

Pattern Susceptibility of Antibiotics against Clinical Isolates from Diabetic Foot Ulcer in Khartoum State

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Abstract

Diabetes constitutes a global emergency with case numbers increasing exponentially every year. Diabetic Foot Ulcers, DFUs, are one of the major complications of Diabetes Mellitus, DM, and are a significant risk factor for lower extremity amputation.

This study is a cross sectional study was performed in Khartoum state for isolation and characterization of aerobic bacteria and antimicrobial pattern among diabetic patients with foot ulcers. Wound swabs were collected from 50 patients and were cultured and bacterial isolates were identified using standard methods including Gram stain and biochemical test according to Feltham, Cowan and Steel methods. Then Chromogen agar was used as a confirmation for identification. Antibiotic susceptibility Testing was conducted on bacterial isolates. The antimicrobial susceptibility Testing was performed using the agar diffusion method according to Kirby-Bauer.

A total of 70 bacteria were isolated from 50 clinical samples, which revealed 47, [94%] growth and 3 [6%] no growth, among all isolates, 8 [11.4%] were Gram-positive bacteria, including *Staphylococcus aureus* 6 [9%], *Staphylococcus saprophyticus* 1[1%], *Enterococcus faecalis* 1[1%]. While Gram-negative bacteria constituted 62 [88.6%] of all the isolates, including *Proteus spp* 25 [40.3%], *Pseudomonas spp* 15[24.2%], *Klebsiella spp* 8 [12.9%], *E. coli* 6 [9.7%], *Citrobacter spp* 6 [12.7 %] and *Serratia spp* 2 [3.2%]. The antimicrobial susceptibility test showed 100% resistance to Oxacillin, 56% to Ampicillin/Cloxacillin, 57%, Ampicillin, 54%, Cefixime, 54%, Cephalexin, 35.2 %, Gentamicin, 26%, Ampicillin, 18.3% Doxycycline hydrochloride, 18.3%, Carbenicillin, 14%, Doxycyclin, 12.7%, Piperacillin, 7%, Meropenem and 5.6% to Erythromycin.

Keywords: Oxacillin, Ampicillin/Cloxacillin, Cefixime, Cephalexin, Gentamicin, Meropenem.

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Introduction:

Diabetes Mellitus DM is a chronic disorder that affects a large number of people globally and is a major public health problem ^[1] Diabetics are more susceptible to infections due to increased glucose levels and suppressed immune response as well as the neuropathy and decreased blood flow to extremities that lead to slow-healing wounds ^[2]. The individuals with diabetes have at least a 10-fold greater risk of being hospitalized for soft tissue and bone infections of the foot than individuals without diabetes ^[3]. Wound is defined as any anatomical and physiological disruption in skin structure leading to skin cell damage. Thereby, wound healing is a complex and overlapping process of reaction and interaction among cells and mediators to return natural skin ability, beginning immediately after skin loss. Inflammation, proliferation and remodeling are three overlapping and continuous

wound healing phases. Diabetes wounds infection is major long term impact on the morbidity, mortality and quality of patients ^[4]. DFUs (Diabetic Foot Ulcers) are one of the major complications of DM and are a significant risk factor for lower extremity amputation. Fluctuating blood sugar and hypoxia from poor circulation may impair the ability of white blood cells to destroy pathogenic bacteria increasing infection risk ^[5]. The increasing association of MDR pathogens with diabetic wound further compounds the challenge faced by the Physician or the surgeon in treating diabetic ulcers without resorting to amputation.

Infection with MDR, ([Multi-Drug Resistance), pathogens is also responsible for the increased duration of hospitalization and cost of management ^[6]. MDR profiles can be transmitted to human through the food chain adding serious burden to human health ^[7]. Previously the most commonly

bacteria isolated from DFUs for Gram-positive bacteria were *S. aureus*, followed by *Staphylococcus saprophyticus*, *Staphylococcus epidermidis*, *Streptococcus agalactiae*, and *Streptococcus pneumonia* and the most commonly isolated Gram negative bacteria were *Proteus spp* followed by *E.coli*, *Pseudomonas spp*, and *Citrobacter spp*. Today MDR has rendered most of the original antibiotics obsolete for many infections. The emergence of pathogenic bacteria resistant to most, if not all, currently available antimicrobial agents has become a critical problem in modern medicine, particularly because of the concomitant increase in immune-suppressed patients [8].

Materials and methods:

Study area

Study was confined on diabetic patients who have wound infection in different hospital Khartoum state [Arabian specialized hospital,

Gaber Abualez Center and Al eamtiaz Center].

