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Bacterial Infection profile and Antibiotic sensitivity of Diabetic Foot Ulcer in Zefta Hospitals, Egypt

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Abstract: One of the cause's insulin patients are sent to clinics is diabetic foot ulcers (DFU), and when this happens, empirical antibiotic therapy is required. In order to evaluate the bacteriological profile and susceptibility pattern of these infections, we undertook a retrospective study of patients with DFU who needed hospitalization. We evaluated 100 patients were collected over a period of 5 months starting from October, 2018 until March 2019 on patients attending the outpatient clinic of diabetic foot unit, Zefta Hospitals**,** Gharbay, Egypt. The bacteria were isolated from different age, from 62 male there age ranged from 45 to 70 years and from 38 female there age ranged from 40 to 65 years. Gram-positive bacteria were responsible for 52.8% of the isolates, while Gram-negative were responsible for 47.2%. *Staphylococcus aureus* was the most prevalent pathogen, followed by *Escherichia coli*. Regarding the susceptibility profile, we found all bacterial strains were sensitive to IPM, except *Pseudomonas aureginosa* was resistant. IPM was the effective antibiotics against all Gram positive bacteria. All isolates of *P. aeruginosa* resistant to IPM and susceptible to antibiotics AK, CN. Our result shows *S. aureus* was susceptible to antibiotics AK, IPM with 100%. The *E. coli* was susceptible to antibiotics IPM with 100%. Information of microbiological profile and antibiotics susceptibility of patients with DFU is useful to evidence clinical therapy.

Keywords: Bacterial infection, Antibiotics resistance, Sensitivity, Diabetic foot ulcers

1. Introduction

One of the most frequent reasons for hospitalization in diabetic patients, diabetic foot infection (DFIs) is also a substantial infections are also to blame for the extended use of broad-spectrum antibiotics, which leads to bacterial resistance development [1].

contributor to increased hospitalization and healthcare costs. These

On World Health Day 2016, the World Health Organization (WHO) issued a report defining diabetes has quadrupled since 1980, reaching 422 million in 2014. This reflects an increase in disease-related risk factors. In 2019, diabetes was the direct cause of 1.5 million deaths, 48% of which occurred before the age

diabetes as a global epidemic. According to the report, the number of adults living with of 70 [2]. The increase in the number of people with diabetes has also led to an increase in the incidence of diabetic foot infections and peripheral arterial disease.

Globally, adults with diabetes accounted for 463 million in 2019, and this figure is anticipated to rise to 642 million by 2040 [3]. According to the International Diabetes Federation, Egypt is one of the ten countries with the greatest prevalence of diabetes. The *1*number of diabetic patients in Egypt is likely to rise from 9 million in 2019 to 13.1 million by 2035.3 In Egypt, the frequency of DFUs is significant, ranging from 6.1% to 29.3% [4]. About half of the DFUs will be infected across their lifetime [5]. This infection starts superficially, but if the therapy is delayed and immunity is compromised, it can spread to the deeper tissues and cause gangrene and amputations [6].

Many variables, such as age, sex, geographic location, ulcer severity, and ulcer duration could influence the types of bacteria implicated in DFIs and their patterns of antibiotic susceptibility [7, 8]. As the number of DFUs infected with multidrug-resistant (MDR) bacteria is increasing, doctors are faced with a more difficult challenge when treating DFIs due to a limited number of antibiotic choices.

Clinically, when there are two or more major signs of inflammation, we call it DFI (induration, erythema, increased temperature, increased pain, and purulent discharge) [9]. DFI can be classified as moderate, mild or severe and is usually polymicrobial, with multiple bacteria [9, 10]. Gram-positive microbes such as *Staphylococcus aureus* and Gram-negative organisms such as *Pseudomonas aeruginosa* are the most common pathogens [9]. However, the frequency and prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) has increased, which is related to the Clinicians often must use antibiotics analytically until microbial culture results are available. Misdiagnosis of DFI can lead to unnecessary antibiotic overuse or misuse. In addition, due to the widespread use of broad-spectrum antibiotics and the variation of antibiotic resistance genes, the types of pathogenic bacteria and the drug resistance rate of DFI have increased greatly [12, 13]. Past antibiotic treatment may have influenced the bacterial profile of foot ulcers. On the other hand, pathogenic microorganisms on DFU varied and were related to location, economy, environment, lifestyle, and awareness. Therefore, it is important for physicians to carefully select the appropriate antibiotic when treating DFI. The aim of article is evaluate the bacteriological profile and susceptibility pattern of these infections.

