

Aerobic bacteria isolated from diabetic septic wounds

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Abstract

Diabetic septic wounds are one of the most dangerous complications of diabetes mellitus that remain major cause of morbidity and mortality in diabetic patients.

The present work included collection, purification, identifications of 187 clinical strains isolated from patients with diabetic septic wounds during August 2008 and March 2009. The samples were collected entirely from Jabir Abo Alezz diabetic center in Khartoum State.

The clinical isolates were purified by streaking on suitable selective and differential culture media. They were identified on the basis of the results of microscopical examinations, Gram reactions, cultural characters and biochemical tests. The clinical strains identified as the following:-

Staphylococcus aureus (46%), Enterococcus faecalis(8%), Coagulase- negative Staphylococci (5.9%). Streptococcus pyogenes (2.1%), Pseudomonas aeruginosa(13.4%), Proteus species (9.1%), E.coli (4.8%), Klebsiella Pneumoniae (3.7%), Providencia species (1.1%), Citrobacter freundii(1.6%) and Serratia species (4.3%).

Key Words: diabetic; aerobic; bacteria; wound; infection

{**Citation:** Eithar Mohammed Mahgoub, Mohammed Elfatih A. Omer. Aerobic bacteria isolated from diabetic septic wounds. American Journal of Research Communication, 2015, 3(10): 91-99} www.usa-journals.com, ISSN: 2325-4076.

1.1. Introduction

Diabetes is a significant risk factor in coronary heart disease, stroke and it's the leading cause of blindness, end-stage renal disease, as well as a major contributor to lower extremity amputation, (Porth, 2002).

Foot ulceration occurs as a result of trauma often trivial, in the presence of neuropathy and peripheral vascular diseases, (Boon *et al*, 1999).

Once the protective layers of skin are breached, underlying tissues are exposed to bacterial colonization, (Lipsky *et al*, 2004).

The progression from colonization of a wound to clinical depends on various factors such as the quantity, type and interaction of pathogens present combined with host factors such as immune response and tissues conditions if ischemic or not (Armstrong and Lipsky, 2004).

These sequences of events can be rapid occurring over days or even hours, especially in an ischemic limb. (Lipsky *et al* 2004).

1.2. Diabetic septic foot

Diabetic foot infection is most simply defined as any infra-malleolar infection in patients with diabetes mellitus. These include paronychia, cellulitis, myositis, abscesses, necrotizing fasciitis, septic arthritis, tendonitis and osteomyelitis, (Lipsky *et al*, 2004).

1.3. Causative agents of diabetic septic wounds

Different types of bacteria have been associated with diabetic septic wounds. They range from single gram positive Cocci in acutely infected wound to multiple organisms (3-5 species in chronic infection). In addition, due to impaired host defense around necrotic soft tissues or bone may allow low virulence colonizer such as Coagulase-negative *Staphylococci* and *Corynebacterium* species "*diphtheroids*" to assume a pathological role,(Lipsky *et al* 2004).

Moreover, Anaerobic species should be suspected if the wound has fetid smell, (Armstrong and Lipsky,2004).

Fungi may be isolated from both infected and uninfected foot wound, but rarely require systemic antifungal therapy, (Lipsky, 2004).

Hospitalization, surgical procedures and especially prolonged or broad-spectrum antibiotic therapy may predispose patients to colonization and or infection with antibiotic resistant organisms (MRSA or Vancomycin resistant *Enterococci*, (Lipsky *et al*, 2004).

2.1. Materials

All the culture media are either from Conda Ltd. Spain or Himedia Ltd. India with expiry date until 1 / 2010.

2.2. Epidemiology survey of diabetic septic wounds

Between October 2008 and March 2009, 150 wound samples were collected from diabetic patients admitted to Jabir Abo Alezz diabetic center in Khartoum State-Sudan. The samples were collected from patients suffered from infected ulcer, wound osteomyelitis or previous amputation.

2.3. Specimens

For elimination surface contaminants, the wounds, have been debrides of all necrotic material and mechanically cleansed, before collecting a culture specimen. (Armstrong and Lipsky, 2004).

Adequate amounts of pus was taken on sterile cotton swab, which was inserted on a container of Amies transport medium. The swab stick was broke to allow the bottle top to close tightly. In moderate to severe wounds, the culture materials were either from curettage of debrided ulcer or tissues biopsy, (Apelquist *et al*, 2000) and (Wheat.*et al*,1986).

The labeled specimen was taken to the microbiology laboratory in Faculty of pharmacy in Omdurman Islamic university as soon as possible, maximally within six hours since delay may cause the death of organisms.

