscesses are generally polymicrobial (mixed aerobic and anaerobic). As might be predicted, *Staphylococcus* sp. is the principal isolate in infections (both abscesses and wounds) of the extremities and trunk, whereas anaerobes are more numerous than aerobic facultative species in infections of the genital, peri-rectal, inguinal and, head and neck regions.

In two studies of microbiological investigation *Staphylococcus aureus* is the single causative bacterium found in approximately 25 % to 30 % of cutaneous abscesses [33, 34]. *Staphylococcus aureus* was recognized as the most frequent isolate in superficial infections seen in hospital accident and emergency departments. However, other studies revealed that approximately 30 % to 50 % of cutaneous abscesses [33, 32], 50 % of traumatic injuries of varied etiology [35, 36] and 47 % of necrotizing soft tissue infections [37] have polymicrobial aerobic and anaerobic microflora. Necrotizing soft tissue infections involve the skin (eg: clostridial and non-clostridial anaerobic cellulitis), subcutaneous tissue to the muscle fascia (necrotizing fasciitis) and muscle tissue (*Streptococcus myositis* and *Clostridium myonecrosis*).

2.2.3 Cellulitis:

Cellulitis is an acute and invasive infection of the skin that extends deeper into the subcutaneous tissues. Group A Streptococci or *Staphylococcus aureus* are the most common etiological agents. Previous trauma (laceration, puncture wound) or an underlying skin lesion (furuncle, ulcer) predisposes the development of cellulitis. Occasionally cellulitis results through blood-borne spread of infection to the skin and subcutaneous tissues; rarely cellulitis occurs by direct spread from subjacent infections (subcutaneous abscesses and fistulas from osteomyelitis).

Cellulitis is a serious disease because of the propensity of infection to spread via) lymphatics and blood stream. Cellulitis of the lower extremities in older patients is complicated by thrombophlebitis conditions. A polymorphonuclear leucocytosis is usually present regardless of the bacterial etiology. Data from bacterial culture of needle aspirates of cellulitis provided first-hand information on the most likely pathogens to be found [38, 39].

A pathogen was isolated in 30 % of 284 patients; of which, 79 % represented Gram-positive bacteria (mainly *Staphylococcus aureus*, group A Streptococci, group B Streptococci, *Streptococcus viridans* and *Enterococcus faecalis*) and the remaining were Gram-negative bacteria (Enterobacteriaceae, *Hemophilus influenza*, *Pastuerella multocida*, *Pseudomonas aeruginosa* and *Acinetobacter* species).

Broader spectrum of pathogens was isolated from deep wounds or debris tissue in diabetic patients with limb threatening infections including cellulitis. Nearly 56 % were Gram-positive aerobes comprising pathogens like *Staphylococcus aureus*, *Enterococcus* species and various streptococcal species, while Gram-negative aerobes constitute about 22 % with microbes like Enterobacteriaceae, *Acinetobacter* sp. and *Pseudomonas aeruginosa* and the remaining 22 % were anaerobes like *Bacteriodes* sp. and Peptococcus sp. When deciding on the empirical antibiotic choices for treatment, similar broad-spectrum pathogens will hold the same as in the case of cellulitis progressing to complicated decubitus ulcers and in the case of patient hospitalized patient, resistant nosocomial pathogens should also be considered.

2.2.4 Chronic wounds:

Chronic wounds remain one of the most expensive unsolved problems in health care until to-date. Leg ulcers, pressure ulcers, ischemic ulcers and diabetic foot ulcers are examples of common chronic wound infections.

Open wounds are categorised into one of the four states at the time of observation based on the level of bio-burden: bacterial contamination normal but short lived state, colonization - normal state, critical colonization- abnormal state and infection-abnormal state. When the open wound progresses in the directions towards the two abnormal states rather than the order of healing the resultant outcome is the development of chronic wound. The cost of treating a chronic wound infection will thus

depend on various predisposing factors like wound bio-burden, diversity of the microflora, microbial toxins, wound infection's anatomical position, shape and invasiveness and the underlying health condition of the patient including pathology, foreign body debris found in the wound infection, hematoma and necrotic tissues.