Study design

Cross sectional study hospital based for isolation of an aerobic bacteria associated with diabetic foot ulcers wound infections.

Sample size and study population

Study population is all Diabetic patients which has wound infection in Al Khartoum State all group of age. Size of sample include 50 inclusion criteria patients who suffer wound infections and another group that had no diabetics foot ulcers.

Data collection

Data were collected by using designed in structured questionnaire to obtain data from the patients.

Ethical clearance: The research approved and received ethical clearance from the management of hospital. Permissions were taken from patients and the research ethic committee. Sample obtained were

only diabetic wound infection patients.

Methods

Specimens Collection:

Clinical samples were collected in this study included 50 wound cotton swabs by using sterile cotton swabs then Deeping in amies' transport media. All steps were performed under aseptic Technique.

Culture media:

All Collected specimens were cultured directly on blood agar and MacConkey agar for primary isolation. Then plate were inoculated and incubated for 24hours [hrs] at 37 ° C. Gram stain and other biochemical tests were also performed according to conventional identification and then identified according to Feltham and Cowan Steel methods [9]. Then Chromogen agar was used as confirmation of identification Gram- positive and negative bacteria.

ISSN: 1858-6147

Antimicrobial susceptibility test:

All isolated bacteria were examined for their susceptibility to a number of antibiotics by disk diffusion technique [Kirby-Bauer method]. The isolated bacteria were subcultured onto nutrient agar and incubated at 37 ° C for 24 hrs. Then were made bacterial suspension [by taken a different colony from nutrient agar and emulsified in normal saline] and matched with, 5 McFarland standard [BaSO₃ was formed by mixing Bacl and H₂so₄] then sterile cotton swab was immersed and inoculated on a plate of Muller-Hinton agar. The inoculation was evenly distributed all over the plate. Commercially prepared antibiotic discs of HI-media., bioanalyses were placed on the surface of the media using sterile forceps. Discs were gently pressed to ensure full with the medium. Plates were incubated at 37 ° C for 24 hrs. The zone of growth inhibition around each disc was measured in millimeters and

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the result was reported sensitive, intermediate or resistant. Antibiotics was used in susceptibility test, Antibiotics used for Gram positive bacteria include Ampicillin [10µg], Oxacillin[1µg], Amoxicillin[25µg], Gentamycin [10µg], Cefotaxime [30µg], Erythromycin [15µg]. Furthermore antibiotics were used for Enterobacteriaceae Ampicillin [10µg], Amoxicillin [25µg], Gentamycin [10µg], Cefotaxime [30µg], Cephalexin [30µg], Cefixime [5µg]. On other hands antibiotics were used for *Pseudomonas spp* Carbenicillin [100µg], Meropenem [10µg], Doxycycline [30µg], and Piperacillin [100µg].

Results

A total of 50 wound swab of diabetic patient with DFUs were Collected. The specimens were collected from different hospital in Khartoum state during a period of December to March. Out of the 50 patients there is 31 male

and 19 female, 30 specimens from male yield growth and 17 specimen from female yield growth as showing in Table [1]

Out of the 50 patients specimens from 47 patients were positive bacterial growth 3 patients were sterile [no growth] ass showing in fig1.

Overall number of clinical isolated from 47 [95.7] specimens was 70 bacteria were isolated. Number of Gram positive was 8 [11.4] where remaining was Gram negative 62 [88.6] Table [3].

Gram positive bacteria including *Methicillin* resistance *staphylococcus aureus* 6 [75%], *S. saprophyticus* 1 [12.5%] and *Enterococcus faecalis* 1 [12.5%] Table [2]. Gram negative bacteria include *proteus spp* 25 [40.3%], *pseudomonas spp* 15[24.2%], *klebseilla spp* 8 [12.9%], *E. coli* 6 [9.7%], *citrobacter spp* 6 [12.7 %] and *serratia spp* 2 [3.2%] Table [5]. The results of

antimicrobial susceptibility test are shown in Tables 6, 7 and 8.

