Prevalence of bacterial resistance with diabetic foot patients in MMC

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Article information

Key words
Diabetic foot infections, antimicrobial profiles, and multi-drug resistant bacteria.

Abstract

Diabetic patients may experience a variety of complications, including immunodeficiency, blood ischemia and microbial infections, which can increase the risk of diabetic foot wounds becoming chronic and difficult to treat. Diabetic foot patients are subjected to long periods of antimicrobial therapy, which leads to an increase in antibiotic-resistant strains. Multidrug resistance organisms have spread throughout the world in diabetic foot infections (DFIs). This type of infection requires prompt and effective antimicrobial therapy to reduce the complications associated with such infections. The detection rate and identification of common microbial pathogens, as well as their antimicrobial susceptibility patterns, were the focus of this study in DFIs. Forty swabs (specimens) were collected from none repeated diabetic foot patients attending MMC for medical services. Standard microbiological methods were used to identify microbial pathogens. The Clinical and Laboratory Standards Institute's standard guidelines were used to determine antimicrobial susceptibility testing and resistant profiles (CLSI). According to the study's findings, bacterial growth was found in 80 % of the specimens. *Staphylococcus aureus* (30%) and *Pseudomonas aeruginosa* (22.5%) were the most frequently isolated bacterial pathogens. Gram-positive isolates were generally highly sensitive to Cefuroxime (72%), Azithromycin (61%), and Clindamycin (57 %). There was a high MRSA prevalence (75 %). The majority of Gram-negative isolates were susceptible to Azithromycin (63%) and Amikacin (59%), but highly resistant to Augmentin (81%) and Ceftriaxone (63%). DFIs are common cases in MMC's surgery OPD, and the majority of them are associated with multi-drug resistant strains.

I. INTRODUCTION

The primary function of intact skin is to control microbial populations that live on the skin surface and to prevent underlying tissue from becoming colonized and invaded by potential pathogens [1]. In addition, the exposure to subcutaneous tissue following a loss of skin integrity (wound) provides a moist, warm, and nutritious environment that is conducive to microbial colonization. The exposed damaged tissue is at risk of infection with endogenous patient flora [2], and exogenous infection [3]. Wound colonization is most frequently poly-microbial, involving numerous microorganisms that are potentially pathogenic, therefore any wound is at risk of becoming infected [4]. The incidence rate of wound infection may vary depending on the patient's characteristics (age, pre-existing illness, immunological status) [5].

A diabetic foot is one of the most complications of diabetes [6], diabetic foot patients have many disorders of peripheral neuropathy, immunodeficiency**,** and vascular diseases (ischemic), leading to the development of gangrene, which even may require amputation of a foot [7]. In diabetic foot infections, patients are initially treated empirically many times, which may improve the outcome [8]. However, the causative agents of infection are sometimes resistant to the used empirical treatments which could worsen the diabetic foot infection. In previous studies, Gram-positive cocci are the predominant organisms responsible for DFI, with *Staphylococcus aureus* the most commonly isolated pathogen [9,10,11]**.**

Diabetic foot ulcer patients are often infected with multidrug-resistant organisms (MDRO) due to inappropriate antibiotic treatment, chronic course of the wound, and frequent hospital admission [12]. Furthermore, there is poor penetration of antibiotics into the lower limb tissue, due to ischemic status, law in immunity and prevalence of polymicrobial pathogens, thereby increase of MDRO infections.[13]. This study was conducted to know about to invstigate the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) and multidrug-resistant organisms (MDRO) from DFIs and their antimicrobial susceptibility pattern at Misrata Medical Center (MMC), Libya.

II. MATERIALS AND METHODS

2.1. Study design and location

A cross-sectional study was conducted at MMC which is located 200 Km away from the east of the capital, Tripoli, North West Libya**.**

2.2. Study sample and collection

The participants in this study were forty patients who attended the department of surgery at MMC for medical treatment for three months (from February to April 2021). A questionnaire form and consent form were given to the patient during sample collection to obtain the age, gender, history and any other clinical details. All the wounds were judged as infected by the presence of purulent material. The wound surface has been cleaned from exudate and contaminants by using sterile wet gauze and iodine to reduce the chance of contamination. Swabs (samples)were collected aseptically by the surgeon, using sterile cotton swabs (Amies transport media), by rotating the swab over each wound for five seconds, after that samples were transported directly to the laboratory (Misurata Reference Laboratory) in ice.

