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PREVALENCE AND ANTIBIOTIC RESISTANCE OF PATHOGENIC BACTERIA ISOLATED FROM DIABETIC FOOT INFECTIONS

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Abstract

The increasing incidence of antibiotic resistant pathogen as cause of diabetic foot infection makes selecting empiric antibiotic therapy more difficult. Those who treat these patients are well aware of the growing problem of methicillin-resistant *Staphylococcus aueus* (MRSA). The aim of this work was to study the relative frequency of bacteria isolated from diabetic foot infection and assess their comparative susceptibility to the commonly used antimicrobial agents. A total number of 50 cases of diabetic foot infection patients attending at Khartoum state hospitals were investigated to isolate bacterial pathogens responsible for diabetic foot infection and to test their antimicrobial susceptibility patterns. Samples were collected by swabbing from all studied patients and examined by Gram stain, cultured on blood agar, mannitol salt agar, macConkey agar. Biochemical and antimicrobial susceptibility tests were done . The species of bacteria isolated were: *Staphylococcus aureus* 32% , *Pseudomonas aeruginosa* 14%, *Klebsiella pneumoniae* 12%, *Escherichia coli* 10%, *Proteus vulgaris* 10%, *Proteus mirabilis* 8%, *Staphylococcus epidermidis* 8%, *Citrobacter freundii* 4%, *Enterobacter species* 2%. The antimicrobial susceptibility testing revealed that amikacin was the most effective drug against both gram positive and gram negative bacteria followed by gentamycin. 62.5% of the *S. aureus* isolates were resistant to methicillin. All *S. epidermidis* isolates were resistant to methicillin, erythromycin, ceftriaxone, gentamicin, and trimethoprim. All gram negative isolates were resistant to ampicillin

Keywords: Diabetic foot infections**,** pathogenic bacteria**,** Prevalence and antibiotic resistance

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Introduction

Diabetes mellitus (DM) is a serious and complex disease affecting almost all the vital organs in the body. About 347 million people in the world are diagnosed with DM. The incidence of DM is on the rise and it has been predicted that it will increase by a double by the year 2030. DM is known to have many complications and one of the most distressing is Diabetic Foot Ulcer (DFU) which affects 15% of people with diabetes (Singh et al., 2013).

Diabetic foot ulcers (DFUs) are complex, chronic wounds, which have a major long-term impact on the morbidity, mortality and quality of patients' lives (Abetz et al., 2002). Individuals who develop a DFU are at greater risk of premature death, myocardial infarction and fatal stroke than those without a history of DFU (Brownrigg et al., 2012). Unlike other chronic wounds, the development and progression of a DFU is often complicated by wide-ranging diabetic changes, such as neuropathy and vascular disease. These, along with the altered neutrophil function, diminished tissue perfusion and defective protein synthesis that frequently accompany diabetes, present practitioners with specific and unique management challenges (NIHCE, 2011). Foot complications are common among diabetic patients; foot ulcers are among the more serious consequences. These ulcers frequently become infected, with potentially disastrous progression to deeper spaces and tissues. If not treated promptly and appropriately, diabetic foot infections can become incurable or even lead to septic gangrene, which may require foot amputation. Diagnosing infection in a diabetic foot ulcer is based on clinical signs and symptoms of inflammation. Properly culturing an infected lesion can disclose the pathogens and provide their antibiotic susceptibilities. Specimens for culture should be obtained after wound debridement to avoid contamination and optimise identification of pathogens (Armstrong and Lipsky, 2004). The common pathogens in diabetic septic foot infection are: *Staphylococcus aureus* (methicilin sensitive and methicilin resistant), coagulase negative *Staphylococci, Enterococcus species (Streptococcus faecalis* and group D *Streptococci), Corynebacterium species (Diphthroid), Protues species, Escherichia coli, Klepbsiella species* and *Pseudomonas aeruginosa* (Cheesbrough, 2000). Patient who had received prolonged, inappropriate broad-spectrum antibiotic or had length hospitalization; chronic wound procedure were most likely to have infection and/or colonization with methicillin-resistant *Staphylococcus aureus* (MRSA) (Hartemann-Heurtier et al., 2004). The increasing incidence of antibiotic resistant pathogen as cause of diabetic foot infection makes selecting empiric antibiotic therapy more difficult. Those who treat these patient are well aware of the growing problem of methicillin-resistant *S. aueus* (MRSA) (Dang et al., 2003 ; Eady and cove, 2003).
The selection of empiric antibiotic therapy depends on various factors such as: severity, previous antibiotic treatment, antibiotic activity…etc. Proper identification of causative agent, appropriate antibiotic and management of complication of diabetes foot infection remain essential to the achievement of a successful

outcome (Abdulrazak et al., 2005). Optimal management of diabetic foot infections can reduce morbidity, hospitalization and amputation rates but these infections are frequently not managed appropriately; to help with optimal management, a multidisciplinary team approach is helpful (Pecoraro et al., 1990). The team managing these infections should include, or have ready access to, an infectious diseases specialist or a medical microbiologist (Lipsky et al., 2006).