Table [1]: Cross tabulation between Gender and Growth

Gender	Frequency	Number of Growth plate	Percentage %
Male	31[62%]	30	96%
Female	19[38%]	17	89%
Total	50[100%]	47	

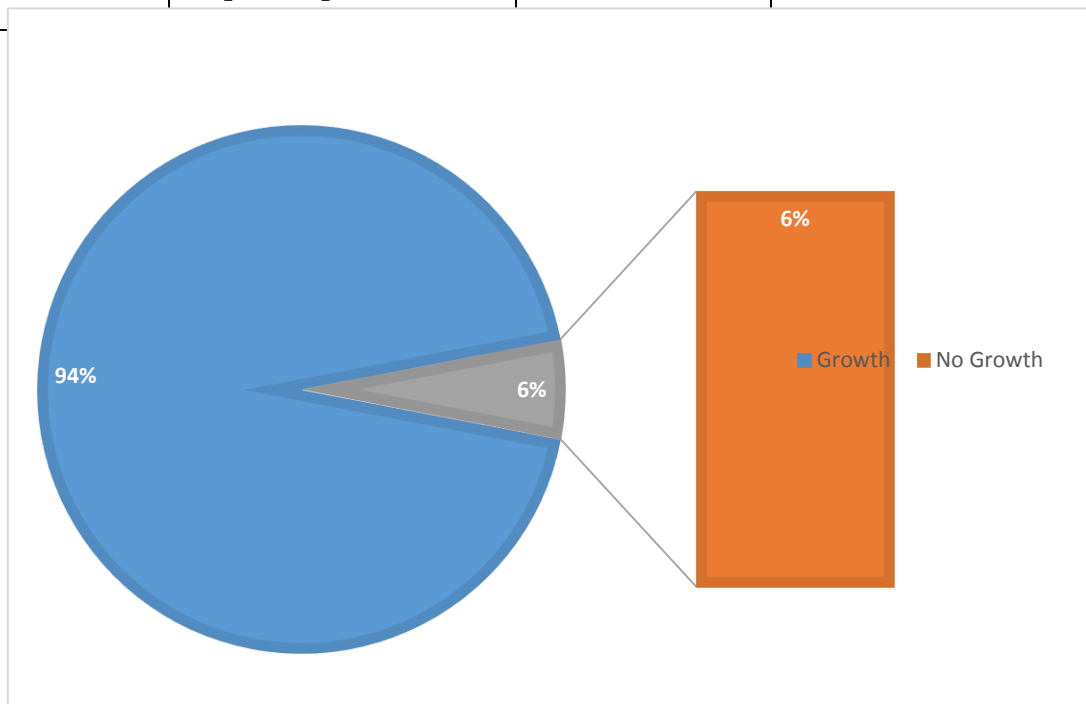


Figure [1]: Percentage of yields growth of cultured swabs from diabetic food ulcer

$P= 0.54$ [Insignificant more than 0.05 Level]

Table [2]: Cross tabulation between Age and cultivation

Age	cultivation		Total [percentage]
	Growth	NO Growth	
from 20 to 30	2	0	2[4.0%]
from 30 to 40	3	0	3[6.0%]
from 40 to 50	15	1	16[32.0%]
from 50 to 60	10	2	12[24.0%]
from 60 to 70	17	0	17[34.0%]
Total	47[94.0%]	3[6.0%]	50[100.0%]

$P= 0.35$ [Insignificant more than 0.05 Level]

Table [3]: frequency and percentage of Gram reaction for bacterial isolates from diabetic food ulcer

Types of bacteria	Frequency	Total %
Gram positive	8	11.4%
Gram negative	62	88.6%
Total	70	100%

Table [4]: Frequency and percentage of Gram positive bacteria isolated from diabetic food ulcer

Types of bacteria	Frequency	Gram-positive %	Total%
<i>MRSA</i>	6	75%	9%
<i>S.saprophyticus</i>	1	12.5%	1%
<i>E. faecalis</i>	1	12.5%	1%
Total	8	100%	11%

Table [5]: Frequency and percentage of Gram negative bacteria isolated from DFUs:

Types of bacteria	Frequency	%	Total %
<i>Proteus spp</i>	25	40.3%	36%
<i>Pseudomonas aeruginosa</i>	15	24.2%	21%
<i>Klebseilla spp</i>	8	12.9%	11%
<i>E. coli</i>	6	9.7%	9%
<i>Citrobacter spp</i>	6	9.7%	9%
<i>Serratia spp</i>	2	3.2%	3%
Total	62	100%	89%

Table [6]: Antimicrobial susceptibility pattern of Gram positive bacteria isolated from diabetic foot ulcers by using Kirby Bauer techniques:

