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Original article

Phenotypic and genotypic characterization of the most prevalent microbial pathogens in diabetic patients' clinical samples

Yara El-Sayed Marei [*1], *Marym Saied Abo Al-khair* [2], *Mohamed Abd Al-Razek* [2]

1- *Department of Medical Microbiology and Immunology, Faculty of Medicine, Suez Canal University, Ismailia, Egypt.*
2- *Department of Microbiology, Faculty of Science, Suez Canal University, Ismailia, Egypt.*

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ABSTRACT

Background: Diabetes mellitus (DM) is one of the known risk factors for many infections due to the uncontrolled hyperglycaemia that causes immunocompromised state of patients. This study was thus carried out to compare the most prevalent pathogenic organisms and their antimicrobial susceptibility patterns in both diabetic and non-diabetic patients. **Methods:** We collected 169 non-duplicate clinical isolates from different clinical samples. Identification of the isolates up to spp. level and antimicrobial susceptibility profiles were performed by VITEK® 2 compact system. The extensively drug-resistant (XDR) and pan drug-resistant (PDR) isolates were selected for the detection of beta-lactamase genes using PCR. **Results:** 55.6 % of all isolates were collected from diabetics, while 44.4 % were collected from non-diabetics. Gram-negative bacteria were the most prevalent (80.5%), followed by *Candida species* (10.7%), then Gram-positive bacteria (8.9%). Most of the Gram-negative bacteria in diabetic patients showed a high resistance rate to ciprofloxacin (80.8%) and cefazolin (78.2%). However, in non-diabetic patients, high resistance rate was found to ampicillin (70.7%), ceftriaxone (67.2%) and cefepime (65.5%). Most of the Gram positive bacteria in diabetic patients showed high resistance rate to benzyl penicillin (71.4%). 72.2% of the isolates showed resistance to ≥ three antibiotics; 60.7% were from diabetics and 39.3% were from non-diabetics. The frequency of beta-lactamase genes among isolates from diabetics was found to be 68.6% but only 46.4% among isolates from non-diabetics. High frequencies of *blaOXA-48-like* (84.9%) were found. **Conclusions:** Antibiotic abuse and immunocompromised state of uncontrolled diabetics were highly associated with multidrug resistance.

Introduction

Diabetes mellitus (DM) is considered a metabolic disease that is associated with impaired secretion of insulin or insulin resistance. It is regarded as one of the most important emergent health problems in the 21st century [1].

Diabetes mellitus is one of the known risk factors for many infections due to the uncontrolled hyperglycaemia that causes immunocompromised state of patients [2]. Hyperglycaemia causes immune dysfunction (e.g., neutrophil dysfunction, reduced T-cell response, depression of the

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* *Corresponding author:* Yara El-Sayed Marei
E-mail address: yarayara253@hotmail.com

antioxidant system and humoral immunity dysfunction), so, DM predisposes the diabetic patients to many infections compared to non-diabetics [1].

Blood, skin and soft tissue, respiratory tract, gastrointestinal and genitourinary tract infections are more common in diabetic patients that may lead to irreversible complications. Drug resistant profiles are particularly common in the diabetic patient group. Generally, diabetic patients are susceptible to common resistant phenotypes as vancomycin resistant enterococci, extended-spectrum β-lactamase-producing *E.coli*, carbapenem resistant enterobacteriaceae and non-fermenting Gram-negative rods. Resistance to antimicrobial drugs imposes a major therapeutic challenge [3].

The most common infection sites in diabetic patients are skin and soft tissues, including diabetic foot infections (DFIs) and surgical site infections, caused mainly by *Staphylococcus aureus (S. aureus)* [4]. Lower respiratory tract infections are also prevalent in diabetics, including *Streptococcus pneumonia*, *Mycobacterium tuberculosis*, *S. aureus*, *Candida albicans*, and influenza virus [5].