The microbiological characteristics of diabetic foot infections have not been extensively studied in Sudan. This study investigated the microbiology of diabetic foot infections and their resistance to antibiotics in patients with diabetic foot infections in Khartoum State.

Materials and Methods:

Microbiological evaluation:

A total of 50 wound swabs were collected from diabetic foot ulcer patients from 3 tertiary care hospitals in Khartoum state. All samples were cultured in blood agar, MacConkey's agar and mannitol salt agar incubated at 37˚C for 24 hours, Gram stained and subjected to further biochemical tests according to procedures mentioned by Cowan & Steels (Barrow and Feltham, 2003).

Antibiotic sensitivity testing:

This was performed using the standard disk diffusion method (Kirby Bauer method) in which the organisms under investigation were cultured in Muller Hinton sensitivity testing agar, then 5-7 different antibiotic disks were placed on the media about two centimeters apart. After overnight incubation at 37˚C aerobically the culture was examined for zone of inhibition of bacterial growth around the respective disks which was measured in millimeters. All species isolated were tested for antibiotic sensitivity against commonly used antibiotics: Ampicillin, Methicillin, erythromycin, gentamicin, amikacin, Trimethoprim and ceftriaxone.

1. *Results:*
2. *Bacteriological findings:*
3. ***The total number of bacteria isolated was 50. The organisms isolated were:*** **Staphylococcus aureus *(32%),* Staphylococcus epidermidis *(8%),* Pseudomonas aeruginosa *(14%),* Klebsiella pneumoniae *(12%),* Escherichia coli *(10%),* Proteus vulgaris *(10%),* Proteus mirabilis *(8%),* Citrobacter freundii *(4%),* Enterobacter species *(2%). (Table1).***
4. *Antibiotic sensitivity testing :*

Results showed that 75% *of Staphylococcus* aureus isolates were sensitive to amikacin and 62.5 were sensitive to Gentamycin, 62.5% of *Staphylococcus aureus* isolates were resistant to methicillin, **t**rimethoprim, and **c**eftriaxone. All *Staphylococcus epidermidis* isolates (100%) were resistant to methicillin, erythromycin, ceftriaxone, gentamicin, and trimethoprim (table 2). All gram negative isolates were sensitive to amikacin and resistant to ampicillin (table 3).

Table (1): Species of bacteria isolated (number and percentage).

Bacterial species	No.	Percentage (%)
Staphylococcus aureus	16	32%
Staphylococcus epidermidis	4	8%
Escherichia coli	5	10%
Klebsiella pneumonia	6	12%
Pseudomonas aeruginosa	7	14%
Proteus vulgaris	5	10%
Proteus mirabilis	4	8%
Citrobacter freundii	2	4%
Enterobacter species	1	2%
Total number	50	100%

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Discussion:

Foot ulcers are a frequent complication of patients suffering with diabetes mellitus, accounting for up to 20% of diabetes-related hospital admission. Secondary infection of these ulcers is by far the leading cause of amputation of feet and legs and the polymicrobial nature of diabetic foot infection has been well documented in the literature (Sharma et al., 2006).

In this study the total number of bacteria isolated from three major hospitals were 50 isolates, they were: *Staphylococcus aureus* (32%), *Staphylococcus epidermidis* (8%), *Pseudomonas aeruginosa* (14%), *Klebsiella pneumoniae* (12%), *Escherichia coli* (10%), *Proteus vulgaris* (10%), *Proteus mirabilis* (8%), *Citrobacter freundii* (4%), *Enterobacter species* (2%). This in agreement with other workers EL nazeer, (2003), who found that *S. aureus* was the most common isolate being 48.5% , *Pseudomonas aeruginosa* was 16%, and *Klebsiella* 13.8%. Martínez-Gómez et al. (2009), in their study revealed that the most frequently isolated germ group was gram-positive bacteria with *Staphylococcus aureus* (33%), followed by *Pseudomonas aeruginosa* (12%), *Enterococcus spp*. (9%), and *Escherichia coli* (8%). Also Sharma et al. (2006), in their study found that the most frequent bacterial isolate were *Staphylococcus aureus* (38.4%), *Pseudomonas aeruginosa* (17.5%), and *Proteus* (14%). Aerobic Gram-positive cocci (especially *Staphylococcus aureus*) are the predominant pathogens in diabetic foot infections. Patients who have chronic wounds or who have recently received antibiotic therapy may also be infected with Gram-negative rods, and those with foot ischemia or gangrene may have obligate anaerobic pathogens (Lipsky et al., 2006).