2.3. Culture and identification

All forty samples were cultured and identification of microbial infection. The detected of microbial pathogens were used microbiological standard techniques. Briefly collected swabs were inoculated onto Blood agar, Chocolate agar, and MacConkey agar plates by surface streaking method. Plates of Blood and MacConkey agars were incubated in an aerobic environment at 37°C for 24-48 hours, while chocolate plates were incubated in an additional 5-10% CO2 for microaerophilic enviroment. Identification of isolates associated with pyogenic infection was based on phenotypic characterization of colony shape, changes in physical appearance in differential media, hemolysis on blood agar, enzyme activities of the organisms, and lactose fermenting. In addition, confirmation of isolates using the Gram stain and other biochemical tests such as coagulase test, catalase test and the analytical profile index (API 20E) for Enterobacteriaceae**.**

2.4. Antimicrobial susceptibility testing

The antimicrobial susceptibility testing was performed following the Kirby-Bauer method by using Mueller Hinton agar (MHA) plates and according to criteria set by the Clinical and Laboratory Standards Institute (CLSI) 2018 [14]. Briefly, the inoculum was prepared by comparison with the opacity standard on McFarland 0.5 Barium Sulfate. Antibiotic disks were placed onto the agar medium and then incubated at 37°C for 18-24 hours. diameters of the zone of inhibition around the disks were measured to the nearest millimeter using a ruler and classified as susceptible, intermediate, and resistant according to the standardized table supplied by CLSI, 2018. The anti-microbial agent's disks tested for both gram-negative and gram-positive bacteria were used with quinolones: (ciprofloxacin5µg), augmentin (30 µg), carbapenems (meropenem 10 µg), cephalosporins: (ceftriaxone 30 µg and cefuroxime 30 µg), doxycycline (30 µg), azithromycin (15 µg), amikacin (30 µg). erythromycin (15 µg) and clindamycin (2 µg), and for Methicillin resistance *S. aureus* were used cefoxitin (30 µg) as a confirmatory test. Antimicrobial agents were selected based on the availability and clinician's prescription frequency of these drugs in the study area**.**

2.5. Phenotypic test for methicillin-resistant (MRSA) and inducible clindamycin-resistant (iMLSB)*S. aureus*

Methicillin-resistant *S. aureus* (MRSA) isolates were detected by the cefoxitin disk (30 μg) method of CLSI, *S. aureus* isolates were judged as methicillin-resistant when the zone inhibition for cefoxitin was ≤21 mm [14]. Similarly, inducible macrolide-lincosamide streptogramin-B (iMLSB) resistance was detected in *S. aureus* strains by disk diffusion approximation using clindamycin (2 μg) and erythromycin (15 μg) on MHA plates, after overnight incubation, isolates with the flattened zone of inhibition adjacent to the erythromycin disk (referred to as a "D" zone) were considered to exhibit inducible clindamycin resistance [14].

III. RESULTS

A total of forty specimens were collected from patients with clinical evidence of wound infection (wound with symptoms of discharge, pain, swelling, foul-smelling and chronic wound) from February to April 2021. The study samples included 26 (65%) males and 14 (35%) females, and the ages of the patients ranged from 30 to 77 years.

3.1. Prevalence of bacterial isolates

The forty specimens were cultured on traditional media, thirty-two (80%) showed growth of the bacterial pathogen, while 8 (20%) were bacteriologically sterile. Polymicrobial growth was observed in 25% of specimens. A total of forty bacterial isolates were obtained and the proportion of gram-positive isolates was 45%, while the percentage of gram-negative was 55%The most common causative organisms associated with DFIs were *S. aureus* isolates (30%), followed by *P. aeruginosa* (22.5%), *Klebsiella pneumoniae* (10%), *Proteus mirabilis* (10%), *Escherichia coli* (7.5%), *Coagulase-negative staphylococci* (CoNS) was (5%), *Enterococcus faecalis* (5%), *Acinetobacter baumannii* (5%) *Streptococcus pyogenes* (2.5%), and *non-hemolytic Streptococci* (NHS)(2.5%)(Fig.1)**.**