Urinary tract infections (UTIs) are more frequent in diabetic people and may cause severe symptoms and/or complications. The most common isolated pathogens are *Escherichia coli (E.coli)*, *Klebsiella* spp., *S. aureus*, *Enterococcus* spp and *C. albicans*. The main pathogenic mechanisms include reduced chemotaxis and phagocytic activity, immobility of polymorphonuclear leukocytes, diminished interleukin production in response to infection; glycosuria and urinary dysmotility. Hyperglycemia also increases the virulence of several pathogens [6].

Acute pyelonephritis with bilateral renal involvement is more prevalent in diabetics compared to the non diabetics. *Escherichia coli* and *Proteus species* are the most causative agents. Fungal cystitis may cause difficulties such as urinary tract obstruction. Moreover, individuals with DM are at a raised probability of problems such as renal and perinephric abscesses, emphysematous pyelonephritis, and renal papillary necrosis [7].

Any infection, including UTIs, pneumonia, or skin wounds, can cause septic shock and sepsis. Additionally, diabetic patients may develop complications with their management due to their susceptibility to infections, including post-operative infections, malignant external otitis, chronic periodontitis, emphysematous cholecystitis, gangrenous cholecystitis, rhinocerebral mucormycosis and others [1,8]. Therefore, decreasing DM complications and mortality requires efficient preventive measures, such as vaccination or early detection and rapid treatment of diabetic infections [9].

This study was thus carried out to compare the most prevalent pathogenic organisms and their antimicrobial susceptibility patterns in both diabetic and non-diabetic patients admitted at Suez Canal University Specialized Hospital in Ismailia.

Methods

One hundred and sixty-nine (169) non duplicate clinical isolates were collected under aseptic techniques between December 2020 and April 2022 from hospitalized patients in different clinical wards and intensive care units at Suez Canal University Specialized Hospital and transported immediately to the microbiology laboratory for further processing.

These isolates were collected from various sources as urine, sputum, blood and pus from diabetic and non-diabetic patients. We obtained an informed consent from each patient to include their data in this research.

This study has taken the approval of the Research and Ethical Committee of Faculty of Science, Suez Canal University (Committee No. 8 dated 9-27-2020 Code REC42/2020). This study adheres to the ethical standards of the Declaration of Helsinki.

Isolation and purification

All samples were cultured on blood agar, MacConkey agar, and sabouraud dextrose agar media (Himedia, Mumbai), and then incubated for 24-48 hours at 37°C. Microscopic examination of Gram-stained samples was then performed to identify the colony as being Gram positive, Gram negative or Candida.

Identification and antibiotic susceptibility testing (AST) by VITEK® 2 compact system

Further identification of all isolates up to spp. level and antimicrobial susceptibility profiles were performed at the microbiology laboratory at Suez Canal University Specialized Hospital by using VITEK® 2 compact system (bioMérieux, Marcy l'Etoile, France) [10].

Suspension preparation:

A sufficient number of colonies from pure culture were transferred using a sterile swab or applicator stick, and the microorganism was then suspended in a 12 x 75 mm clear plastic (polystyrene) test tube containing 3.0 mL of sterile saline (aqueous 0.45% to 0.50% NaCl, pH 4.5 to 7.0). Using a turbidity metre known as the DensiChek™, the turbidity was adjusted and measured according to the following [10]:

Product	McFarland Turbidity Range
Gram Negative	0.50 -0.63
Gram Positive	0.50 -0.63
Yeast	1.80 – 2.20

Inoculation and interpretation:

The test tube containing the microorganism suspension was inserted into a special rack (cassette). The identification card was inserted into a nearby slot while the transfer tube was inserted into the corresponding suspension tube. Up to ten tests can fit on the cassette. The filled cassette was manually inserted into a vacuum chamber station. After the vacuum was applied and the air was re-introduced into the station, the organism suspension was forced through the transfer tube into micro-channels that fill all the test wells.

Finally, the identification results were available in 10 hours, and calculations were performed on raw data and compared to thresholds to determine reactions for each test [10].