This has been well - established that open wound pathogens are aerobic microbes like Staphylococci and Streptococci, however, anaerobic species like *Peptostreptococcus* sp., *Prevotella* sp., *Porphyromonas* sp. and *Bacteroides* sp. has recently been isolated with a potential role to play in clinical manifestation of chronic wound infections. They may act synergistically to invade tissue without penetration into the deep wound compartment [40]. Recent *in vitro* research [41] shows that anaerobic species delay healing by inhibiting fibroblast and keratinocyte proliferation; keratinocyte wound repopulation; and endothelial tubule formation.

In addition, a third group of micro- organisms, Gram- negative bacteria like *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella* sp., *Proteus* sp., *Acinetobacter* sp. and *Enterobacter* sp. establishes in open wound at approximately 4 weeks after symptomatic initiation. This group of microbes does not penetrate but add to the wound bio-burden. However, Gram-negative bacteria possess antiphagocytic and adherence mechanisms, endotoxins and exotoxins that make asepsis difficult and toxins participates in prolonged wound inflammatory responses. *Pseudomonas* sp., for example, secretes the exotoxin pyocyanin that can cause sepsis of wound infections without cellulitis. On reaching the required numbers, these microbes initiates quorum sensing or chemical communication that expresses virulence factors and encourages biofilm formation, which is a much worse condition than classic cellulitis of open wound. Hence, chronic infected wounds are polymicrobial with both aerobic and anaerobic microbes that exhibits co-habitation on intact skin and synergistic mechanism of infection with delayed healing.

Another instance of species-specific infection on the wound is beta-hemolytic Streptococci, in specific *Streptococcus pyogenes*, which are pathogenic at numbers significantly lower than many other species. Other species, eg: *Staphylococcus aureus*, *Proteus* sp. and *Escherichia coli*, may have a positive effect by provoking inflammatory response that accelerates wound repair by stimulating blood flow [42, 43].

Trengrove *et al* [44] support the notion that the presence of multiple species (four or more) delays healing. In general, fewer species and numbers are better for normal healing progress. A diagnosis of critical colonization has two main symptoms: cessation/delay in healing (despite receiving an effective therapy) and absence of cellulitis. Nevertheless, corroborative signs include a wet rather than moist wound, abnormal smell, change in exudate colour, dull dark red or overly bright red discoloration of granules and an oedematous wound base that does not have a granular appearance.

2.2.5 Diabetic foot ulcer infections:

Diabetic patients frequently suffer from foot ulcerations and this complication became more prevalent with advancement in diabetic medical care that prolonged the life expectancy of diabetic patients. Despite progress in the treatment of diabetic ulcerations, prevention and treatment of established ulcerations is a significant challenge. The foremost requirement is identification of the predisposing factors to diabetic foot disease, which is truly multifactorial. Within a single patient, a single factor may dominate over all or some of the other predisposing factors. The various factors involved are: neuropathy, macrovascular and microvascuar diseases, infections, connective tissue abnormalities and hematological disturbances. Identification of the dominant causative factors in each case is essential to plan treatment and the recent developments in neuropathic foot, neuroischaemic foot and ischemic foot is useful for effective treatment.

S. Fredenburg stated that an altered immune response, peripheral vascular disease and neuropathy are the key factors of infection [45] W. S. Joseph stated that the three main factors responsible for diabetic foot infections are neuropathy, angiopathy and immunopathy [46]. L. J. Wheat *et al*., stated that successful treatment of diabetic foot infection requires accurate assessment of the extent and etiology of infections

that thus often involves a broad antibiotic coverage and surgery [47].

A diverse range of Gram-positive and Gram-negative aerobes and anaerobes [47, 48, 49, 50] like *Staphylococcus aureus*, *Bacteroides* sp., *Proteus* sp., *Enterococcus* sp., clostridia and *Escherichia coli* causes the infection. Of these, B. A. Lipksy *et al* described aerobic Gram-positive cocci are the major pathogens. Aerobic Gram-negative bacilli or anaerobes are present mainly in chronic or previously treated infections [51].