2.4. Cultivation of isolates on different type of media

For isolation and purification of bacteria isolates swabs from the transport media were inoculated on suitable sterile different culture media. The plates were incubated under aerobic condition at 37°C for 24 hours. The media used includes:-

Nutrient agar, Blood agar, Cetrimide agar , MacConkey's agar, Mannitol salt agar and Eosin methylene blue agar.

The purified bacterial isolates were preserved in the refrigerator at 4°C until they were used.

2.5. Identification of isolated bacteria strains

Identification of isolates was done according to cultural characteristics, gram staining properties & results of different biochemical tests, (Cheesbrough,M.(2000).

3. Results & discussion

3.1 Site of diabetic septic wounds infection

In this study as shown in figure No.1, the commonest primary site of infection was the foot (98.7%) followed by other sites of body (1.3%). This agrees with the findings of a study done by (Abdalrhman, 1999).

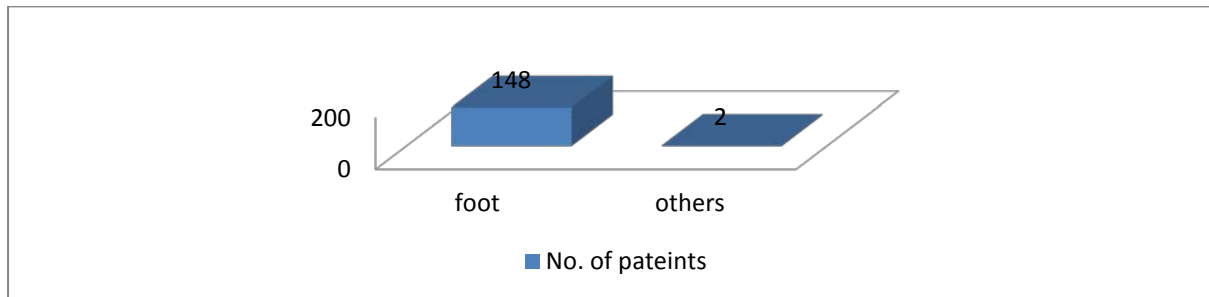


Figure 1: Sites of diabetic septic wounds infection.

This may be explained by the fact that the lower limbs are more affected by peripheral neuropathy and vascular insufficiency that makes the lower limbs more prone to trauma and then infection.

3.2. Types of microbial infection of diabetic septic wounds

From 150 diabetic patients with septic wounds 187 organisms were recovered indicating that there were two types of bacterial infections; monomicrobial infection – which is the major (77.3%) and polymicrobial infections (22.7 %) figure No.2.

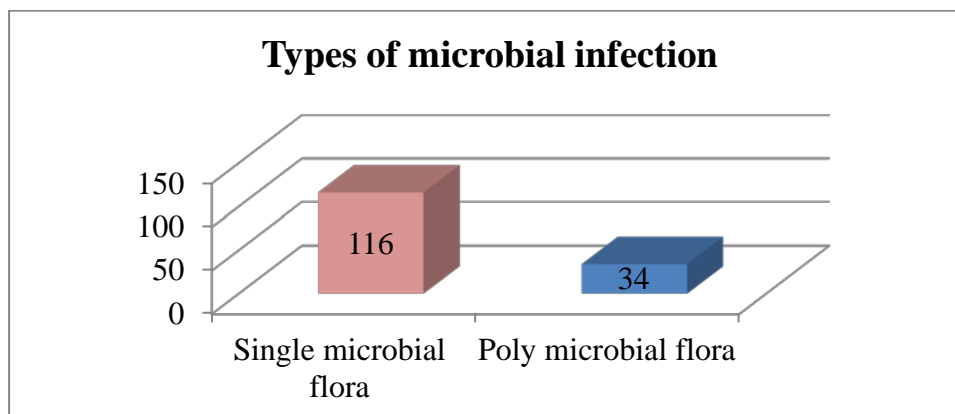


Figure 2: Types of microbial infection of diabetic septic wounds.

The average numbers of isolates is 1,2 per case, similarly (Abdalrahman , 1999) found that the number of isolates per case was 1.39 and single infection was in 63% patients. It is also not far away from the study done in Malaysia, which documented that diabetic septic wounds were monomicrobial rather than polymicrobial, the number of isolates per case was 1.47, (Raja, 2007). A greater number of isolates per case was 1.6 & 3 was reported by (Abdulrazak *et al*, 2005) and (Goldstein *et al*, 1992).

