Journal of Infectious Diseases and Therapy
ISSN: 2332-0877

Babandina et al., J Infect Dis Ther 2018, 6:3
DOI: 10.4172/2332-0877.1000361

Research Article Open Access

Phenotypic Profile and Antibiogram of Pathogens Isolated from Diabetic Patients Attending National Hospital in Abuja, Nigeria

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Received date: April 16, 2018; **Accepted date:** May 8, 2018; **Published date:** May 15, 2018

Abstract

Diabetic patients are at increased risk of wound infection after minor or major surgery due to the role diabetes play in metabolic function by impairing inflammatory process leading to increased risk of infection and impaired wound healing. This study investigated the phenotypic profile and antibiogram of pathogens isolated from diabetic patients attending National Hospital in Abuja, Nigeria After approval from the Ethics and Research committee of National Hospital, Abuja. Wound swab sticks were carefully collected from consented 40 post-operative diabetic subjects and 40 post-operative non-diabetic subjects. These samples were analyzed using standard microbiological techniques for isolation, identification and the antibiograms of pathogens. Distribution of bacterial isolate in this study revealed that *Staphylococcus spp* (both coagulase positive and coagulase negative) are the most common pathogen from post-operative septic diabetic patients, 15 (37.5%); followed by *Escherichia coli*, 10 (25.0 %); *Klebsialla spp* and *Pseudomonas aeruginosa* has 6 (15.0%) each. However, *Staphylococcus spp* in septic non-diabetic wounds was 35.0%, followed by *Escherichia coli* and *Proteus spp* (27.5%) while *Klebsiella spp*, *Streptococcus spp* and *Pseudomonas aeruginosa* comprised of 16.3%, 3.7% and 2.5% respectively. The antibiotic susceptibility pattern of respective isolates showed Ceftriazone, Levofloxacin, Ofloxacin as more susceptible compared to Amoxicillin-Clavulanate and gentamycin. The pattern also suggests multidrug resistance in *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas spp* compared with no antibiotic resistance in isolates such as *Proteus spp*, *Klebsiella spp* and coagulase negative *Staphylococcus* (CoNS).

Conclusion: Findings from this study indicated that unprecedented bacterial infection is mostly supported by diabetes which may lead to delayed wound healing. It further revealed that more antibiotics resistance in septic diabetic subject than septic non-diabetics.

Keywords: Diabetic wound; Bacterial pathogen; Antibiotic resistance; Nigeria

Introduction

Diabetic patients are at increased risk of wound infection after minor or major surgery due to the role diabetes play in metabolic function by impairing inflammatory process leading to increased risk of infection and impaired wound healing. Septic operation in tropical countries is seen to have contributed to high rate of mortality especially in diabetes and non-cardio logical critically ill patients, with as many as 20 million cases of sepsis and a mortality rate of around 35% reports worldwide annually [1].

Diabetes and surgery increases the risk of infection and impaired wound healing [2]. Diabetes mellitus and invasive procedures (surgery) both play a significant role in alteration of host defense [3]. Furthermore, post-operative infection associated with diabetes are the second most common healthcare associated problems resulting in prolonged hospitalization, higher cost of medical care with increase morbidity and mortality [4].

Type 2 diabetes is increasingly common, primarily due to an increase prevalence of sedentary lifestyle which has significantly lead to an observable increase in body mass index (BMI), and as a result considerable number of adult and children are obese [5]. Diabetes promotes path physiology to infection by altering both the innate and adaptive immune system by suppression of innate and cellular immunity seems to be one of the principal underlying mechanisms for increased risk of infection [6].

Neutrophils are crucial phagocytes in the innate immune responses; these cells are recruited first to inflammatory site and are essential for illuminating [7]. People with diabetes have a decrease neutrophil chemotaxis, phagocytosis and dihydroamine oxidation [8]. It also plays a major role in the defense against bacterial infection, hence its dysfunction may expose patient to an increased risk of complication after invasive procedure [9]. Complement system is an important part of the innate, humoral immune system and play a central role in opsonisation, anaphylatoxin. Activation of complement cascade helps

to clear invading pathogens [10]. Although, the immunological mechanism or role of neutrophils and complement down regulations ware not fully understood, there malfunction poised a life threatening consequences in treatment with which prolong hospitalization in post-operative septic diabetic and non-diabetics patient due to heavy infection and development of septic operation [11].