Bacteria isolated		<i>S.aureus</i>	<i>S.saprophyticus</i>	<i>Enterococcus faecalis</i>	Total
Antibiotics					
AX	S	16.6%	0%	0%	12.5%
	I	0%	0%	0%	0%
	R	83.4%	100%	100%	87.5%
AMP	S	33.4%	0%	0%	25%
	I	0%	0%	0%	0%
	R	66.4%	0%	100%	75%
OX	S	0%	100%	0%	12.5%
	I	0%	0%	0%	0%
	R	100%	87.50%	100%	87.5
CTX	S	33.4%	0%	0%	25%
	I	0%	0%	0%	0%
	R	66.4%	100%	100%	75%
AX	S	16.6%	0%	0%	12.5%
	I	0%	0%	0%	0%
	R	83.4%	100%	100%	87.5%
GEN	S	33.4%	0%	0%	25%
	I	0%	0%	0%	0%
	R	66.4%	100%	100%	75%
E	S	16.6%	0%	0%	12.5%
	I	0%	0%	0%	0%
	R	83.4%	100%	100%	87.5%

AX= Amoxicillin

AMP= Ampicillin

OX = Oxocillin

CTX= Cefotaxime

GEN=Gentamycin

CFM= Cefoxime

E= Erythromycin

Table [7]: Antimicrobial susceptibility pattern of Gram negative bacteria isolated form DFUs by using Kirby Bauer techniques:

Antibiotic		E.coli	<i>Klebisella Spp</i>	<i>Citrobacter SPP</i>	<i>proteus spp</i>	<i>Seratia spp</i>	Total
AX	S	0%	25%	16.4%	28%	0%	21.3%
	I	0%	0%	0%	8%	0%	4.3%
	R	100%	75%	83.6%	64%	100%	74.4%
CFM	S	0%	25%	0%	40%	50%	27.7%
	I	33%	25%	0%	16%	0%	17%
	R	67%	50%	100%	44%	50%	55.3%
CTX	S	17%	37.5%	33.4%	40%	50%	36.2%
	I	0%	0%	16.6%	8%	0%	6.4%
	R	83%	62.5%	50%	52%	50%	57.4%
E	Not effective		Not effective	Not effective	Not effective	Not effective	Not effective
GEN	S	67.8%	63%	50%	72%	100%	68.1%
	I	0%	0%	33%	4%	0%	6.4%
	R	32.2%	37%	17%	24%	0%	25.5%
CN	S	33%	37.5%	33.3%	48%	100%	44.7%
	I	0%	0%	0%	0%	0%	0%
	R	67%	62.5%	66.7%	52%	0%	55.3%

AX= Amoxicillin CFM= Cefoxime CTX= Cefotaxime E= Erythromycin
 GEN=Gentamycin CN= Cephalexin

Table [8]: Antimicrobial susceptibility pattern of pseudomonas spp isolated from DFUs by using Kirby Bauer techniques:

Antibiotic	Sensitive	Intermediate	Resistance
MEM	60%	0%	40%
DO	13.4%	0%	86.6%
PY	33.3%	0%	66.7%
PRL	33.3%	0%	66.7%
GEN	33.3%	0%	66.7%
CTX	33.3%	33.3%	33.4%
CN	*	*	*
CFM	*	*	*
E	*	*	*
AMP	*	*	*