2. Materials and methods

2.1. Sample collection

The 100 diabetic foot wound swap samples were collected over a period of 5 months starting from October, 2018 until March 2019 on patients attending the outpatient clinic of

inappropriate use of antibiotics and non-restrictive regulations to control antibiotic abuse [11].

diabetic foot unit, Zefta Hospitals**,** Gharbay, Egypt. The bacteria were isolated from different age, from 62 male there age ranged from 45 to 70 years and from 38 female there age ranged from 40 to 65 years.

2.2. Diabetic Foot Ulcer Bacteria Isolation

Various category of media were used for sample inoculation; namely blood, MacConkey, chocolate and mannitol salt (MSA) (Oxoid, Basingstoke, UK). All plates were incubated at 37°C for 48 hours until growth was observable. Cultures with any size of bacterial growth on at least one inoculated plate were noted as lab positive. When indicating the presence of bacterial growth, we did not take the lower or upper ranges of bacterial growth into account.

2.3. Isolates identification

Note each growing colony characteristics. Pathogens are identified using Gram stain and supplementary biochemical tests. In addition, all isolates were identified at the species level using the VITEK® 2 compact system (bioMérieux, France) according to the manufacturer's instructions antibiotic Susceptibility for clinical isolates

Susceptibility testing of the isolated bacteria to antibiotics was performed according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) was examined using a disc diffusion method [14]. The zone diameter (mm) of the sensitivity was interpreted based on the CLSI guidelines. 10 antibiotics belonging to groups of beta and non-beta-lactam agents by the standard disk diffusion method [15]. The beta-lactam antibiotic discs are penicillins: amoxicillin/clavulanicacid (AMC) (30ug) and Ampicillin/sulbactam (SAM) (20ug). Cephalosporins: cefoperazone (CEP) (75ug), ceftriaxone (CRO) (30ug) and Cefadroxil (CFR) (30 ug). Carbapenems: Imipenem (IPM) (10ug). The non-beta-lactam antibiotic discs are: aminoglycosides: amikacin (AK) (30ug) and Gentamicin (CN) (10ug). Quinolones: Gtifloxacin (GAT) (5ug). Macrolides: Azithromycin (AZM) (15ug).

3. Results and Discussion

3.1.Bacterial isolates distribution from DFU

The bacterial isolation results from 100 diabetic foot patient were divided to 70 had a signal bacterial growth, 22 had double bacterial infection and 8 samples with nonbacterial infection show figure 1.

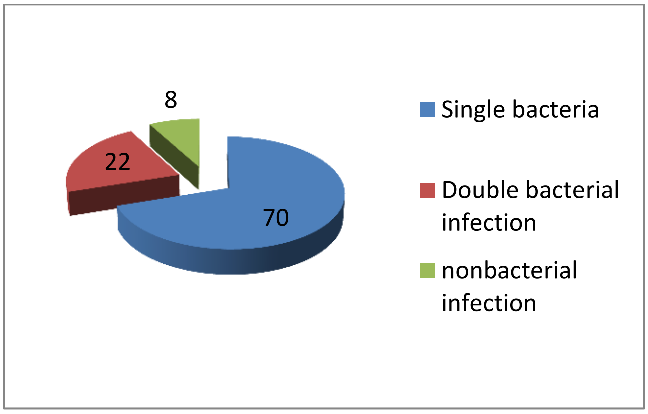

Figure 1: The bacterial isolation results from 100 diabetic foot patient

3.2.Bacterial identification

Gram stain results classified the 70 DFU had with signal bacterial infection to 37 gram-positive and 33 gram-negative isolates. According to the culture characteristics of isolates and deferential media mention at materials and methods section as well as biochemical reactions. Identification of gram-positive and negative bacteria performed using the VITEK® 2 compact system. Results reported that *Staphylococcus aureus* was the most common pathogen for DFU that recorded 25/70 isolates. The second pathogen in DFU was *Escherichia coli* that recorded 14/70 isolates. Results indicated that presence of seven strains another in clinical case were studied as *Streptococcus* 6/70, *Staphylococcus epidermis* 5/70, *Klebsiella pneumonia* 6/70, *Pseudomonas aeruginos* 4/70, *Proteus*

Vulgaris 4/70, *Enterococci* 3/70 and .*Acinetobacter* 2/70. The distribution percentage of pathogenic bacterial strains from diabetic foot ulcer showed at figure 2 and 3.