In view of these, the present study sought to investigate the phenotypic profile and antibiogram of pathogens isolated from diabetic patients attending National Hospital in Abuja, Nigeria.

Materials and Methods

Study design

Between 7th August, 2016-19th February, 2017, this case-control study was conducted on 40 post-operative septic diabetic and 40 post-operative septic non-diabetic patients as controls.

Study area

This study was conducted at National Hospital in Federal Capital Territory, Abuja, Nigeria. The hospital offers full complement of services to patients and also provides diagnostic services for proper management of patients with immunological disorders. Neutrophil phagocytic test and complement function tests were conducted at the hospital.

Informed consent and ethical approval

Participants enrolled into this study were tutored about the study and gave their written consent after meeting the inclusion criteria for the study. Patients' biodata was obtained using structured questionnaire. Patients who didn't undergo any form of surgery but with etiology similar to disease under investigation were not included in the study. Ethical approval was obtained from the Ethics and Research committee of the National Hospital, Abuja. Data generated were anonymously analyzed throughout the study.

Sample size calculation

The sample size for this study was determined using data from a prevalence studies conducted at Kano State, Nigeria with 9.0% prevalence rate of post-operative surgical site infections in patients as demonstrated by Aisha et al. [12]. Thus, the minimum sample size required for this study was 70 using a 5% error margin and 95% confidence interval. However, statistical credence was given to this study by increasing the sample size to 80.

Sample collection and preparation

Wound swabs were carefully collected from various sites of the body of study participants. These samples were immediately sent to the microbiology laboratory of National hospital, Abuja for cultures, identification and antibiotics susceptibility tests.

Analytical laboratory procedures

Microscopy and Culture: Swabs were cultured on arrival at microbiology laboratory on chocolate agar, cysteine electrode deficient agar and Mc-Conkey agar and incubated anaerobically using anaerobic jar and aerobically at 37°C for 24 hours. A smear was made on a clean greased free slide for a primary Gram reaction.

Gram Staining Techniques: Smears of 15 by 15mm was made, air dried and heat fixed, smears were stained by flooding with crystal violet for 1 minute, rinsed with a gentle flow of water, flooded with lugos iodine for 1 minute, rinsed with gentle flows of water, briefly differentiated using acetone for 30 seconds and rinsed immediately, smear was again flooded with neutral red for 1 minute, rinsed and allow to air dry. The stained smear was examined using X100 oil immersion objective for possible Gram positive and Gram-negative organisms.

Plate Reading and bacterial identification: Isolates were morphologically identified based on size, elevation, and colour changes due to fermentation of lactose on Mac-Conkey and CLED agar while haemolysis was observed on chocolate. Bacterial biochemical tests conducted included catalase, coagulase, triple sugar iron, urea, citrate and indole. The tests results were compared with bacterial identification provided by the U.S. Clinical and Laboratory Standard Institute guideline [13].

Antibiogram: Antibiotic susceptibility of pure culture of confirmed isolate was performed on diagnostic sensitivity test agar (Mueller Hinton agar) by the Kirby Bauer disc diffusion method, using the appropriate Gram positive and Gram negative discs. Isolates were considered sensitive after incubation for 24 hours at 37°C by measuring zone of inhibition with meter rule which was then compared with zone diameter interpretative to National committee for clinical Laboratory standard (CLSI chart) for different organisms and different antibiotics [13]. To guarantee precision and reliability of antibiogram data, quality control strains of *Staplylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853 supplied by department of Pharmaceutics, Obafemi Awolowo University, Ile-Ife, Nigeria were used.

Statistical analysis: Data were analyzed using statistical package for social sciences (SPSS) version 21.0. Discrete variables were expressed as percentages and proportions were compared using the Chi-square test. Statistical significant difference was considered at value of $P<0.05$.

Results

The distribution of bacterial infection in relation to surgical intervention reveals that 46.3% of the subject had orthopaedic surgery, while 5.0% had diabetic foot amputation. Also we reveal that 75.0% of the surgical cases had deep tissue incision. All subject responded that medical negligence as the cause of post-operative infections with more incidence in secondary public health facilities (Table 1).