Antimicrobial susceptibility cards were processed until the minimal inhibitory concentrations (MICs) were obtained and interpreted according to the CLSI guidelines [11]. Isolates were then categorized into multidrug-resistant (MDR), extensively drug-resistant (XDR) or pandrug-resistant (PDR). Multi drug resistant was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories, XDR was referred to as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories and PDR was defined as non-susceptibility to all agents in all antimicrobial categories [12].

Storage of the isolates

After isolation and identification from clinical samples as previously described, all isolates were labeled and stored in glycerol broth at -20 ºC for further processing.

Detection of beta-lactamase genes by PCR

The PDR and XDR isolates were screened for the presence of beta-lactamase genes by PCR using reaction conditions and specific set of primers as described by **table (1).**

QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) was used to extract the genomic DNA based on the manufacturer's instructions.

Polymerase chain reaction was done at a final volume of 25 µl containing 6 µL of extracted DNA as a template, 1 µl forward primer, 1 µl reverse primer, 12.5 µl of 2× Master Mix (including 1.5 × PCR buffer, 0.5 mmol/L of dNTPs, 4 mmol/L of MgCl2, and 0.08 IU of Taq DNA polymerase), and 4.5 µl nuclease free water.

The DNA was amplified in the thermal cycler (Eppendorf Co., Germany) using the following protocol: initial denaturation (95 ºC for 5 minutes), followed by 35 cycles of denaturation at 95 ºC for 1 min, annealing (54˚C for 45 seconds for *Bla TEM* and 56˚C for 1 minutes for *bla Z, Bla OXA-48-like* and *Bla KPC*) and extension (72 ºC for 1 minutes), with a single final extension of 10 minutes at 72 ºC.

The amplified products were then visualized by electrophoresis on 2% agarose gels stained with ethidium bromide and then visualized under ultraviolet (UV) illumination. The produced amplicons were compared to a DNA ladder with sizes ranging from 100 to 1000 bp (Fermentas, Germany).

Statistical analysis

The data were analyzed by SPSS version 22 for windows (SPSS Inc., Chicago, IL, USA). Categorical values were represented by using numbers and percentages. Chi square test and Fisher exact test were used to measure the significance. The results were considered significant at p value $\leq$ 0.05 (confidence level of 5%).

Results

One hundred and sixty-nine clinical isolates were collected from different sources, including urine (n = 96), sputum (n = 30), blood (n = 21), and pus (n = 22). Ninety-four isolates (55.6 %) were collected from diabetic patients (male = 47; female = 47) including urine (n = 50), sputum (n = 18), blood (n = 13), and pus (n = 13), while seventy-five isolates (44.4 %) were collected from non-diabetic patients (male = 39; female = 36) and divided into urine (n = 46), sputum (n = 12), blood (n = 8), and pus (n = 9). Patients over 60 years old;

either diabetic or non-diabetic; constitute the largest frequency of infection (71%).

Gram-negative bacteria were the most prevalent group (n= 136, 80.5%), followed by *Candida species* (n= 18, 10.7%), then Gram-positive bacteria (n=15, 8.9%) in both diabetic and non-diabetic patients' samples.

In diabetic patients, *K. pneumoniae* was the most isolated (30/94; 31.9%), followed by *E. coli* (17/94; 18.1%) among Gram negative bacteria. While *Enterococcus faecalis* was the most isolated (3/94; 3.2%), followed by *S.aureus* (2/94; 2.2%) among Gram positive bacteria.

On the other hand; in non-diabetic patients, *E. coli* was the most isolated and more prevalent (25/75; 33.3%) followed by *K. pneumoniae* (20/75; 26.7%) among Gram negative bacteria. While *Staphylococcus haemolyticus* was the most isolated (3/75; 4%), followed by *S.aureus* (2/75; 2.7%) among Gram positive bacteria.

The most prevalent isolated *Candida species* in both diabetic and non-diabetic patients was *Candida albicans* and represented 6.4 % (6/94) and 5.3 % (4/75), respectively.

