Prevalence of bacterial types in Wagner grade three of diabetic foot ulcer

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Abstract:

Diabetic foot ulcer (DFU) is one of the chronic wound infection and leads to non-traumatic lower limb amputation. Advances in diabetes research are significant and much needed because diabetes is on the rise worldwide and is considered by some experts already to be at an epidemic level. Among diabetic patients 70% were males and 30% were females. Aerobic bacteria 104(82%) were the most frequently isolated than anaerobic bacteria 23(18%). Among aerobes Gram negative bacteria 67(64.4%) were more commonly isolated than Gram positive 37(35.5%). The three most frequently found that *S. aureus* 28(22%) followed by *P. mirabilis* 22(17.3%) then *E. coli* 14(11%). Among anaerobic bacteria Gram negative bacteria 16(69.5%) were predominantly isolated than Gram positive bacteria 7(30.4%). The most probably isolated *Veilonella* spp. 10(7.9%) followed by *Peptostreptococcus anaerobius* 6(4.7%).

Keywords: bacterial types, foot ulcer.

انتشار انواع البكتريا في قروح الدرجه الثالثه لاقدام مرضى السكري

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الخلاصه:

قرحه قدم السكري هي واحده من الاصابات المزمنه وتؤدي الى بتر العضو المصاب التقدم في بحوث السكري مهمه وضروريه لان مرض السكري اصبح منتشر عالميا وبعض الخبراء يعتبرونه وبائي . من بين مرضى السكري عدد الذكور (70%) اكثر من عدد الاناث (30%). البكتريا الهوائيه 104(82%) هي الاكثر شيوعا من البكتريا اللاهوائيه 23(18%). من بين البكتريا الهوائيه البكتريا السالبه67 (64.4%) هي الاكثر شيوعا من البكتريا الموجبه 37(35.5%). البكتريا السائده ثلاثه وهي *S. aureus* 28(22%) ، ويليها *P. mirabilis* 22(17.3%) ثم *E. coli* 14(11%). من بين البكتريا اللاهوائيه البكتريا السالبه هي الاكثر سياده 16(69.5%) من البكتريا الموجبه 7(30.4%). اكثر البكتريا المعزوله هي *Veilonella* spp. 10(7.9%) وتليها *Peptostreptococcus anaerobius* 6(4.7%).

Introduction:

Diabetes is one of the most common chronic communicable diseases .It is a disease of complications popularly known as Iceberg disease [1]. It is rapidly emerging as a global health problem that threatens to reach pandemic levels by 2030; the number of people with diabetes worldwide is projected to increase from 171 million in 2000 to 366 million by 2030 [2, 3]. Deeper

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limb threatening infections are usually poly microbial and caused by aerobic Gram positive cocci including *Staphylococcus aureus*, coagulase negative *Staphylococci* and *Streptococcus* species. Gram negative pathogens include *Escherichia coli, Klebsiella* species, *Proteus* species and other species of *Enterobacteriaceae. Peptostreptococcus* species, *Bacteroides* species are the most commonly isolated anaerobes [4]. Infection in stage 3-4 ulcers is polymicrobial and may involve exposed deeper structures [5].

Materials and methods:

Specimens' collection

Between November 2012 and May 2013, fifty clinical specimens were collected from diabetic patients with foot ulcer from Diabetes and Endocrinology center and Diabetes clinic. Two samples were taken from each patient and were subjected for aerobic and anaerobic culture. The first sample (swab) was immediately inoculated into brain heart infusion broth. The second sample (biopsy) was immediately inoculated into thyoglycolate broth, taking aseptic precautions by using parafilm that overlaid to prevent contamination and labeled.

Isolation and identification of the bacteria

The collected specimens were streaked directly on MacConky agar, blood agar for aerobic bacteria and streaked on Brucella blood agar for anaerobic bacteria [6]. The inoculated plates are immediately placed onto an aerobic and anaerobic environment jars and are incubated at 37C^{o} for 24 h and at 37 C^{o} for 48 h, respectively. Different shape of colonies were selected and subcultured on another MacConky agar, blood agar and Brucella blood agar to obtain isolated colonies. The direct smear is dried, fixed, and Gram stained.

The identification of the isolate included morphological characteristics and biochemical tests such as catalase test, oxidase test, coagulase test for gram positive bacteria and IMVC test, TSI test, urease test, motility test, API test, Vitek 2 Compact device for gram negative bacteria and sodium poly anethol sulphonate disc test, bile test, special potency discs test, nitrate reduction test for anaerobic bacteria [6-9].

Detection of methicillin resistant *Staphylococcus aureus* (MRSA)

All the isolates were subjected to cefoxitin D.D. test using a 30 µg disc. A 0.5 McFarland standard suspension of the isolate was made and lawn culture was done on Muller-Hinton agar plate. Plates were incubated at 37ºC for 18 hr. and zone diameters were measured. An inhibition zone diameter of ≤ 19 mm was reported as Methicillin-resistant and ≥20 mm was considered as methicillin- sensitive [10].

Results and discussion

From fifty specimens, 70% were males and 30% were females. The male-female ratio was 2.5:1. The current work depicted that patient's age ranged from (40-75) years with an average of 58 years. Also, the maximum number of patients having diabetic foot infections belonged to the age group of 50-68 years. Of the fifty cases studied, most of patients belonged to fourth and fifth decades of life as shown in figure (1).The reason of that might be due to a genetic factor influence the infection.

