Identification and antimicrobial susceptibility of bacterial species obtained from diabetic foot lesion

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Abstract:

Introduction:

Diabetic foot is a major problem lead to a serious complications. Immune impairment in this group of patients predispose to bacterial infections. Different bacterial species were identified from samples taken from diabetic foot including gram positive and gram negative species. Antimicrobial considered important part of treatment regime and selection of effective antimicrobial will lead to better recovery results. This study was aimed to identify bacterial species infected diabetic foot and their antimicrobial patterns.

Sterile cotton swab applied on the lesions and transferred to the laboratory immediately. Standard microbiology techniques were used to identify bacterial species whereas different media were used; MacConkey agar, Chocolate agar and blood agar. Multiple biochemical API 20 E test was used to identify gram negative bacteria. Catalase and coagulase test also used for gram positive collection identification. Disc diffusion method used to evaluate the antimicrobial susceptibility of collected identified isolates.

Polymicrobial infections were 47.5% (19/40). Dominant combination of identified bacterial species was gram positive and gram negative group 15 (78.9). The highest prevalent bacterial species among gram positive isolates was *S. aureus* 31.7% (20/63) followed by coagulase negative staphylococci (CNS)15.9% (10/63) while *Pseudomonas aeruginosa* 17.5% (11/63) was the most prevalent among gram negative bacterial species. Methicillin resistant isolates was detected in 55% (n=11/20) of identified S. aureus collection. Antimicrobial resistance, Amikacin

showed the highest effect against gram negative bacteria and ciprofloxacin against gram positive bacteria.

High prevalence of polymicrobial infection in diabetic foot increase the concern of serious complications. *S. aureus* and CNS infection occurrence was high and their resistance to methicillin was a major concern. Most combination of polymicrobial infection consisted from gram positive and negative bacterial species which require antimicrobials effective against both groups.

Introduction

In diabetic patient any wound bacterial contamination may lead to serious complications, including extremity amputation (Boulton, Vileikyte, Ragnarson-Tennvall, & Apelqvist, 2005). Epidemiological data recorded 40-70% of lower extremities amputation related to diabetes (http://www.idf.org/position-statement-diabetic-foot). Many factors; neuropathy, vascular insufficiency and neutrophil function inhibition contribute to impair diabetic patient immune response against wound bacterial contamination (B. A. Lipsky, 2004). Impaired immunity against bacterial wound invasion lead to spread of infection deeper into subcutaneous tissue (Delamaire et al., 1997).

Previous studies showed different bacterial species were identified from samples obtained from diabetic foot wounds (Abdulrazak, Bitar, Al-Shamali, & Mobasher, 2005; Ramakant et al., 2011). Most common pathogenic bacteria were identified; aerobic gram positive bacteria; *Staphylococcus aureus* (*S. aureus*) and Streptococci (group A and B) and gram negative bacteria; *Escherichia coli*, *Proteus* species and *Klebsiella* species. Anaerobic bacteria including Bacteroides species, Clostridia species and Peptococcus species were moreover identified (Abdulrazak et al., 2005; Gadepalli et al., 2006). Methicillin-resistance *S. aureus* (MRSA) commonly isolated from hospitalized patient or who have treated with antibiotics. Due to more prevalence of MRSA in the community, isolation of MRSA from patient rather than population at risk was increased (King et al., 2006; Tentolouris et al., 2006). Polymicrobial infection was 40% in a study conducted by Khairul and others whereas 52% were showed monomicrobial infection and 6% had no growth on cultural media. The most commonly isolated bacterial species were; *S. aureus* (10.2%),

Pseudomonas aeruginosa (*P. aeruginosa*) (19.4%) and Streptococcus species (19.3%) (ABDUL KADIR, Satyavani, & Pande, 2012).

