



Research Article

Microbial isolates in diabetic foot ulcers: culture & sensitivity patterns and antibiotic resistance

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Abstract

Objective: Diabetic foot leads to diabetic ulcer infections and these infections are most devastating complication of diabetes. This study was conducted to analyze the spectrum and sensitivity of microbial to commonly available antibiotics at a peripheral hospital in Nasik.

Material and Methods: The study included all patients with diabetic foot ulcer infection who reported to the surgical outpatient department. We sent appropriately obtained specimens for culture prior to starting empirical antibiotic therapy in all participants. All diabetic foot ulcers were included in the study and wound swabs and/or slough for culture and sensitivity was sent. The 101 wound swabs were collected from diabetic foot ulcer patients.

Results: Due to analyses, the *Staph aureus* was the most commonly isolated bacteria (58.41%). The bacterial isolates exhibited a high degree of resistance to the antibiotics tested with most isolates showing resistance from 5.56 % as in Chloramphenicol to 90.90% as seen in Imipenem. High resistance levels were found against to Imipenem, Ampicillin, Augmentin, Cloxacillin, Carbenicillin, Ceftriaxone, Ceftazidime. Moderate resistance to Ofloxacin, Ciprofloxacin, Azithromycin and Ticoplanin and low antibiotic resistance with Chloramphenicol, Gentamycin, Amikacin, Clindamycin, Erythromycin, Levofloxacin, Linezolid, Polymyxin B, Piperacillin, Tobramycin, Vancomycin, Tetracyclin and Netilmycin have been observed.

Conclusion: As it seen in our study, prescribed antibiotics for the management of diabetic foot ulcers should be considered in a broad range spectrum against to Gram-negative and Gram-positive pathogens to achieve successfully treatment of diabetic foot ulcer patients.

Keywords: Diabetic foot infection, Diabetic foot ulcer, wound swab, slough culture, culture and sensitivity, Antibiotic resistance.

Introduction

Patients with diabetes mellitus are at risk for considerable morbidity as a result of chronic foot ulceration and foot infection including limb loss. Diabetic foot infections are usually a consequence of skin ulceration from ischemia or trauma to

neuropathic foot. Recurrent infections are common and 10-30% of affected patients eventually require amputation⁽¹⁾. *Staph aureus* is the most important pathogen in diabetic foot infections⁽¹⁾. It is often present as a monomicrobial infection but usually it is also an important

pathogen in polymicrobial infections. Among gram negative bacilli, bacteria of Enterobacteriaceae family are common and *Pseudomonas aeruginosa* may be isolated from wounds⁽¹⁾. Agents that have been shown to be effective for therapy of Diabetic foot infections include Cephalosporins, B- lactamase inhibitors, Fluoroquinolones, Clindamycin, Carbapenems, Vancomycin and Linezolid⁽¹⁾. Common practice is to treat mild infection for 1 week whilst serious infections are given antibiotics for 2 weeks. Adequate debridement, resection, or amputation may shorten the duration of antibiotic requirement.

As summary, the aim of this study was to analyze wound swab samples from patients with diabetic foot infections and study their culture and sensitivity reports and report the antibiotic resistance levels.

Materials and Methods

In this retrospective study which records of wound swab culture and /or slough culture and sensitivity reports of patients being treated for diabetic foot infections at the department of Surgery in a peripheral hospital in Nasik were analyzed.

Exclusion and Inclusion Criteria

All diabetic foot patients were included in the study. Very ill patients with sepsis, patients already on antibiotics for long and patients having osteomyelitis were excluded.

Sample Collection

Sample was collected by the operating / treating Surgeon in the Operating room under sterile conditions and after cleaning the wound with Normal Saline to prevent surface contamination followed by debridement of superficial tissue exudates. Commercially available cotton swabs were used and the sample was transported to the lab within one hour of collection to prevent drying of the swabs. Slough from diabetic foot patients who underwent wound debridement was sent for culture and sensitivity in sterile containers.

Prevalence of wound infection:

A total of 101 wound swab/ slough culture samples were analyzed for bacterial growth and sensitivity tested for antibiotics in the agar culture. Normal saline was applied to moisten the head of the swab to increase the adherence of bacteria and the swab passed over the wound from centre of the wound outward to the edge of the wound in a zigzag motion while twisting the swab so that the entire head of the swab comes into contact with the wound surface. Anaerobic media were inoculated in an anaerobic holding jar and incubated at 96.8 °F (36 °C) in the absence of oxygen for five to seven days. Aerobic culture included inoculation of sheep blood agar for general growth; chocolate agar for isolation of *Haemophilus*; MacConkey agar for isolation of enteric gram negative bacilli; CNA or PEA blood agar for gram-positive cocci. Cultures were incubated in humid air at 96.8 °F (36 °C) for 48 hours (except for chocolate agar which was incubated in 5-10% carbon dioxide). Cultures were examined each day for growth and any colonies were Gram stained and transferred to appropriate media. The sub-cultured isolates were tested via appropriate biochemical identification panels to identify the species present. Organisms were also tested for antibiotic susceptibility by the microtube broth dilution or Kirby Bauer method. The selection of antibiotics for testing depended upon the organism isolated (i.e., gram-negative versus gram-positive, aerobe versus anaerobe).