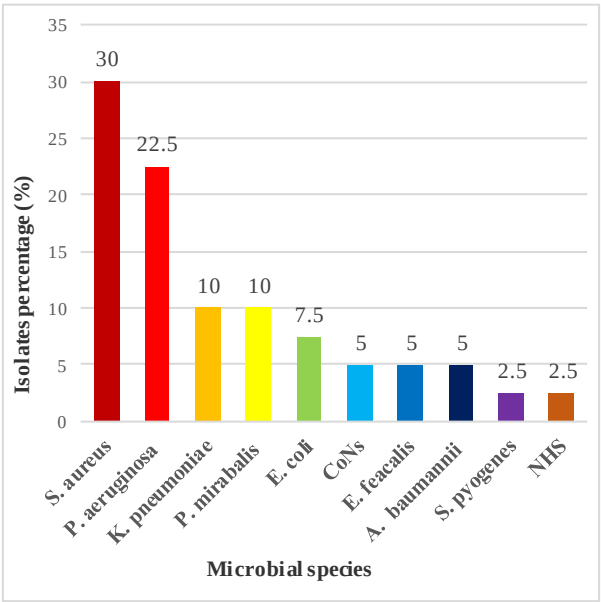

Fig. 1: The percentage of microorganisms isolated from the infected diabetic foot. CoNS: Coagulase-negative staphylococci, NHS: Nonhemolytic streptococci.

Table 1: Antibiotic susceptibility pattern of Gram-positive bacteria isolated from diabetic foot infections.

Numbers of isolates	RXN	The percentage (%) of antimicrobial agents										
		CIP	AUG	CRO	MEM	CXM	DXT	AZM	AK	FOX	E	DA
S. aureus(n=12)	S	50	33.3	25	41.7	75	58.3	58.3	66.7	25	16.7	58.3
	I	8.3	25	16.7	33.3	-	33.3	25	-	-	50	-
	R	41.7	41.7	58.3	25	25	8.4	16.7	33.3	75	33.3	41.7
CoNS (n=2)	S	100	100	50	50	50	50	100	100	-	50	50
	I	-	-	50	50	-	50	-	-	-	50	50
	R	-	-	-	-	50	-	-	-	100	-	-
E. faecalis(n=2)	S	-	50	100	50	100	50	-	-	-	-	-
	I	50	-	-	-	-	50	100	-	-	-	-
	R	50	50	-	50	-	-	-	100	-	-	-
.*S. pyogenes*(n=1)	S	-	100	-	-	100	-	-	-	-	-	-
	I	-	-	-	-	-	100	100	-	-	-	-
	R	100	-	100	100	-	-	-	100	-	-	-
NHS(n=1)	S	-	100	-	100	-	-	-	-	-	-	-
	I	100	-	-	-	100	100	-	-	-	-	-
	R	-	-	100	-	-	-	100	100	-	-	-
Total (n =18)	S	44.4	50	27.7	44.4	72.2	50	61.1	55.6	21.4	14.3	57.1
	I	16.6	16.7	16.7	27.8	5.6	44.4	22.2	-	-	50	7.1
	R	39	33.3	55.6	27.8	.22.2	5.6	16.7	44.4	78.6	35.7	35.8

KEY: CoNS: Coagulase-negative staphylococci, NHS: Non-hemolytic streptococci, S: Sensitive, R: Resistant, I: Intermediate sensitive, CIP: Ciprofloxacin5µg, AUG: Augmentin 30µg, CRO: Ceftriaxone 30µg, MEM: Meropenem 10 µg, CXM: Cefuroxime 30µg, DXT: Doxycycline 30µg, AZM: Azithromycin 15µg, AK: Amikacin 30 µg, FOX: Cefoxitin 30 µg, E: Erythromycin 15 µg, DA: Clindamycin 2µg.

Table 2: Antibiotic susceptibility pattern of gram-negative bacteria isolated from Diabetic foot infection.