Antibiotic therapy is crucial to control bacterial infection in diabetic foot wounds. Empirical antibiotic treatment need to be started and evaluated after 72 hours to reconsider the antibiotic regimen protocol. Initiative antibiotic treatment should be effective against MRSA and streptococci (King et al., 2006; Benjamin A Lipsky, 2004). Antibiotic susceptibility and microbial profile of bacterial species isolated from diabetic foot wounds are variable between different geographical areas and times in the same location (Ramakant et al., 2011).

Studies on bacterial infection of diabetic foot wound in Libya is very rare. This study was aimed to identify bacterial species associated with infection of diabetic foot and to evaluate their antimicrobial susceptibility.

Materials and Methods

Study setting and patients' demography

This is a prospective study conducted in Alshefa, Misurata hospital, whereas 40 patients were included from January to May 2016. Cotton swabs were applied on diabetic foot lesions with a pressure and rotation and transferred in ice box immediately to the laboratory as described by Slater and his colleagues (Slater et al., 2004). Each patient was included once in this study, and one cotton swab was taken from each involved patient.

Patient involved in the study (28 male and 12 female; age average 57 years) followed at special diabetic and endocrinology department as an outpatient or non-hospitalized.

Bacterial identification

Tips of Collected cotton swabs were broken into nutrient broth and incubated at 37°C for overnight. Loopful of overnight incubated broth inoculated on MacConkey agar, Chocolate agar and blood agar and incubated at 37°C for overnight. Colony growth on different media investigated morphologically and gram stain was performed and grouped into gram positive and negative groups.

Gram negative bacteria were further identified using oxidase test and API 20E system. Gram positive bacteria were identified by bacterial cell shape and arrangement followed by catalase and coagulase production test. When gram positive bacteria showed catalase and coagulase positive and black colonies on pair parker media identified as *S. aureus*. Group of staphylococci represented coagulase negative results identified as coagulase negative staphylococci (CNS). Gram negative cocci arranged in chain and negative for catalase were identified as Streptococci.

Antimicrobial susceptibility

Evaluation of commonly used antimicrobial effect on collected bacterial species was performed by Kirby-Bauer's Disc diffusion method. Mueller-Hinton agar and Oxoid antibiotic discs were used and results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) recommendations (CLSI, 2006). Collected bacterial isolates were tested for antimicrobial susceptibility of the following types: sulphamethoxazole trimethoprim 25μg (SXT25), erythromycin 30μg (E30) (tested only against gram positive bacteria), Amikacin 30μg (AK30) (tested only against gram negative bacteria), ceftriaxone 30μg (CRO30), gentamicin (CN30) and ciprofloxacin 10μg (CIP10). Methicillin Resistance in staphylococci (*S. aureus* and CNS) was determined by using cefoxitin 30μg (FOX30) antibiotic disc.

Results

Of 40 patient included in the study, 63 bacterial isolates were obtained. Out of 40 patient, 47.5% (n=19) were Polymicrobial infections, whereas 52.5% (n= 21) were monomicrobial infections. In the monomicrobial group 66.6% (n=14/21) were gram positive (*S. aureus*=10 and CNS=4). Grouping of bacteria isolated from polymicrobial infection showed in table 1.

Table 1: Grouping of bacterial combination isolated from patients with polymicrobial infection

Bacterial group	Frequency N (%)
Gram positive and gram positive	0
Gram positive and gram negative	15 (78.9%)
Gram negative and gram negative	4 (21.1%)
Total	19

Gram positive isolates were 49.2% (n=31/63), whereas gram negative isolates were 50.8% (n=32/63). Gram positive and gram negative bacterial species frequencies identified in this study presented in table 2. In this study, the most common identified bacterial species in the collection (63 isolates) were *S. aureus* 31.7% (20/63), *Pseudomonas aeruginosa* (*P. aeruginosa*) 17.5% (11/63) and CNS 15.9% (10/63).

Table 2: Source and frequency of bacterial species identified from collected diabetic foot samples.