Result	Septic Diabetic (%) n=40	Septic non-diabetic (%) n=40	Total (%) n=80	P value
Surgery types				
Abdominal	9 (22.5)	17 (42.5)	26 (32.5)	P=0.0758

Breast	1 (2.5)	0 (0.0)	1 (1.3)	
O & G	5 (12.5)	7 (17.5)	12 (15.0)	
Orthopaedic	21 (52.5)	16 (40.0)	37 (46.3)	
Diabetic foot amputation	4 (10.0)	0 (0.00)	4 (5.0)	
Surgical site infection class				
Superficial	12 (30.0)	8 (20.0)	20 (25.0)	p=0.3047
Deep tissue/organ	28 (70.0)	32 (80.0)	60 (75.0)	
What contributes to SSI				
Strikes	2 (5.0)	0 (0.0)	2 (2.5)	p=0.3189
Un-qualified nurse	5 (12.5)	4 (10.0)	9 (11.2)	
Use of fake drugs	9 (22.5)	16 (40.0)	25 (31.3)	
Un-sterilized equipment	9 (22.5)	6 (15.0)	15 (18.7)	
Medical negligence	15 (37.5)	14 (35.0)	29 (36.3)	
O&G=Obstetrics and Gynaecology; SSI=Surgical Site Infection.				

Table 1: Distribution of surgical intervention and post-operative risk factor to SSI.

Results	Septic diabetic (%) n=40	Septic non-diabetic (%) n=40	Total (%)
Escherichia coli	10 (25.0)	12 (30.0)	22 (27.5)
Klebsiella spp	6 (15.0)	7 (17.5)	13 (16.3)
Proteus spp	3 (7.5)	1 (2.5)	4 (5.0)
Pseudomonas spp	6 (15.0)	4 (10.0.)	10 (12.5)
Staphylococcus spp	15 (37.5)	13 (32.5)	28 (35.0)
Streptococcus spp	0 (0.00)	3 (7.5)	3 (3.7)
Total	40 (100)	40 (100)	80 (100)
χ^2=4.802; df=5; p=0.4406; CI=0.238; spp=species			

Table 2: Frequency distribution of bacterial isolates among post-operative septic diabetic and non-diabetic subjects.

Table 2 shows frequency distribution of bacterial isolates among post-operative septic diabetic and septic non-diabetic patients. Study revealed that *Staphylococcus spps* (both coagulase positive and coagulase negative) 28 (35.0%) was the highest bacterial isolates and *Streptococcus spp* (3.7%) in septic non-diabetics subject were the least isolates.

Drugs	Septic diabetics (n=40)						
	E.coli (12)	Kleb (6)	*Proteus* (3)	Pseudo (6)	*Staphylococus spp* (15)		*Strept* (0)
	(%)	(%)	(%)	(%)	SA (10%)	NSA (5%)	(%)
Levofloxacin (5 µg)	9 (75.0)	6 (100)	3 (100)	2 (50.0)	4 (25.0)	5 (100)	0 (0.00)
Gentamycin (10 µg)	5 (45.6)	5 (83.3)	3 (100)	0 (0.00)	5 (38.4)	5 (100)	0 (0.00)
Ceftriazone (30 µg)	8 (91.6)	5 (83.3)	3 (100)	2 (50.0)	10 (76.9)	5 (100)	0 (0.00)
Augumentin (30 µg)	3 (25.0)	5 (83.3)	2 (66.6)	0 (0.00)	4 (30.7)	2 (40.0)	0 (0.00)

Ciproflox (10 µg)	7 (83.3)	4 (66.6)	3 (100)	1 (25.0)	7 (53.8)	5 (100)	0 (0.00)
Ofloxacin (5 µg)	8 (66.6)	6 (100)	3 (100)	0 (0.00)	7 (53.8)	2 (40.0)	0 (0.00)
Resistance	4 (33.3)	1 (16.6)	1 (33.3)	4 (25.0)	5 (46.1)	1 (20)	0 (0.00)

χ^2=23.942; df=30; p=0.7746; SA=Coagulase positive *staphylococcus aureus*; NSA=Non coagulase positive *staphylococcus*; *E.coli=Escherichia coli*; Kleb=*Klebsiella spp*; *Proteus=Proteus spp*; Pseudo=*Pseudomonas aureoginosa*; *Strept=Streptococcus spp* and Spp=Spacies; Ciproflox=Ciprofloxacin

Table 3: Antibiotic susceptibility pattern of the bacterial isolates among septic diabetic subjects.