Resistance patterns for the Gram-negative bacteria isolated from diabetic and non-diabetic patients' samples was demonstrated in **table (2)**. Most of the Gram negative bacteria in diabetic patients showed high resistance rate to ciprofloxacin (80.8%) and cefazolin (78.2%) followed by cefepime (74.4%), ceftriaxone (73.1%), ampicillin/sulbactam (70.5%), ampicillin (60.3%) and aztreonam (60.3%). However, in non-diabetic patients, high resistance rate was found to ampicillin (70.7%), ceftriaxone (67.2%), cefepime (65.5%), ampicillin/sulbactam (63.8%) and aztreonam (63.8%).

Resistance patterns for Gram-positive bacteria isolated from diabetic and non-diabetic patients' samples was demonstrated in **table (3)**. Most of the Gram positive bacteria in diabetic patients showed high resistance rate to benzyl penicillin (71.4%), while high resistance rate was found to tetracycline (75%) and erythromycin (62.5%) in non-diabetic patients.

Only one *Candida krusi* isolate, which was obtained from the diabetic patient, showed resistance against fluconazole and flucytosine.

Overall, 72.2% (n=122) of all isolates were resistant to three or more antibiotics; 60.7% (n=74) were from diabetic and 39.3% (n=48) were from the non-diabetic source. Among these isolates, 35.2% (n=43) was *K. pneumoniae*, 10.7% (n=13) was *A. baumannii* and 9% (n=11) was *P. aeruginosa*. **Table 4** divides the drug resistance isolates into PDR, XDR, and MDR from diabetic and non-diabetic sources. Statistical significant differences were observed between both groups in drug resistance patterns for all isolates as $p < 0.001$.

All the PDR (n=15) and the XDR (n=38) isolates of *K. pneumoniae*, *A. baumannii*, and *P. aeruginosa* were screened for the presence of beta-lactam genes by PCR as illustrated in **figures (1 & 2)**. The frequency of beta-lactamase genes among isolates from diabetics was found to be 68.6% but only 46.4% among isolates from non-diabetics. Distribution of resistance genes was slightly higher in *K. pneumoniae* (65.3%), *P. aeruginosa* (60%), and then *A. baumannii* (58.3%). High frequencies of *blaOXA-48-like* (84.9%) were found. *blaTEM*, *bla Z*, and *blaKPC* genes were present at a relatively close percentage (56.6%, 60.4%, and 49.1% respectively) for all isolates. *bla Z* gene wasn't detected in any *P. aeruginosa* isolates but *bla KPC* was present in all isolates. The *bla TEM* gene wasn't detected in any *A. baumannii* isolates but *bla OXA-48-like* was present in 91.7% of the *A. baumannii* isolates **(Table 5)**.

Table 1. Oligonucleotide primers sequences (Biobasic (Canada)).

Target gene	Primers sequences	Amplified segment (bp)	Reference
blaTEM	ATCAGCAATAAACCAGC0	516	[13]
	CCCCGAAGAACGTTTTC		
bla Z	CAAAGATGATATAGTTGCTTATTCTCC	610	[14]
	TGCTTGACCACTTTTATCAGC		
blaOXA-48-like	TTGG TGGC ATCG ATTA TCGG	597	[15]
	GAGC ACTT CTTT TGTG ATGG C		
blaKPC	AAA ACG GCA AGA AAA AGC AG	340	[15]
	AAA ACG GCA AGA AAA AGC AG		

Table 2. Antibiogram for all of the Gram-negative bacteria (n=136).