A culture with polymicrobial flora from diabetic foot ulcer does not reveal which microorganism is pathogenic. In fact bacteria are thought to be synergistic and form biofilm at the surface of chronic wound, thus allows anaerobes to survive on the wound surface and support the growth of bacteria not normally considered pathogenic, (Williams *et al* ,2004).

In contrasts to monomicrobial infections poly microbial infection is reported in serious infection that fail to response to previous antibiotic therapy (Armstrong and Lipsky, 2004).

3.3. Type of bacterial isolates in diabetic septic wounds

The type of bacteria involved in multiple infections was shown in figure No.3.

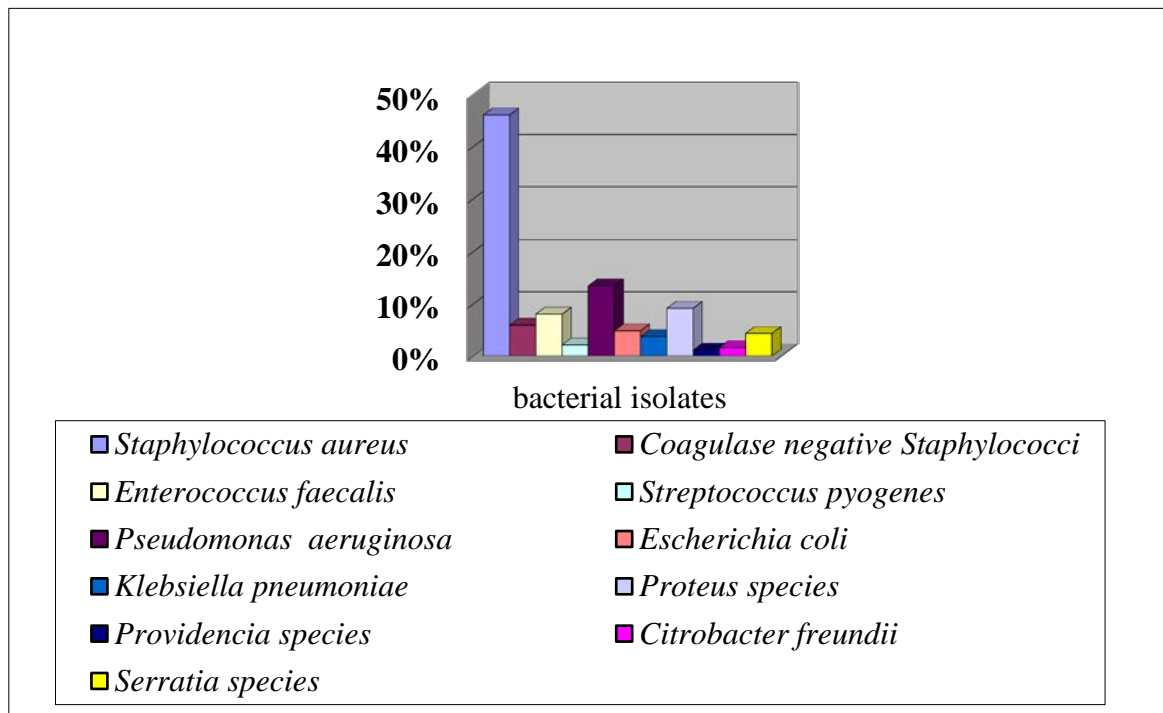


Figure 3: Bacteria isolated from diabetic septic wounds.

The commonest isolated organisms in the present work were *S. aureus* (46%), These results agree with many studies done worldwide particularly in that *S. aureus* is the most frequent organism encountered as a causative agent of diabetic septic wounds.

Similarly Abdalrhman, (1999) identified 139 clinical isolates from patients with diabetic septic wounds. They included *S.aureus* (52%), *Streptococcus pyogenes* (0.71%), *Proteus mirabilis* (25%), *Pseudomonas aeruginosa* (9%), *Klebsiella pneumoniae* (8%) and *E.coli* (4%).

In addition, a more recent study in Sudan; found that the most common isolates from diabetic septic wounds were *S. aureus* that amount for 53.7% of all isolates. The strict aerobic *Pseudomonas aeruginosa* constituted 3.6% of all isolates, other isolated Gram-negative bacilli included, *Proteus mirabilis* 4.5% *Proteus vulgaris* 3.6%, *Klebsiella pneumoniae* 11.8% and *E.coli* 8.2%. *Streptococcus pyogenes* isolates were the least frequent, with prevalence rate of 1.8%, (Adam, 2007).