The bacterial isolates exhibited a high degree of resistance to the antibiotics tested with most isolates showing resistance from 5.56 % as in Chloramphenicol to 90.90% as seen in Imipenem. High resistance levels were found with Imipenem, Ampicillin, Augmentin, Cloxacillin, Carbenicillin, Ceftriaxone, Ceftazidime. The study showed moderate resistance to Ofloxacin, Ciprofloxacin, Azithromycin and Ticoplanin. Low antibiotic resistance were seen with Chloramphenicol, Gentamycin, Amikacin, Clindamycin, Erythromycin, Levofloxacin, Linezolid,

Polymyxin B, Piperacillin, Tobramycin, Vancomycin, Tetracyclin and Netilmycin.

Degree of susceptibility of micro-organisms isolated to antibiotics expressed in percentage resistance:

Staph Aureus

Table 1: Sensitivity and Resistance of Staph Aureus against antibiotics

| Antibiotic | Sensitivity | Resistance |
|---------------|-------------|------------|
| Cotrimoxazole | 33.33% | 66.66% |
| Erythromycin | 62.26% | 37.73% |
| Gentamycin | 72.72% | 32.14% |
| Levofloxacin | 84.21% | 15.78% |
| Linezolid | 90.74% | 9.25% |
| Ampicillin | 30.76% | 69.23% |
| Augmentin | 19.60% | 80.39% |
| Amikacin | 84.61% | 15.38% |
| Ciprofloxacin | 41.17% | 58.82% |
| Clindamycin | 87.93% | 12.06% |

Pseudomonas

Table 2: Sensitivity and Resistance of Pseudomonas against antibiotics

| Antibiotic | Sensitivity | Resistance |
|---------------|-------------|------------|
| Amikacin | 85.71% | 14.28% |
| Ciprofloxacin | 75% | 25% |
| Gentamycin | 85.71% | 14.28% |
| Piperacillin | 57.14% | 42.85% |
| Ceftazidime | 20% | 80% |
| Tobramycin | 66.66% | 33.33% |
| Ceftriaxone | 0% | 100% |

E Coli

Table 3: Sensitivity and Resistance of E Coli against antibiotics

| Antibiotic | Sensitivity | Resistance |
|---------------|-------------|------------|
| Ampicillin | 22.22% | 77.77% |
| Augmentin | 16.66% | 83.33% |
| Amikacin | 85.71% | 14.28% |
| Ciprofloxacin | 53.84% | 46.15% |
| Gentamycin | 87.5% | 12.5% |
| Netilmycin | 100% | 0% |
| Tobramycin | 66.66% | 33.33% |
| Cotrimoxazole | 14.28% | 85.71% |

Proteus

Table 4: Sensitivity and Resistance of Proteus against antibiotics

| Antibiotic | Sensitivity | Resistance |
|---------------|-------------|------------|
| Ampicillin | 25% | 75% |
| Augmentin | 0% | 100% |
| Amikacin | 87.5% | 12.5% |
| Ciprofloxacin | 100% | 0% |
| Gentamycin | 80% | 20% |
| Cotrimoxazole | 40% | 60% |

Results

Culture sensitivity patterns from wound swabs sent from diabetic foot wounds were studied and it was found that in 59 (58.41%) patients, the culture sensitivity report showed growth of Staph aureus, in 6 (5.94%) patients the report showed growth of Proteus, in 11 (10.89%) patients the report suggested the growth of Klebsiella, in 15 (14.85%) patients the report revealed growth of E Coli and in 10 (9.90%) patients the report suggested growth of Pseudomonas.

So to infer Staph aureus was the most common organism isolated from diabetic foot wounds and Proteus was the least common organism isolated.

The bacterial isolates exhibited a high degree of resistance to the antibiotics tested with most isolates showing resistance from 5.56 % as in Chloramphenicol to 90.90% as seen in Imipenem. High resistance levels were found with Imipenem, Ampicillin, Augmentin, Cloxacillin, Carbenicillin, Ceftriaxone, Ceftazidime.

The study showed moderate resistance to Ofloxacin, Ciprofloxacin, Azithromycin and Ticoplanin.

Low antibiotic resistance were seen with Chloramphenicol, Gentamycin, Amikacin, Clindamycin, Erythromycin, Levofloxacin, Linezolid, Polymyxin B, Piperacillin, Tobramycin, Vancomycin, Tetracyclin and Netilmycin.