Gram stain group	Bacterial species	Source of isolate(s)		Frequency Total N=63 (%)
		Polymicrobial	Monomicrobial	
Gram positive N=31	*S. aureus*	10	10	20 (31.7)
	CNS	5	5	10 (15.9)
	Streptococci	0	1	1 (1.6)
Gram negative N=32	*Pseudomonas aeruginosa*	9	2	11 (17.5)
	Klebsiella pneumonia	2	0	2 (3.1)
	Klebsiella oxytoca	3	0	3 (4.8)
	Providencia stuartii	3	0	3 (4.8)
	Proteus mirabilis	1	2	3 (4.8)
	Serratia marcescens	2	1	3 (4.8)
	Enterobacter cloacae	2	0	2 (3.1)
	Proteus vulgaris, *Proteus penneri, Serratia odorifera, Enterobacter sakazaki, and Morganella morganii*.	One isolate of each bacterial species N= 5	0	One isolate of each bacterial species N= 5 (7.9)
Total		42	21	63

Prevalence of MRSA among *S. aureus* isolates collection was 55% (11/20)m while Methicillin resistance in CNS group was 80% (8/10). Gram positive bacteria showed high resistance to sulphamethoxazole trimethoprim, erythromycin and ceftriaxone, while they were more susceptible to gentamicin and ciprofloxacin (table3).

Table3: Antimicrobial resistance profile of gram positive bacteria

Bacterial species / Antimicrobial	*S. aureus* N=20		CNS N= 10 N (%)	Streptococcal species N=1 N (%)	Total N=31 N (%)
	MRSA N= 11 N (%)	MSSA N=9 N (%)			
sulphamethoxazole trimethoprim	4 (36)	5 (55.5)	4 (40)	1 (100)	14 (45.2)
Erythromycin	6 (54.5)	5 (55.5)	7 (70)	0	18 (58)
Ceftriaxone	3 (27.3)	5 (55.5)	1 (10)	0	9 (29)
Gentamicin	2 (18.2)	2 (22.2)	2 (20)	0	6 (19.3)
Ciprofloxacin	1 (9)	0 (0)	0 (0)	0	1 (4.8)

In gram negative bacterial species antimicrobial sensitivity patterns was showed high resistance to sulphamethoxazole, whereas more susceptible to other antimicrobial types used in this study (table4).

Table 4: **Antimicrobial resistance profile of gram negative bacteria**

Antimicrobial / Bacterial species	sulphamethoxazole trimethoprim N (%)	Amikacin N (%)	Ceftriaxone N (%)	Gentamicin N (%)	Ciprofloxacin N (%)
Pseudomonas aeruginosa **N=11**	8 (72.7)	3 (27.3)	4 (36,3)	3 (27.3)	2 (18)
Klebsiella pneumonia **N=2**	1 (50)	1 (50)	0 (0)	0 (0)	0 (0)
Klebsiella oxytoca **N= 3**	3 (100)	1 (33.3)	1 (33.3)	1 (33.3)	2 (66.6)
Providencia stuartii **N= 3**	2 (66.6)	1 (33.3)	2 (66.6)	2 (66.6)	1 (33.)
Proteus mirabilis **N= 3**	2 (66.6)	0 (0)	0 (0)	1 (33.3)	2 (66.6)
Serratia marcescens **N= 3**	1 (33.3)	0 (0)	0 (0)	0 (0)	0 (0)
Enterobacter cloacae **N=2**	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Enterobacter sakazakii **N=1**	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Morganella morganii **N= 1**	1 (100)	0 (0)	0 (0)	0 (0)	1 (100)
Serratia odorifera **N= 1**	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)
Proteus penneri **N= 1**	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)
Proteus vulgaris **N= 1**	1 (100)	0 (0)	0 (0)	1 (100)	0 (0)
Total N= 32	21 (65.6)	6 (18.7)	7 (21,8)	8 (25)	8 (25)