In a study of 111 patients with diabetic septic foot infections, Gram-positive Cocci were collected from 40% of patients; among them *S. aureus* was the most frequently isolated bacteria (28% of wounds). Gram-negative aerobes were obtained from 47% of the cases, among them *Pseudomonas aeruginosa* accounted for 22% and *Proteus mirabilis* 18%. Gram-negative anaerobes were found in 7% of the patients. Likewise the present work *Enterococcus* was isolated, suggesting a relationship to prior antibiotics (especially cephalosporins) exposure,(El-Tahawy ,2000).

Likewise Armstrong and Lipsky, (2004) noted that most commonly isolates from diabetic septic wounds were *S. aureus*, other frequently isolated aerobes included various *Enterobacteriaceae*, *Streptococci* especially group A and B, *Enterococci*, *Proteus species*, *S.epidemidis* , *Pseudomonas aeruginosa* and *Corynebacterium*. Anaerobic species were found less frequently but have been isolated in 13.5% to 36% in some studies.

In consistent with the present work, Tentolouris *et al*, (1999) found that Gram-positive aerobic bacteria were the commonest isolated bacteria (56.7%) followed by Gram-negative aerobic bacteria (29.8%) and anaerobes (13.5%)., where *S. aureus* was the most frequently isolated microorganism; with some strains were Methicillin resistant *S. aureus* (MRSA). Other Gram-positive aerobes isolated include *coagulase negative Staphylococci* (4.8%), *Streptococci species* (10.6%), *Enterococci species* (5.8%),*Corynebacterium* (29.8%).Among Gram-negative, *Enterobacteriaceae* species were the most frequent pathogen (29.8%), where as *Pseudomonas aeruginosa* accounted for (3.8%).

Similarly a study done in Kuwait, the most frequently isolated Gram-positive Cocci were *S.*

aureus (53%), *Enterococcus* species (17.2%) and group B *Streptococci* (12.5%). *Pseudomonas aeruginosa* and *Proteus mirabilis* were the predominant species among Gram-negative aerobes. In consistent with the present work *Serratia marcescens* was isolated from diabetic septic wounds with prevalence rate of 5.9 %. Moreover, *Candida* species were associated with (7%) of infections, (Abdulrazak *et al*, 2005).

Nonetheless, The present work disagrees with the study done in Malaysia, which arrived at that the most frequently isolated pathogens were Gram-negative bacteria (52%) including *Proteus spp*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *E.coli*. Gram-positive bacteria accounted only for (45%) of all bacteria isolates, (Raja, 2007).

Also, the present work also disagrees with the study done in India which found that among aerobic pathogens *Enterobacteriaceae* accounts for (48%) while *Staphylococcus* species accounts only for (18.2%), *Streptococci* (16.8%) and *Pseudomonas aeruginosa* (17%) of all isolates. (Viswnathan *et al*, 2002)

Methicillin resistant *Staphylococcus aureus* (MRSA) has emerged as serious and common problem with diabetic foot ulcers. Infections or colonization with MRSA may result in prolonged hospital stay and excessive economic cost. In the present work 79% of *S. aureus* isolates were MRSA, with 36.3% prevalence rate among pathogenic microorganisms associated with diabetic septic wounds, which is highly alarming. Similarly 38.2% and 30 of isolated *S.aureus* was MRSA, (Adam, S.O.(2007) & El-Tahawy, A.T,(2002).

In a study done in U.K the prevalence of MRSA in diabetic foot clinics was 15% and associated with prolonged healing time for diabetic ulcer and with antibiotic use, (Tentolouris *et al*, 1999). Investigators in a clinic in Manchester, England reported that the prevalence of MRSA infection among patients with diabetic foot infection was 30.2% and this indicated that the rate of infection with MRSA had doubled over the period of 3 years (Dang *et al*, 2002).

Data suggested that the infection with antimicrobial resistant pathogens might play a role in lower extremity amputation and other complications of lower extremity ulcer.

4. Conclusion

The primary site of infection was foot which was 98.7%.

The major type of diabetic septic wounds infection was monomicrobial which approached 77.3%.

Different types of aerobic bacteria were isolated from diabetic septic wounds. Including Gram positive Cocci and gram negative rods.

Staph.aureus was not only the prominent isolate, but also 79 % of it was Methicillin resistant *Staphylococcus aureus* (MRSA) which play a role in lower extremity amputation and other complications of lower extremity ulcer.

Acknowledgements

We are heartily thankful to Professor Mohammed Elfatih Ahmed Omer, for his encouragement, guidance and support.

My thanks are conveyed to the director and staff of JABiR Abo alezz diabetic center, from where all the samples were collected, for their great assistance during collecting samples Also I am very grateful to staff of Omdurman Islamic university for their co-operation.

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