Antimicrobial	***No. of isolates (%)***		***p-value***
	Diabetic (n=78)	***Non-diabetic (n=58)***	
	R	*R*	
Ampicillin	47 (60.3%)	41 (70.7%)	0.245
Ampicillin/Sulbactam	55 (70.5%)	37 (63.8%)	0.108
Cefazolin	61 (78.2%)	33 (56.9%)	0.092
Ceftriaxone	57 (73.1%)	39 (67.2%)	0.229
Cefepime	58 (74.4%)	38 (65.5%)	0.257
Aztreonam	47 (60.3%)	37 (63.8%)	0.627
Ertapenem	21 (26.9%)	7 (12.1%)	0.382
Imipenem	38 (48.7%)	20 (34.5%)	0.726
Meropenem	39 (50%)	20 (34.5%)	0.098
Amikacin	27 (34.6%)	12 (20.7%)	0.343
Gentamicin	41 (52.6%)	23 (39.7%)	0.110
Tobramycin	46 (59%)	16 (27.6%)	0.099
Ciprofloxacin	63 (80.8%)	37 (63.8%)	0.198
Moxifloxacin	40 (51.3%)	25 (43.1%)	0.366
Tigecycline	23 (29.5%)	6 (10.3%)	0.228
Nitrofurantoin	26 (33.3%)	8 (13.8%)	0.388
Trimethoprim/Sulfamethoxazole	40 (51.3%)	23 (39.7%)	0.082

*statistically significant as $p<0.05$

Chi-square test used.

Table 3. Antibiogram for the isolated Gram-positive bacteria (n=15).

Antimicrobial	***No. of isolates (%)***		***p-value***
	Diabetic (n=7)	***Non-diabetic (n=8)***	
	R	***R***	
Benzyl penicillin	5 (71.4%)	4 (50%)	0.891
Ampicillin	0	1 (12.5%)	0.972
Oxacillin	3 (42.9%)	2 (25%)	0.902
Gentamicin	2 (28.6%)	1 (12.5%)	0.782
Streptomycin	0	1 (12.5%)	0.972
Ciprofloxacin	2 (28.6%)	3 (37.5%)	0.871
Levofloxacin	2 (28.6%)	1 (12.5%)	0.782
Moxifloxacin	1 (14.3%)	1 (12.5%)	0.726
Erythromycin	3 (42.9%)	5 (62.5%)	0.425
Clindamycin	1 (14.3%)	3 (37.5%)	0.291
Quinupristin/Dalfopristin	3 (42.9%)	0	0.208
Linezolid	0	0	1.00
Vancomycin	1 (14.3%)	1 (12.5%)	0.726
Tetracycline	3 (42.9%)	6 (75%)	0.307
Tigecycline	0	0	1.00
Nitrofurantoin	0	0	1.00
Rifampicin	1 (14.3%)	2 (25%)	0.561
Trimethoprim/Sulfamethoxazole	3 (42.9%)	1 (12.5%)	0.456

*statistically significant as $p<0.05$

Fisher exact test used.

Table 4 Distribution of drug resistance patterns for all isolates (n=122) obtained from diabetic and non-diabetic patients.

Organism	Diabetic			Non-Diabetic			*p*-value
	PDR	XDR	MDR	PDR	XDR	MDR	
Klebsiella pneumoniae	4	17	7	4	6	5	<0.001*
Acinetobacter baumannii	1	9	1	0	2	0	
Pseudomonas aeruginosae	5	3	1	1	1	0	
Others	1	3	22	2	4	23	
Total	11	32	31	7	13	28	
	74			48			

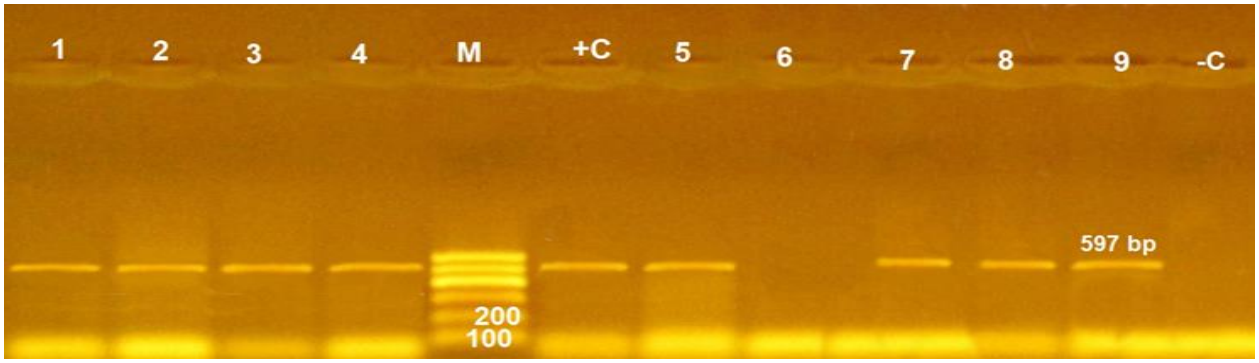
PDR: (pan drug resistance), XDR: (Extensively drug resistance), MDR: (multidrug resistance).
*statistically significant as $p<0.05$
Fisher exact test used.