Staphylococcus aureus is the most common bacterial species isolated while anaerobic bacteria comprised only 10% of the isolates in a study by E. W. Jones [52]. Anaerobes are occasionally isolated in the osteomyelitis of the foot in diabetic foot infections [51]. Armstrong DG *et al* reviewed that anaerobic species were isolated in only 5% of all cultures [53].

Despite the predominance of a single isolate, antibiotic treatment can be valuable only when the infection is local or superficial. The choice of drug should take account of the polymicrobial nature of these lesions. There is evidence that prolonged antibiotic treatment is effective for small ulcerations until there is tissue damage due to infection and is secondary to surgical debridement. In these cases, the use of broad-spectrum antibiotics will have an important role to play. Bamberger DM *et al*. reviewed that diabetic foot infection in absence of extensive necrosis or gangrene usually responds to antimicrobial therapy without the need for an ablative surgical procedure [54]. Peterson L.R. *et al* suggested that ciprofloxacin offers promise for the improved outcome of patient with the severe diabetic foot infections [55].

On the other hand, conservative treatment includes culture guided parenteral and oral antibiotics effectively without amputation on a large proportion of diabetic patients admitted for foot ulcers [48, 56]. However, with optimal treatment involving debridement of devitalized tissue, the use of appropriate dressings and pressure relief wound infection can be minimized. Boultonj *et al* [57] reported an infection rate of 2.5 % in diabetic wounds treated with a moisture retentive hydrocolloid dressing compared with a 6 % infection rate under a traditional gauze dressing. Laing [58] also observed a similar infection rate (2 %) in diabetic foot ulcerations treated with hydrocolloid dressing despite the number of species increasing during treatment.

Cellular therapy like adjuvant therapy using G-CSF that increases the release of neutrophils from the bone marrow and improves neutrophil function (as neutrophils have bactericidal activity is impaired in diabetic) is effectively used for the treatment of severe diabetic foot infections [59]. Other alternative adjunctive therapy using hyperbaric oxygen and topical growth factors can be helpful in aiding the treatment of diabetic foot infections [60]. Self -foot care behavioural regime, besides the foot care given by health care providers reduces the prevalence of lower extremity clinical diseases in patients with diabetes.

3. Wound–sampling methods:

3.1 Wound tissue sampling:

The acquisition of deep tissue during biopsy follows initial debridement and cleansing of superficial debris and is recognised as the most useful method for determining the microbial load and the presence of invasive pathogens [61]. Another novel, less invasive technique involves dermabrasion that enables the acquisition of deeper tissue in an easier manner than traditional invasive biopsy method [62].

3.2 Wound fluid sampling:

When a copious volume of wound fluid exists, sampling by needle aspiration is deployed. This is the most useful procedure for sampling purulent fluid from intact cutaneous abscesses. However, in cavity wounds like pressure sores, irrigation with sterile saline and gentle massaging will exude and accumulate enough fluid for aspiration.

3.3 Wound swabbing:

Most frequently involves the use of a cotton tipped swab to sample superficial wound fluid and tissue debris for semi-quantitative and qualitative analyses of the wound microflora. Johnson *et al* [63] demonstrated superior isolation of anaerobic bacteria from infected diabetic foot ulcers is rather effective using a swab technique than a needle aspiration technique. Studies by Bowler and Davies [40] demonstrated the efficacy of the swab sample

in isolating anaerobes from various acute and chronic wounds.

4. Specimen transport:

Following the acquisition of wound fluid or tissue for microbiological analyses prompt delivery of the specimen to the laboratory is considered to be of utmost importance particularly if anaerobic bacteria are under investigation. Aspirates of purulent fluids and tissue samples are considered to be more preferred to swabs [64] because they are easy to maintain the condition required to sustain microbial viability (a moist and reduced environment) if processed promptly.

However, pre-reduced commercially available transport media are used to transfer the specimen culture if delayed beyond 1-2 hours after collection for isolation and identification of microbes. For specimens that cannot be transferred to the laboratory within 12 hours, storage at room or appropriate temperatures is required for the maintenance of aerobic and anaerobic microorganisms [65].