Numbers of isolates	RXN	The percentage (%) of antimicrobial agents								
		CIP	AUG	CRO	MEM	CXM	DXT	AZM	AK	CT
P. aeruginosa(n=9)	S	66.7	11.1	33.3	55.6	-	11.1	55.6	55.6	11.1
	I	11.1	-	-	11.1	-	11.1	-	-	-
	R	22.2	88.9	66.7	33.3	100	77.8	44.4	44.4	88.9
K. pneumonia (n=4)	S	25	25	25	25	25	25	50	25	100
	I	-	-	-	-	-	-	-	25	-
	R	75	75	75	75	75	75	50	50	-
P. mirabilis (n=4)	S	50	50	100	100	75	50	100	100	25
	I	-	-	-	-	-	-	-	-	-
	R	50	50	-	-	25	50	-	-	75
E. coli (n=3)	S	-	-	-	66.7	-	33.3	66.7	66.7	100
	I	33.3	-	-	-	-	-	-	-	-
	R	66.7	100	100	33.3	100	66.7	33.3	33.3	-
A.baumannii (n=2)	S	-	-	-	-	-	50	50	50	100
	I	-	-	-	-	-	-	-	-	-
	R	100	100	100	100	100	50	50	50	-
Total (n =22)	S	40.9	18.1	36.4	54.5	18.2	27.3	63.6	59.1	50
	I	9.1	-	-	4.5	-	4.5	-	4.5	-
	R	50	81.9	63.6	41	81.8	68.2	63.4	36.4	50

KEY: S: Sensitive, R: Resistant, I: Intermediate sensitive, CIP: Ciprofloxacin 5µg, AUG: Augmentin 30µg, CRO: Ceftriaxone 30µg, MEM: Meropenem 10 µg, CXM: Cefuroxime 30µg, DXT: Doxycycline 30µg, AZM: Azithromycin 15µg, AK: Amikacin 30 µg, CT: Colistin 10µg.

3.2 Antibiotic susceptibilities of gram-positive bacteria

The bacterial isolates of gram-positive were tested against eleven antibiotics disks The isolates varied in their susceptibility to all the antimicrobials used. The most antibiotics that gram-positive isolates were susceptible to azithromycin (61.1%), doxycycline (50%), clindamycin (57.1%), augmentin (50%) and carbapenems (meropenem (44.4%); while the isolates showed a higher resistance to cephalosporins (ceftriaxone, cefuroxime and cefoxitin) (52.1%), amikacin (44.4%), quinolones:(ciprofloxacin) (39%) as shown in Table 1.

3.3 Antibiotic susceptibilities of Gram-negative bacteria

Gram-negative bacteria were tested against selected nine antibiotics disks. The majority of the isolates were susceptible to amikacin (59.1%), meropenem (54.5%), and colistin (50%), while the other isolates were resistant to augmentin (81.9%), doxycycline (68.2%) ceftriaxone, cefuroxime (66%) and ciprofloxacin (50%) (Table 2).

3.4. Multidrug resistance *S. aureus*

The phenotypic characterization of *S. aureus* had shown a high prevalence rate of resistance in this study. The isolates of S. aureus associated with DFIs showed (that 75%) were resistant to methicillin (MRSA); in addition, 41.7% of the isolates were resistant to clindamycin, and the proportion of 5%of the isolates were inducible clindamycin resistant (showed D-shape phenomena) (Fig. 2).

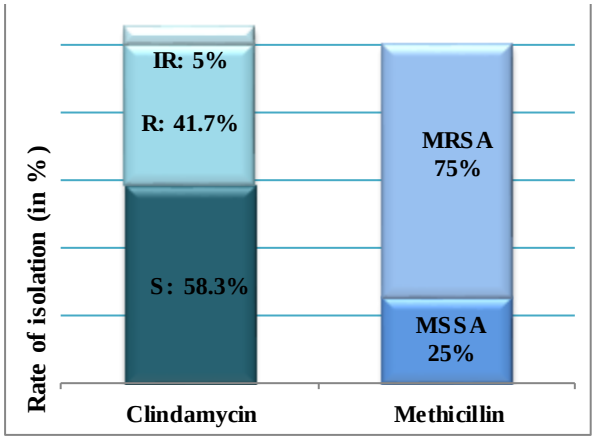

Fig.2: Multidrug resistance *S. aureus* isolated from patients with infected diabetic foot. Key: S: Sensitive, R: Resistant, IR: Inducible resistant (D- shape).