Table 5. Distribution of *blaKPC, blaOXA-48-like, bla Z,* and *blaTEM* genes in the selected isolates among diabetic and non-diabetic patients.

Pathogen	Drug Resistance Genes											
	blaTEM			***bla Z***			***blaOXA-48-like***			***blaKPC***		
	D n (%)	N-D n (%)	***p. value***	D n (%)	N-D n (%)	***p. value***	D n (%)	N-D n (%)	***p. value***	D n (%)	N-D n (%)	***p. value***
Klebsiella pneumonia (n=31)	**18 (58.1 %)**	**6 (19.4 %)**	**0.02***	**18 (58.1 %)**	**6 (19.4 %)**	**0.02***	**19 (61.3 %)**	**7 (22.6 %)**	**0.004***	**4 (12.9 %)**	**3 (9.7 %)**	**0.07**
Pseudomonas aeruginosa (n=10)	**6 (60 %)**	**0**	**0.005***	**0**	**0**	**-**	**8 (80%)**	**0**	**0.001***	**8 (80 %)**	**2 (20 %)**	**0.02***
Acinetobacter Baumannii (n=12)	**0**	**0**	**-**	**8 (66.7 %)**	**0**	**0.003***	**9 (75 %)**	**2 (16.7 %)**	**0.06**	**9 (75 %)**	**0**	**0.02***

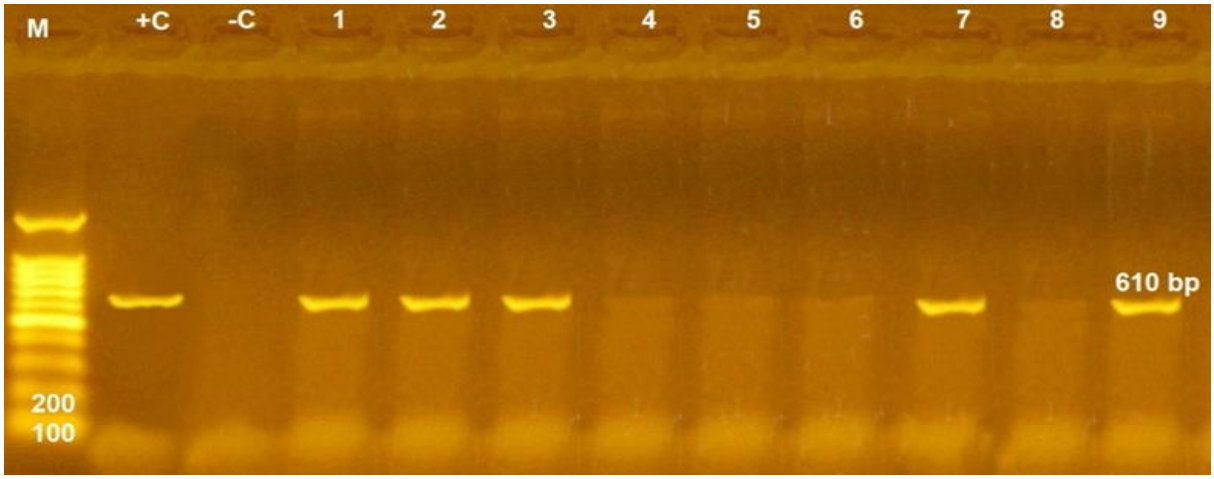
D: (diabetic patient), N-D: (non-diabetic patient)
*statistically significant as $p<0.05$

Figure 1. The results of the *bla OXA-48-like* gene in the tested isolates.