5. Analysis of wound specimen:

Information regarding the type of wound (eg: surgical, traumatic, leg ulcer or pressure ulcer), position of the wound, clinical signs of infection, presence of necrosis, associated malodour and antimicrobials used will greatly assist the microbiologists in predicting the type of microorganisms that are most likely to be involved. This will aid in selecting the appropriate type of culture media and complementary analyses to be adopted. Moreover, knowledge on the current antibiotic treatment will assist the microbiologist in determining the antibiotic regime to be prescribed. Since microbial culture and antibiotic sensitivity result cannot be generated in less than 48h (and may on occasion, take considerably longer), a number of rapid investigations must be considered at the outset for immediate attention and first aid to the patient.

5.1. Gram stain:

Gram's stain is still the most important stain in microbiology [66] and is widely used as a rapid technique for guiding antibiotic therapy in life threatening infections like bacterial meningitis and wound management. Gram staining of a known volume of tissue biopsy homogenate rapidly estimates the microbial load of a wound and thus facilitate successful closure of surgical wounds [67]. However in diabetic foot infection and burn wounds, both of which involve complex microbial ecosystems, a poor correlation between Gram stain and culture results from deep tissue biopsy specimens has been reported.

Meislin *et al* [34] reported that the Gram stain reliably indicates sterile and mixed abscesses, as well as those containing pure *Staphylococcus aureus*. Similarly, this procedure may also facilitate identification of the etiological agent of wound infection following clean surgery when there is a higher probability of infection by a single microorganism like clusters of Gram -positive cocci. With the exception of Gram positive spore forming anaerobes such as *Clostridium perfringens* differentiation between aerobic and anaerobic bacteria is difficult and is further complicated by the fact that many Gram positive anaerobes become Gram variable on exposure to oxygen [68].

5.2. Culture of wound specimen and antibiogram:

Routine analyses of wound specimen normally involves the use of selective and non-selective agar media to culture aerobic bacteria and yeasts; and if a specimen is purulent and/or malodorous, anaerobic bacteria. Although anaerobic bacteria often constitute a significant proportion of the total microflora in wounds, their culture and isolation is prolonged and more resource demanding than investigation of aerobic bacteria that consequently is avoided for analyses unless required. The culture media is assessed qualitatively and semi-quantitatively following incubation under aerobic or anaerobic conditions for 24 to 48 hours. With the exception of *Clostridium* species, anaerobes (if investigated) are likely to be reported as being mixed with aerobic microflora. Antibiograms are frequently screened for aerobic pathogens if they are cultured in abundance and with minimal cohabiting microflora. However, when the aerobes are absent and the wound was reported as clinically infected, then anaerobes are suspected and investigated thoroughly.

6. Extended spectrum beta lactamases:

In recent years there has been an increased incidence and prevalence of ESBL (Amber's class A Penicillinases) that hydrolyze and cause resistance to oxyamino cephalosporins (extended spectrum cephalosporins) and aztreonam [69,70]. ESBLs are now found in a significant percentage of *Escherichia coli* and *Klebsiella pneumoniae* strains. They are also found in *Pseudomonas aeruginosa* and other Enterobacteriaceae strains like *Enterobacter* sp., *Citrobacter* sp., *Proteus* sp., *Morganello morganii*, *Serratia marscens* and *Shigella dysenteriae* [71].

Production of these enzymes are either chromosomally mediated or plasmid mediated with pointed amino acid substitution on the classical plasmid mediated beta lactamases like TEM-1, TEM-2 and SHV-1 that increase the spectrum of activity from earlier generation beta lactams to 3rd generation cephalosporins and monobactams. However, they retain their stability against cephamycins and carbapenems and are inhibited to an extent by beta lactamase inhibitors (clavulanic acid, sulbactam and tazobactam). Today over 575 different ESBLs have been described,[72] of which plasmid mediated enzymes spread faster among various bacteria and are important in infection control and, clinical and therapeutic implications.