Table 3 shows the susceptibility pattern of respective isolates to corresponding antibiotic among septic diabetic and non- diabetic subject. Ceftriazone, Levofloxacin, Ofloxacin proved more susceptible compared to Amoxicillin-Clavulanate and Gentamycin (Table 4).

Drugs	**Septic non-diabetics (n=40)**						
	E. coli	**Kleb**	*Proteus*	**Pseudo**	*Staphylococus spp* **(15)**		*Strept*
	(n=12%)	(n=7%)	(n=1%)	(n=4%)	SA (10%)	NSA (3%)	(n=3%)
Levofloxacin (5 µg)	12 (100.0)	7 (100.0)	1 (100.0)	3(75.0)	10 (100.0)	3 (91.6)	2 (66.6)
Gentamycin (10 µg)	12 (100.0)	5 (71.4)	1 (100.0)	0 (0.00)	10 (100.0)	3 (100.0)	0 (0.00)
Cetriazone (30 µg)	10 (83.3)	7(100.0)	1 (100.0)	2 (50.0)	9 (90.0)	3 (100.0)	2 (66.6)
Amoxicillin-Clavulanate (30 µg)	5 (41.6)	5 (71.4)	0 (0.00)	1 (33.3)	6 (60.0)	3 (66.6)	0 (0.00)
Ciprofloxacin (10 µg)	10 (83.3)	7 (100.0)	1 (100.0)	2 (83.3)	10 (100.0)	3 (91.6)	0 (0.00)
Ofloxacin (5 µg)	11 (91.6)	6 (85.7)	1 (100.0)	1 (33.3)	8 (80.0)	3 (100.0)	1 (33.3)
Resistance	3 (25.0)	0 (0.00)	0 (0.00)	1 (33.3)	3 (30.0)	0 (0.00)	1 (33.3)

χ^2=8.888; df=30; p=0.9999; SA=Coagulase positive *staphylococcus aureus*; NSA=Non coagulase positive *staphylococcus; E.coli=Escherichia coli;* Kleb=*Klebsiella spp*; *Proteus=Proteus spp*; Pseudo=*Pseudomonas aureoginosa; Strept=Streptococcus spp* and spp=Species; Ciproflox=Ciprofloxacin

Table 4: Distribution of antibiotic susceptibility pattern of the isolate among septic non- diabetic subject.

Sensitivity of isolate	**p-value**
Escherichia coli	0.5525
Klebsiella spp	0.9936
Proteus spp	0.7919
Staphylococcus aureus	0.9976
Other *Staphylococcus spp*	0.3975
Streptococcus spp	0.5001

Table 5: Difference between frequency of bacterial isolate among post-operative diabetic and non-diabetic participants.

Table 5 above shows no significant difference between the frequencies of bacterial isolates from post-operative diabetic and non-diabetics.

Sensitivity of isolate	**p-value**
Levofloxacin (5 µg)	0.0252*
Gentamycin (10 µg)	0.4341
Ceftriazone (30 µg)	0.2083
Amoxicillin-Clavulanate (30 µg)	0.1896
Ciprofloxacin (10 µg)	0.2159
Ofloxacin (5 µg)	0.0001*

Table 6: Difference between antibiotic susceptibilities of antibacterial in post-operative diabetic and non-diabetic participants.

Table 6 above Compares the level of significant difference in antibiotic sensitivity of all the antibiotics to specific *E. coli*, *Klebiella spp*, *Proteus spp*, *Staphylococcus spp* and *streptococcus spp* between post-operative diabetic and non-diabetics. There is a significant difference in the susceptibility of levofloxacin and ofloxacin between the two groups (with p<0.0001). This suggests increase antibiotic resistance in septic diabetics than septic non-diabetics.

Discussion

It has been shown by many reports that diabetic patients suffer from multiple immunological disorders. Nigeria is one of the nations most affected by diabetes mellitus. However, there is paucity of information in regards to innate immune status of diabetic patients especially in those with septic operation. This study reveals the polymicrobial nature associated with septic diabetic and septic non-diabetic wound

infections often characterized by gram positive and gram negative bacterial that causes chronic wound and complex infections as being reported in previous studies [14,15].