The problem of microbial drug resistance is a major public health concern, due to its global dimension and alarming magnitude, although the epidemiology of resistance can exhibit remarkable geographical variability and rapid temporal evolution (Rossolini and Mantengoli, 2008). In this study results revealed that amikacin was the most effective drug against both gram positive and gram negative bacteria followed by gentamycin. 62.5% of the *Staphylococcus aureus* isolates were resistant to methicillin. All *Staphylococcus epidermidis* isolates were resistant to methicillin, erythromycin, ceftriaxone, gentamicin, and trimethoprim

Table (2): results of antibiotic sensitivity testing of gram positive isolated bacteria:

Species /Antibiotic	M		AK		G		CRO		E		AMP		TR	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R
	%		%		%		%		%		%		%	
S. aureus	37.5	62.5	75	25	62.5	37.5	37.5	62.5	43.8	56.2	56.2	43.8	37.5	62.5
S. epidermidis	0	100	25	75	0	100	0	100	100		50	50	0	100

AK= Amikacin, **Tr**= Trimethoprim, **CRO**= Ceftriaxone, **G**= Gentamicin, **AMP**=Ampicillin., **M**= Methicillin, **E**= Erythromycin- S= sensitive, R= resistant

Table (3): Results of antibiotic sensitivity testing of gram negative isolated bacteria:

Antibiotic	Sensitivity test	*Proteus*	*Pseud. aeruginosa*	*K. pneum*	*E.coli*	*Citrobacter freundii*	*Enterobacter sp*
AK	S	100 %	100 %	66.6 %	80 %	100 %	100%
	R	0%	0%	33.4 %	20%	0%	0%
TR	S	22.3 %	14.3%	16.7 %	40 %	0 %	100 %
	R	77.7 %	85.7 %	83.3 %	60 %	100 %	0%
CRO	S	33.4 %	0%	16.7%	20%	0%	05
	R	66.6 %	100%	83.3%	80%	100%	100%
G	S	44.4 %	85.7%	83.3%	40%	50%	0%
	R	55.6%	14.3%	16.7%	60%	50%	100%
AMP	S	11.2%	0%	0%	0%	0%	0%
	R	88.8%	100%	100%	100%	100%	100%

AK= Amikacin, **Tr**= Trimethoprim, **CRO**= Ceftriaxone, **G**= Gentamicin, **AMP**=Ampicillin

The prevalence of MRSA and Methicillin resistant coagulase negative staphylococcus isolates was higher in our population as compared with previous studies. Banashankari et al. (2012), in their study found that MRSA was isolated in 47% of S.aureus and Methicillin resistant coagulase negative staphylococcus was 15%. The genus *Staphylococcus* includes pathogenic organisms in which *S. aureus* is the most important one that has become resistant to most of the therapeutic agents that have been developed in the recent years, and hence the antimicrobial chemotherapy for this species has always been empirical (Jun et al, 2004). MRSA infection is common in diabetes patients with foot ulcers, and is associated with previous antibiotic treatment and prolonged healing. Overuse of antibiotics and the selection of broad, rather than narrow spectrum agents, have contributed to the high

prevalence of methicillin- resistant *S. aureus* (MRSA) colonization in diabetic foot wounds. Many of these MRSA isolates are becoming multidrug resistant, and are susceptible only to glycopeptide antibiotics such as vancomycin. In this study we found that all gram negative isolates were sensitive to amikacin and resistant to ampicillin. Our results are in agreement with other workers. Shen Q et al. **(**2014), found that Enterobacteria were highly resistant to ampicillin, also Raja NS, (2007) and Al Benwan et al. (2012), in their studies reported that amikacin was the most effective treatment for the Gram-negative bacteria. The most alarming resistance trends are those observed for enterobacteriaceae and the Gram-negative non-fermenters, with a generalized increase in rates of resistance to the most important anti-Gram-negative agents (beta-lactams and fluoroquinolones) and the circulation of strains showing multidrug resistance phenotypes.[17] (Rossolini and Mantengoli, 2008).
Our study confirms that MDRO infection is common in hospitalized patients with diabetic foot ulcers. The prevalence of MRSA isolates was higher in our population as compared with previous studies.

Conclusion:
This study indicates that The common organism causing diabetic foot infection in Khartoum state hospitals were: *S.aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *E.coli*, *Proteus sp*. Amikacin was the most effective antibiotic against these isolates. The determination of prevalence and antibiotic sensitivity pattern of pathogenic bacteria screened from diabetic foot ulcer patients will help the clinician for first line treatment in tertiary care hospitals and this will shorten hospital stays and reduce costs.

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