6.1. Detection methods for ESBL: [73]

6.1.1. Double disk synergy test:

A disk diffusion test in which synergy between third generation cephalosporin (3 GC) and clavulanate is detected by placing a disk of amoxicillin/clavulanate (20µg/10µg) and a disk of third generation cephalosporin (3GC) (30µg) 15mm apart (from centre to centre) on a seeded agar plate. The extension of the edge of a clear inhibition zone of the 3 GC toward the disk containing clavulanate is interpreted as synergy indicating the presence of the ESBL.

6.1.2. CLSI recommended methods for ESBL detection: [73]

a. 1. Screening for ESBL producers:

a. 1.1 Disk diffusion method:

The CLSI proposed disk diffusion method to screen ESBL for antibiotic susceptibility and screen for ESBL production based on diameters of zone to identify ESBL production against cefpodoxime, ceftazidime, aztreonam, cefotaxime or ceftriaxone. The diameter of the zone of inhibition lower than the following values should be investigated with confirmatory tests: ceftazidime (<22mm), cefotaxime and aztreonam (<27mm) and cerftriaxone(<25mm). In the case of cefpodoxime the cut off for *Proteus mirabilis* was (<22mm) whereas in the remaining 3 species *E. coli*, *Klebsiella pneumoniae* and *Klebsiella oxytoca* was (< 17mm). Criteria for screening for ESBL production in other Enterobacteriaceae have not been established by the CLSI.

a. 2 Broth dilution method:

This method can also be used for screening for ESBL producers. It is recommended that *Escherichia coli*, *Klebsiella pneumoniae* and *Klebsiella oxytoca* strains with Minimum inhibitory concentration (MIC<2 µg/m1) against cefotaxime, ceftazidime, cefriaxone or aztreonam and MIC <8µg/m1 for cefpodoxime should be investigated using specific phenotypic confirmatory tests for ESBL production. For Proteus mirabilis isolates confirmatory tests should be performed if strains demonstrate MIC >2µg/m1 for cefotaxime, ceftazidime or cefpodoxime.

6.2. Phenotypic confirmatory tests for ESBL production:

6.2.1. Cephalosporin / Clavulanate combination disks:

The CLSI advocates the use of cefotaxime 30 µg or ceftazidime 30 µg with and without clavulanate 10 µg for phenotypic confirmation of the presence of ESBL. The disk test is performed on confluent growth of the seeded isolate on Mueller Hinton agar. A difference of 5mm between the zone diameters of either cephalosporin disks and their respective cephalosporin / clavunate disk is taken to be the phenotypic confirmation of ESBL production.

6.2.2. Broth micro-dilution:

Phenotypic confirmatory testing can also be performed by broth microdilution assays using ceftazidime (0.25 to 128 µg/ml), ceftazidime plus clavulanic acid (0.25 to 128 µg/ml), cefotaxime (0.25 to 64 µg/ml) and cefotaxime plus clavulanic acid (0.25/4 to 64/4 µg/ml). A twofold serial

dilution decrease in MIC of either cephalosporin in the presence of clavulanic acid was compared to MIC of cephalosporin alone.

6.2.3. Implications of positive phenotypic confirmatory tests:

According to CLSI guidelines isolates which have positive phenotypic confirmatory test should be reported as resistant to all cephalosporins (except the cephamycins, cefoxitin and cefotetan) and aztreonam, regardless of the MIC of that particular cephalosporin.

Conclusion

Wound infections are serious threats worldwide and are of prominent occurrence in surgical wounds, diabetic patients and trauma. Wounds infections are initially loaded with aerobic microbes, which on invading the deeper subcutaneous tissues and on development of tissue debridement facultative and anaerobes colonise. Thus from the microbiological perspective for identification of the etiological agents and for treatment purposes will require profiling of only the aerobes, which on failure to heal the wound or in the absence of aerobes will require further investigation for anaerobes. Furthermore, microbial investigation until to-date remains a slow process delaying the start of targeted species-specific antibiotic regime for treating wound infections. This provides scope for identification of antimicrobials that will have a broader spectrum (and that too in the age when the development of resistant strains like the advent of the ESBL Enterobacteriaceae isolates) and are slow releasing; and for innovation in rapid microbiological techniques to identify and isolate microbes.

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