1,2,3: *K. pneumoniae* were positive for *bla OXA-48-like* gene at 597 base pair, 4,5: *P. aeruginosa* were positive for *bla OXA-48-like* gene at 597 base pair, M: marker, +C: positive control, 6: *P. aeruginosa* were negative for *bla OXA-48-like* gene, 7,8,9: *A. baumannii* were positive for *bla OXA-48-like* gene at 597 base pair, -C: negative control.

Figure 2. The results of the *bla Z* gene in the tested isolates.



M: marker, +C: positive control, -C: negative control, 1,2,3: *K. pneumoniae* showing positive for *bla Z* gene at 610 base pair, 4,5,6: *P. aeruginosa* showing negative for *bla Z* gene, 7,9: *A. baumannii* showing positive for *bla Z* gene at 610 base pair, 8: *A. baumannii* showing negative for *bla Z* gene.

Discussion

In this study, we found that patients over 60 years old; either diabetic or non-diabetic; constitute the largest frequency of infection (71%) which agreed with studies conducted by **Macfarlane et al.** [16]**, Rosser et al.** [17] and **Caskurlu et al.** [18]**,** who reported that the incidence of the infection increases sharply with increasing age. This may be attributed to immunocompromisation, antibiotic abuse, several hospital admissions and the associated hospital-acquired infections.

Gram-negative bacteria represented 80.5% of all types of infections and it was the most prevalent group among diabetics and non-diabetics and this is in agreement with **Bonadio *et al.*** [19]**, Aswani et al.** [20]**, Okojie and Omorokpe** [21]**, Assefa et al.** [22] Disagreeing with our study, **Mohammed et al** [23] and **Shrestha et al.** [24] reported that the Gam positive bacteria were the predominant pathogens in their study on the blood steam infections (BSI) in diabetic patients (100%, 70% respectively), while in our study the predominant pathogens were Gram negative bacteria (92.3%) on the BSI in diabetic patients.

In diabetic patients, *K. pneumoniae* was the most frequent isolate (31.9%) in this study, followed by *E. coli* (18.1%). This result was different from other study conducted by **Chiţă et al.** who found that the most common organism was *E. coli* (68.9%) followed by *K. pneumonia*e [25]. **Bonadio et al.** found that the most prevalent causative organism in diabetics were: *E. coli* (56.1%) followed by *Proteus sp.* (7.9%) [19]. Also, a cross-sectional descriptive study was carried out on UTI among diabetic patients in Nepal. This study revealed that *E. coli* was the most common isolated organism followed by *Klebsiella* [26]. The differences may be due to the difference in regions and therefore different habitats and strains distributed.

On the other hand, in non-diabetic patients, *E. coli* was the most prevalent (33.3%) followed by *K. pneumoniae* (26.7%). This is slightly similar to the results of **Ramrakhia et al.,** who reported that the most frequent causative agents were *E. coli* (72%) followed by *K. pneumoniae* (11.1%) [27].

Since the use of antimicrobials agents is more frequent in diabetic patients than non-diabetics, drug-resistance is mainly widespread in this group. This also agreed with another study performed by **Signing et al.,** who found that there was a significant association between antibiotic resistance profile and diabetic status ($p < 0.001$) [28].

Most of Gram negative bacteria in diabetics showed a high resistance patterns to some antibiotics compared to non-diabetics, such as cefazolin (78.2%, 56.9% respectively), ampicillin/sulbactam (70.5%, 63.8% respectively), ceftriaxone (73.1%, 67.2% respectively), cefepime (74.4%, 65.6% respectively), and ciprofloxacin (80.8%, 63.8% respectively). **Saber *et al.*** reported a significantly ($p<0.05$) higher resistant to ceftriaxone and ciprofloxacin in Gram negative bacteria isolated from diabetic patients compared to those isolated from non-diabetic patients [29].