IV. DISCUSSION

Diabetes mellitus is a metabolic disorder that impedes the normal wound healing process leading to severe infections. A diabetic foot ulcer is one of the dreaded complications of diabetes and is the leading cause of the increase in morbidity among diabetic patients. The prevalence of diabetic foot ulcers among male subjects was found to be (65%) against (35%) in females and their ages ranged from 30 to 77 years, this may be due to a higher level of outdoor activity among males compared to females. This is in support of the study findings of Ahmed and Hayat colleagues [15, 16]. The current study showed the proportion of poly microbial infection was25%, while the monomicrobial infection was 75%. These findings disagree with previous studies [17,18]. The reason may be due to the type of causative agent causing the infection, the availability of health care, and geographical variation. Overall, gram-negative microbes where the most predominant pathogens were isolated (55%), and a similar result was observed in other studies carried out in India [19] [20] [21]. Therefore, doctors at the MMC should take into their priority the selection of the appropriate antibiotic to cover gram-negative bacterial infections. The commonest organism was frequently isolated in the present study was Staphylococcus aureus, as well as in other studies from India and other countries showed Staphylococcus aureus as the commonest isolated organism from infected diabetic foot ulcers [19,22]. These findings could be due to Staphylococcus aureus one of the most common commensals available on the skin, unlike other isolated gram-negative bacteria. This study revealed that multidrug-resistant (MDR) organisms were very common in patients with diabetic foot ulcers. This is in line with the report of Gadepalli, et al [19]. High rates of antimicrobial resistance among the pathogenic bacteria associated with the DFIs were a major concern of this study. Patterns of antimicrobial resistance among pyogenic bacterial isolates usually exhibit variability according to the geographic areas, and endemicity of resistant pathogens in the locality, among Gram-positive bacteria. Staphylococcus aureus in this study was the most resilient organism to develop resistance, our isolates were highly resistant to augmentin, and ceftriaxone this finding is in agreement with the previously reported studies; while it was highly susceptible to amikacin, and

cefuroxime [23]. The bacterial MRSA isolation rate (75%) in this study was similar to Khanal *et al* finding (68%) [24]. However, in other studies done by Acharya et al (22.5%) isolation rates were lower than in these studies [25]. The reason for this might be due to the misuse of antibiotics in this study region as antibiotics can be easily obtained without a prescription. Another reason beyond that could be the excessive prescribing of empirical antibiotics by clinicians rather than the basis of microbiological reports. Including chronic infections in the studies may increase the higher isolation rate of multidrug-resistant bacteria due to the long exposure to antimicrobial agents. Similar results (5%) were obtained for inducible clindamycin resistance (iMLSB) compared to previous studies [26,27]. In routine tests for sensitivity to clindamycin, a good susceptibility pattern may appear, but it may have the inducible resistance of the S. aureus towards clindamycin, which should be performed routinely by all clinical microbiologist in medical laboratories to guide the clinicians about the iMLSB phenotype of S. aureus to prevent misuse of antibiotics. Alongside, our findings indicated the high incidence of drug resistance among Gram-negative isolates. In this study, E. coli and A. baumannii were highly resistant to cephalosporins (ceftriaxone and cefuroxime 100%), and P. aeruginosa also was resistant to cefuroxime (100%). These findings of the susceptibility pattern of our Gram-negative isolates were in agreement with other previous reports from this region [25,28]. In general, Gram-negative isolates were highly resistant to augmentin (81.9%) and ceftriaxone (63.6%), probably the reason beyond the increase of the resistance, is the empirical widespread prescription of these antibiotics. our findings indicated the existence of multi-drug resistant bacteria in DFIs, which may be due to the longer duration of prophylactic antimicrobial exposure which may contribute to bacterial development of resistance.

V. Conclusion

Diabetic foot infections were mainly caused by *S. aureus* and *P. aeruginosa*. High levels of multi-drug resistance among both Gram-negative bacteria were observed, Continuous surveillance is necessary to update the knowledge of antimicrobial susceptibility profiles of isolates to provide the most appropriate treatment for DFIs and to limit the expanding menace of drug resistance

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