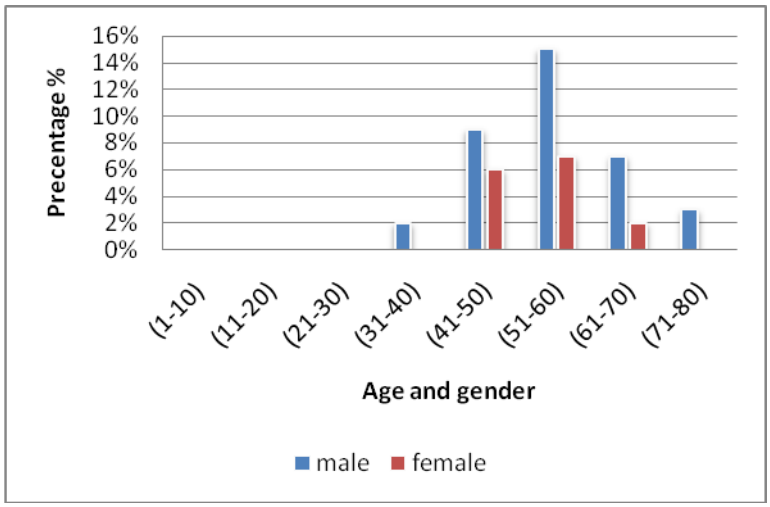

Figure 1- Age and gender distribution of cases under study.

From 127 isolates, aerobic bacteria (82%) were the most frequently isolated than anaerobic bacteria (18%). Among the aerobes, 35.5% were Gram positive cocci and 64.4% were Gram negative bacilli and the ratio of Gram negative to Gram positive organisms isolated from diabetic foot ulcers was 2:1. Among Gram positive, *S. aureus* is the most frequently isolate (22%) and found in phenotypic resistance as methicillin resistant *Staphylococcus aureus* (MRSA) and represent 6% of all isolates. Coagulase-negative *S. epidermidis* found in low percentage (4.7%); *Streptococcus* spp., which are well-recognized pathogens in DFI, were infrequently isolated in 2% patient. *S. aureus* is the common isolate then followed by *Proteus mirabilis.* This study showed that wound infections are polymicrobial and in most cases associated with *S. aureus, Proteus* spp. and *E. coli* [11]. *Proteus* had the highest frequency of occurrence among the Gram negative bacteria isolated. At species level *P. mirabilis* (17.3%) was the commonest followed by *P. vulgaris* (7.9%). *E. coli* also found in high frequency after *Proteus* species. *Pseudomonas aeroginosa* is not as frequently isolated in these infections, despite its ubiquity. The preponderance of Gram negative bacteria due to wounds exposed to fecal sources for instance, may be contaminated mainly by members of the *Enterobacteriaceae* [11]. Among anaerobes the ratio of Gram negative to Gram positive organisms was 2.3:1. Among the anaerobes 30.4% were Gram positive cocci, and 69.5% were Gram negative. The predominant anaerobe was *Veilonella* spp. 8.2% followed by *Peptostreptococcus anaerobius* 4.9%. Most Gram negative anaerobes are *Veilonella* spp., *Bacteroides fragilis* and *Fusobacterium* spp. It should be noted that in this study we did not isolate anaerobic bacteria as the only organism in any of the cultures. This was also the case in previously published papers. It is presumed that these bacteria act synergistically with other more virulent bacteria that cause progression in soft tissue infections [12]. In 8% of patient's only one pathogen was isolated, while 40% were infected with two pathogens and 52% were infected with more than two pathogens. It is very important to mention that these discrepancies could be partly due to differences in the causative organisms occurring over time, geographical variations, or the types and severity of infection included in the studies [13- 15]. In other ward, these different observations due not only to geographic variation but also sources of clinical specimens, genetic background and the collection site of isolates. Table-1 shown the percentage of aerobic and anaerobic bacteria.

Table 1- Percentage of aerobic and anaerobic bacteria.

Bacteria	No. of isolates(Percentage)
Aerobic gram positive bacteria	
Staphylococcus aureus	28(22%)
Staphylococcus epidermidis	6(4.7%)
α- hemolytic *streptococci*	1(0.8%)
β- hemolytic *streptococci*	2(1.6%)
Aerobic gram negative bacteria	
Escherichia coli	14(11%)
Enterobacter cloacae	1(0.8%)
Enterobacter spp.	2(1.6%)
Citrobacter fruendii	5(3.9%)
Citrobacter spp.	2(1.6%)
Klebseilla pneumonia	7(5.5%)
Proteus mirabilis	22(17.3%)
Proteus vulgaris	10(7.9%)
Pseudomonas aeroginosa	1(0.8%)
Salmonella enterica diarizonae	1(0.8%)
Acinetobacter baumanni	1(0.8%)
Achromobacter dinirificans	1(0.8%)
Anaerobic bacteria	
Anaerobic Gram negative bacteria	
Bacteroides fragilis	5(3.9%)
Fusobacterium spp.	1(0.8%)
Veilonella spp.	10(7.9%)
Anaerobic Gram positive bacteria	
Peptostreptococcus anaerobius	6(4.7%)
Peptostreptococcus assacharolyticus	1(0.8%)

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