Staph aureus was found to be most sensitive to Linezolid (90.74%) and most resistant to Augmentin (80.39%). (Table 1)

Pseudomonas was found to be most sensitive to Amikacin (85.71%) and most resistant to Ceftriaxone (100%). (Table 2)

E Coli was found to be most sensitive to Netilmycin (100%) and most resistant to Cotrimoxazole (85.71%). (Table 3)

Proteus was found to be most sensitive to Ciprofloxacin (100%) and most resistant to Augmentin (100%). (Table 4)

Discussion

Non-traumatic lower limb amputation is the most common devastating complication of diabetes, primarily due to diabetic foot ulcers (DFU) and diabetic foot infections (DFI). Diabetes-associated foot ulcers, followed by infection causes substantial morbidity and dreaded complications like systemic toxicity, gangrene, and lower extremity loss. Diabetic peripheral neuropathy and peripheral arterial disease are the key etiologic agents in foot ulceration. Although current guidelines recommend antibiotic treatment be initiated when obvious clinical signs of infection develop², these signs may not appear until destruction of underlying tissue and bone triggers a systemic inflammatory response. Patients with diabetes, however, may not express clinical signs of infection, despite high levels of bacteria in local DFU tissue², because peripheral vascular disease, poor metabolic control, and neuropathy dampen first-line inflammatory responses³.

Common DFU pathogens include *Staphylococcus aureus*⁴, methicillin-resistant *S. aureus* (MRSA)⁵, Gram-negative bacilli⁶, β -hemolytic *Streptococcus*⁴, and obligate anaerobes⁴, these microbes are targeted for antibiotic treatment in DFUs with moderate to severe clinical signs of infection².

If cultures are negative, empirical antibiotic therapy should usually be stopped after no more than 48-72 hours¹. Unnecessary antibiotic therapy increases risk of multi drug resistance infection, so prolonged therapy with negative cultures is usually unjustifiable. The high degree of resistance may be attributed to the widespread abuse of antibiotics, practicing self medication, indiscriminate use of antibiotics as oral prophylaxis, lack of lab services and guidelines/

protocols regarding the selection of antibiotics⁷. Neu said "Bacteria are cleverer than men" as they have capacity to adapt in every environmental niche on the planet and now adjusting to a world laced with antibiotics⁸.

The high proportion of *S. aureus* as evident in this study, might be because of endogenous source of infection or contamination from the environment such as contamination of surgical instruments with the disruption of natural skin barrier as these bacteria are a common bacterium on surfaces, easily finds their way into wounds⁹. The common bacterial pathogens responsible for wound infections are *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and bacteria belonging to family Enterobacteriaceae¹⁰. Since the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) in 1960, there have been reports of increasing rate of infection by MRSA and this superbug has established itself as the common cause of nosocomial as well as community acquired infections¹⁰.

The increased prevalence of drug resistance mainly methicillin resistance among the strains of *S. aureus* has impelled the usage of macrolide–lincosamide–streptogramin B (MLS_B) antibiotics mainly clindamycin for the treatment of the infections caused by *Staph aureus*¹¹. Clindamycin is considered as one of the drugs of choice for treatment of the infection caused by MRSA¹².

Antibacterial agents for empirical use¹:

- Antipseudomonal: Piperacillin – Tazobactam, Cefipime, Ceftazidime, Imipenem-cilastatin
- Gram positive: Vancomycin, Linezolid
- Gram negative: Third generation Cephalosporin, Polymyxin B
- Anti- anaerobic: Metronidazole, Carbapenems
- Broad spectrum: Piperacillin- Tazobactam, Carbapenems, Fluoroquinolones, Tigecycline
- Anti MRSA: Linezolid, Vancomycin, Tigecycline, Minocycline

Enforcing a strict adherence policy in the healthcare sector to reduce the development and spread of drug-resistant bacterial strains goes hand in hand with nationwide antimicrobial surveillance. Routine clinical diagnostic laboratories can contribute to the national surveillance network by sharing routine antibiograms from clinical samples¹³.

Conclusion

Severe antimicrobial resistance in diabetic foot infection was observed among patients treated by department of Surgery in a peripheral hospital in Nasik. There is a need for serious and urgent intervention to stem the spread and further evolution of this antibiotic resistance. A rigorous infection control policy along with rational use of antibiotics will go a long way in fighting against antibiotic resistance. It is recommended that Linezolid, Amikacin, Ciprofloxacin and Netilmycin be used in preference to Ampicillin, Amoxicillin and Ceftriaxone for treatment of diabetic foot infection. Antibiotics prescribed in the management of diabetic foot ulcers should be broad spectrum covering both Gram-negative and Gram-positive pathogens to improve the healing status and should also target multidrug-resistant strains which are a compounding trouble in treatment of diabetic foot infection. Serial wound debridements and daily dressings plays a pivotal role in the management of diabetic foot infection and can't be substituted by the use of antibiotics .

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