In both diabetics and non-diabetics, most of Gram-positive bacteria showed a low resistance

rate to antibiotics, while linezolid, nitrofurantoin, and tigecycline were the most active drugs. This result agreed with **Wadekar et al**. as they reported that the efficacy of linezolid against Gram-positive bacteria in diabetics and non-diabetics was 92.5% [30].

In this study, we found that 72.2% of all isolates were MDR. The frequency of drug-resistant isolates was significantly higher in diabetic than non-diabetic patients and represented 60.7% and 39.3% respectively. However, a lower ratio was reported by **Wright et al.** who found that MDR was seen in 37% of isolates [31]. **Assefa et al.** reported in their study that the prevalence of MDR was found to be 72.2% [22] and this is nearly similar to the studies conducted in Egypt (76.2%) [32] and Ethiopia (76%) [33].

Klebsiella pneumoniae, *A. baumannii*, and *P. aeruginosa* were the most resistant species and represented 35.2%, 10.7%, and 9% respectively. **Arbianti et al.** also found that the most common multidrug-resistant isolates from diabetic patients were *K. pneumoniae* and *A. baumannii* (3.3% and 1.6% respectively) [34]. **Assefa et al.** reported a high level of MDR among *K. pneumoniae* (42%) [22].

The presence of beta-lactamase genes was screened by PCR for detection of *bla*KPC, *bla*OXA-48-like, *bla*Z, and *bla*TEM. It was found that the frequency of resistance genes in the isolates from diabetics was higher than those from non-diabetics and represented 68.6% and 46.4% respectively. Distribution of resistance genes was slightly higher in *K. pneumoniae* (65.3%), *P. aeruginosa* (60%), and then *A. baumannii* (58.3%) with higher frequencies of *bla*OXA-48-like (84.9%) than other genes. **Codjoe and Donkor** disagreed with us, as they found *bla*CTX-M-1 was the highest (95.45%) compared to other genes [35].

In this study, *bla*KPC was present in 100% of *P. aeruginosa*; whereas it was not found in any *P. aeruginosae* isolate in the study conducted by **Amini and Namvar** [36] . In our study, *bla Z* gene wasn't detected in any *P. aeruginosa* isolates. The *bla TEM* gene and *bla OXA-48-like* were detected in (60%, 80% respectively) of *P. aeruginosa* isolates. **Hosu et al.,** reported a similar percentage for the *bla*TEM in *P. aeruginosa* isolates (79.3%) [37].

The *bla TEM* gene wasn't detected in any *A. baumannii* isolates in our study, but **Jafari-Sales et al.** reported in their study that 31.3% of *A. baumannii* isolates had the *bla TEM* gene [38].

In conclusion, Gram-negative bacteria were the most prevalent in diabetic and non-diabetic patients compared to Gram-positive bacteria and *Candida species*. Antimicrobial resistance was higher in diabetic patients than non-diabetic patients and common in older patients. The frequency of resistance genes in the isolates from diabetics was higher than those from non-diabetics.

Therefore, antibiotic abuse and immunecompromised state of uncontrolled diabetics were highly associated with multidrug resistance and caused a huge burden on health and the economy.

The study has certain limitations. First, the DM cases were not classified into types, i.e., type 1 or 2. However, these did not significantly affect the outcome and interpretations. Further studies may consider that and ascertain significant associations with such classifications. Second, we did not consider patient treatment variables in order to stratify the degree of diabetic control that could have an influence on infection. Third, the aspects of personal hygiene, socioeconomic status, immunocompromised patients and concurrent medications were not considered in our analysis.

Disclosure of potential conflicts of interest

Conflict of interest

The authors report no conflicts of interest.

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Authors' contributions

All the authors were involved in the study conception and design. They contributed to the methodology, writing the manuscript, data acquisition, analysis and interpretation. All authors read and approved the final version of the manuscript.

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