Distribution of bacterial isolate in this study suggests that *Staphylococcus spp* (both coagulase positive and coagulase negative) 15 (37.5%) is the most common pathogen isolated from post-operative septic wounds and in septic diabetic subject followed by *Escherichia coli* 10 (25.0 %), *Klebsialla* and *Pseudomonas aeruginosa* has 6 (15.0%). Similarly, *Staphylococcus spp* in septic non-diabetic has (35.0%), followed by *Escherichia coli* and *Proteus spp* were 27.5% while *Klebsiella spp*, *Streptococcus spp* and *Pseudomonas aeruginosa*, have 16.3 %, 3.7 % and 2.5%, respectively. This work agrees with Abid et al. [16] who reported 50.3% *Staphylococcus spp*, 16.3% *Pseudomonas spp*, 14.37% *Escherichia coli*, 11.8% *Klebsiella spp* and 1.3% *Proteus spp*. This also acquiesces with the study of Kassam et al. [17] in Tanzania, where *Staphylococcus aureus* was the most common isolate (16.0%) from infected wounds. This is not far-fetched as *S. aureus* is normal flora of the skin and anterior nares; therefore, they can easily contaminate wounds and cause infections coupled with their vast numbers of virulence factors that increase their ability to cause infections when compared to other bacteria in immune laden diabetic patients.

In a similar study by Aisha et al. [12] reported *Staphylococcus aureus* as the common pathogen (22.0%), followed by *Pseudomonas spp* (19.9%), *Escherichia coli* (14.7%) and *Proteus* (14.5%). Mathangi et al. [18] reported that out of 22 cultured isolates obtained from diabetic foot of infected patients 9 isolates were predominantly *Staphylococcus aureus* thereby indicating its prominence amidst other isolates including *Pseudomonas aeruginosa*, *Morganella morganii* and *Acinetobacter baumanii*. This may be due to the frequent and predominant colonization pattern of coagulase negative *Staphylococus aureus* among the other organisms from septic surgical diabetic foot infections [19]. This study also, agrees with Amlsha et al. [20] who reported common isolate as being *Staphylococcus aureus* (39.7%) among surgical site infections.

The antibiotic susceptibility patterns of respective isolates showed Ceftriazone, Levofloxacin, Ofloxacin as more susceptible compared to Amoxicillin-Clavulanate and Gentamycin. This observed susceptibility pattern is an indication that these agents may serves as alternate options for effective therapeutic output and clinical management of post-operative diabetic wound infections in affected patients [21]. However, there is an increased level of antibiotic resistance for bacterial pathogens isolated in this study in post-operative septic diabetic and septic non-diabetic patients against Gentamycin, Ceftriazone and Amoxicillin-Clavulanate.

The pattern also suggests multidrug resistance in *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas spp* to Amoxicillin-Clavulanate, Gentamicin, Cephalosporin (Ceftriazone) and fluoroquinolones (Levofloxacin and Ofloxacin). This is closely related to most studies conducted in Sub-Saharan African Countries [22]. Reasons for such resistance patterns are often due to easy affordability and accessibility of these agents being readily administered for a wide range of infections in the community. This study agrees with a similar work by Hossain et al. [23] in Bangladesh that reports an increased resistance in *Pseudomonas* and *Staphylococcus spps* to over 12 groups of antibiotics in septic diabetic foot infections compared to reduced resistance in 8 groups of antibiotics in septic non-diabetic foot infections. Also, low resistance observed in coagulase negative *Staphylococcus aureus* may be due to possible external contamination.

Increased antimicrobial resistance for *Escherichia coli* and *Pseudomonas spp*. observed in this study can be associated with extended spectrum beta lacta mase enzymes that are sometimes produced by these bacterial pathogens. This takes the advantage of suppressed immunity caused by wound trauma [24]. However, increased antibiotic susceptibility or zero resistance was observed in isolates such as *Proteus spp*., *Klebsiella spp*. and coagulase negative *Staphylococci*. It further suggests an increased resistance in septic post-operative diabetic subjects against septic non-diabetics.

Conclusion

In clinical management of post- operative septic diabetic patients, care must be taken in prescribing antibiotics, while a more effective broad-spectrum antibiotic with wide therapeutic margin against polymicrobial wound pathogens may be a better option. Also, studies to harness the genetic mechanisms of antibiotic resistance with resultant immunological effects in post-operative septic diabetics against septic non-diabetics are suggested.

Acknowledgement

Authors wish to appreciate the technical support provided by staff of medical microbiology laboratory, University of Abuja Teaching Hospital and National Hospital, FCT, Abuja, Nigeria.

Conflict of Interest

None

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