Discussion

Monomicrobial and polymicrobial, It has been reported polymicrobial infection of diabetic foot lead to more serious complication than monomicrobial infection (Dang, Prasad, Boulton, & Jude, 2003; Kathirvel, Jayarajan, Sivakumar, & Govindan, 2018; Benjamin A Lipsky, 2004). In this study, 47.5% were polymicrobial and will lead to sever or moderate diabetic foot infection. Compared to a study conducted in India polymicrobial diabetic foot infection were 66% higher than results reported in this study (Ramakant et al., 2011). Similar results were reported in another study, where polymicrobial infection was 40% of included patients (ABDUL KADIR et al., 2012).

Gram positive and negative; In this study prevalence of gram positive were similar to gram negative bacterial species isolates; 49.2% (31) and 50.8% (32) respectively, which is different from finding of another study that reported 67% of bacterial species identified from diabetic foot were gram negative isolates (ABDUL KADIR et al., 2012). In Korea, researchers found similar results reported in our study where the prevalence of gram negative was 40% and 57.5% reported for gram positive bacteria (Son, Han, Lee, Namgoong, & Dhong, 2017). Of previous variable results obtained from different studies may attributed to these studies were conducted in different countries and location which lead to this variation.

Among gram negative bacteria, *P. aeruginosa* was the dominant bacterial species were identified (34.4%; 11/32) in this study isolates collection. This was also reported in other studies conducted in Korea and India (ABDUL KADIR et al., 2012; Ramakant et al., 2011; Son et al., 2017). The most dominant gram positive bacteria in this study was *S. aureus* 31.7% (20/63) followed by CNS 15.9% (10/63). This is similar to result finding in other studies investigated diabetic foot bacterial infections (ABDUL KADIR

et al., 2012; Bader, 2008; Son et al., 2017). Methicillin resistance among *S. aureus* isolates was 55% (11/20) and this has been reported in different previous studies (Ramakant et al., 2011; Son et al., 2017; Xie et al., 2017). Our study showed 15.8% (10/63) CNS prevalence and methicillin resistance among this collection was 80% (8/10), similar results was founded in other studies (Son et al., 2017; Xie et al., 2017). High prevalence of Methicillin resistance among CNS highlighting a major problem as it may play a role as a possible source of local transferring antibiotic resistance gene to Methicillin susceptible *S. aureus* and evolved to MRSA (Hanssen, Kjeldsen, & Sollid, 2004). According to our knowledge, this is the first study recorded isolation of *Enterobacter sakazakii* from diabetic foot infection.

From previous discussion we can conclude, In agreement with other studies *P. aeruginosa* and *S. aureus* were the most common bacterial species identified from samples obtained from diabetic foot infections.

Antimicrobials profile showed good effect against bacterial species isolated in this study this may due to the not hospitalized patients were included. High resistance to sulphamethoxazole trimethoprim in gram positive and gram negative isolates, while ciprofloxacin showed highest effect against gram positive bacteria and amikacin was the most effective antimicrobials against gram negative bacteria. As gram positive and gram negative combination polymicrobial infection was the most common occurrence in diabetic foot in this study, patient should treated with antibiotic covered both gram positive and negative bacteria.

In conclusion, polymicrobial infection was common, as reported in previous studies it was preloaded to sever or moderate diabetic foot complications. The most dominant gram positive bacterial species in this study collection was *S. aureus* and *P.*

aeruginosa was the highest prevalent among gram negative bacteria. In antibiotic sensitivity test, better results were obtained by using effective antibiotics covering gram positive and gram negative bacteria like ciprofloxacin and amikacin. Methicillin resistance in *S. aureus* and CNS was high and more studies to understand their genetic background and evolutionary history is needed for effective control measures.

Study limitation

Due to limited resources anaerobic bacteria did not included in this study and only aerobic bacteria were detected.

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