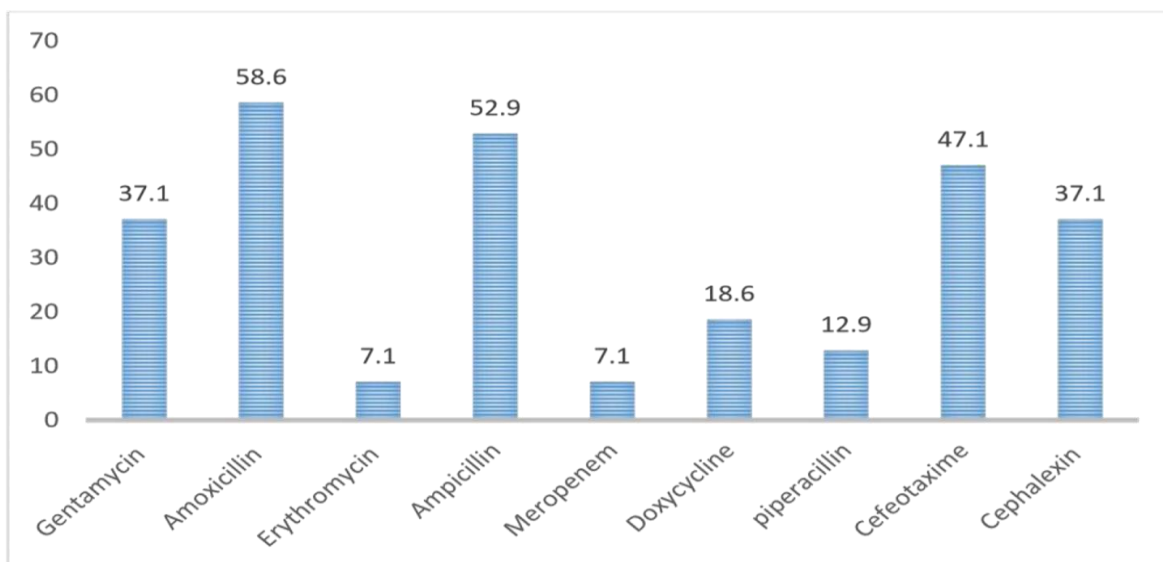


Fig 2 Resistance of bacterial isolates from diabetic foot ulcer

Discussion

DFUs are one of the major complications of DM and are a significant risk factor for lower extremity amputation, the increasing association of MDR pathogens with diabetic wound further compounds the challenge faced by the physician or the surgeon in treating diabetic ulcers without resorting to amputation [6, 10]. Today MDR has rendered most of the original antibiotics obsolete for many infections, the emergence of pathogenic bacteria resistant to most, if not all, currently available antimicrobial agents has become a critical problem in modern medicine, particularly because of the concomitant increase in immune-suppressed patients [11].

Although specimens from 94% of our diabetic patients had positive bacteria growth and 6% had no growth because of prior used of antibiotics. Out of the total isolated bacteria Gram negative were the predominated isolates; it accounted [88.6%], while the remaining [11.4%] were Gram positive this result approximately in agreed with previous study was done in Sudan, Egypt and India by [12, 3, 13] respectively. Our findings results were disagreed with that reported in Pakistan that Gram positive bacteria were high prevalence [14], the variance may be due to selection of patients that exhibited sensory neuropathy and septic conditions during collection specimens. Interestingly out of total the most frequent bacterial strains in the present study was *Proteus spp* it is accounted 25 [35.7%] followed by *Pseudomonas aeruginosa* 15[21.4%]. Out of all Gram positive *S.aureus* is most common isolated 6 [75%] and they were MRSA, this results were agreed with studies was done in Brazil, USA, Iran [3, 15, 16] respectively. Our present study showed that the most common Gram negative bacteria isolate is *Proteus spp* 25[40.3%] followed by *Pseudomonas spp* 15[24 .2%], *klebseilla spp* 8[12.9%], *E. coli* 6[9.7%], *Citrobacter spp* 6 [9.7 %] and

serratia 2 [3.2%] as same observations with study was done in Malaysia^[17].

The Antimicrobial pattern of the current study for Gram positive showed that 87% resistance Oxacillin, Amoxicillin [75%], Erythromycin [75%], Cefotaxime [75%], Ampicillin [62.5%], Gentamycin [37.5%]. Five *Staphylococcus* were isolated and the antimicrobial pattern showed resistance to Oxacillin, Amoxicillin, Ampicillin, Cefotaxime, Erythromycin, and Gentamycin exhibited [100%], [80%], [60%], [60%], [60%] and [60%] respectively. These results were disagreed with study done in Egypt^[6] the different in result may be due to sample size and techniques used and manufactures company of antibiotics and type of strains used. Importantly the *Enterobacteriaceae* [*Proteus spp*, *Citrobacter spp*, *Klebsiella spp* and *E.coli*] the resistant of antibiotics as following Amoxicillin 78.7%, Cephalexin 57.4%, Cefotaxime 55.3%, Cefixime 55.3% and low resistance to Ampicillin 46.8%, Gentamycin 27.6%. This result is agree with Perim et al^[1]. Antimicrobial pattern of *Pseudomonas aeruginosa* show resistant 100% resistant to Erythromycin, Ampicillin, Cefixime, Cefotaxime 66.6%, Gentamycin 73.3%, Meropenem 40%, and Doxycycline 66.6%. The result is disagree with Sivanmaliappan and Sevanan^[18]. Their result of resistance is much lower than our results because to excessive use of antibiotics today.

Conclusion:

According to finding in the study, we conclude thesis:

Different types of aerobic bacteria were isolated from DFUs. Including Gram- positive Cocci and Gram-negative rods. The *Proteus species* was the most frequent bacteria in DFUs followed by *Pseudomonas spp* and,

klebseilla spp, Other less frequent bacteria included: *Staphylococcus aureus*, *Escherichia coli*, *citrobacter spp*, *serratia spp* and *enterococcus faecalis*. All of by *Staphylococcus aureus* isolates were Oxacillin resistant therefore are MRSA. Gentamycin was the most effective antimicrobial therapy against Gram- negative and Gram-positive microorganisms. Meropenium was the most effective antimicrobial therapy against *Pseudomonas spp*.

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