In a study conducted at a tertiary hospital in São Paulo, Brazil, Gram-positive bacteria were reported accounted for 68.1% of the isolates, whilst Gram-positive bacteria accounted for 68.1% of the isolates, and negative accounted for 31.9% of the isolates [16]. DFI *Enterococcus faecalis* was the most popular bacterium, trailed by *Staphylococcus aureus* and coagulase-negative staphylococci. With Gram-negative pathogens, P. aeruginosa is the most popular pathogen [16]. In a recent 2021 study at Mansoura University Hospital in Egypt, aerobic bacterial infections were detected in 78.8% of DFU. The isolation rate of Gram-negative bacilli (GNB) (56.1%) was higher than that of Gram-positive cocci (GPC) (43.9%) [17]. The most frequently isolated bacteria were *Klebsiella pneumoniae* (26.8 percent), *S. aureus*, and coagulase-negative staphylococci (22 percent each) [17].

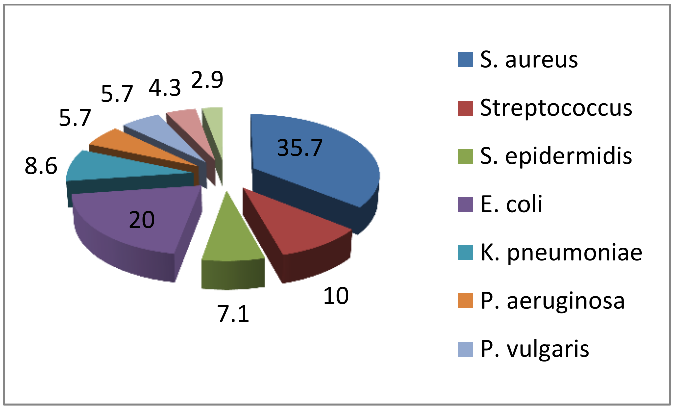

Figure 2: The distribution percentage of 70% single bacterial infection from DFU

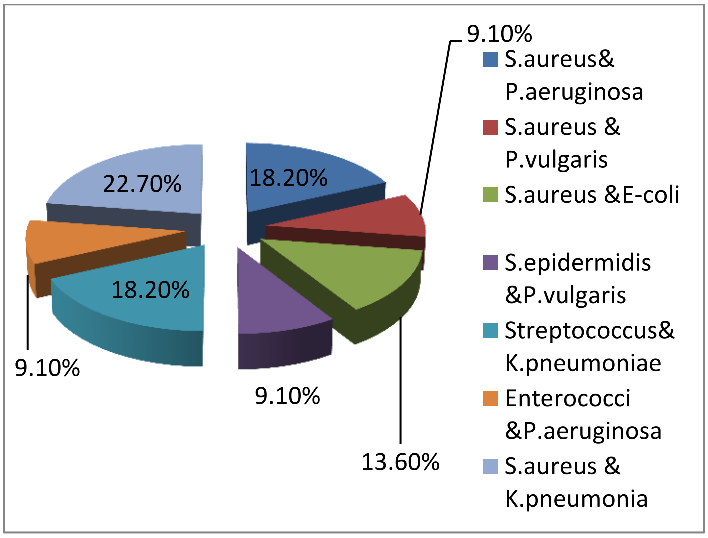

Figure 3: The distribution percentage of 22% double bacterial infection from DFU

3.3.Antibiotic Susceptibility of DFU bacterial strains

The bacterial strains showed variation in the inhibition zone diameter (mm) on different types of antibiotic discs and selected according to their antibiotic resistant against the beta-lactams groups. All bacterial strains were sensitive to IPM, except *Pseudomonas aureginosa* was resistant. IPM was the effective antibiotics against all Gram positive bacteria. All isolates of *P. aeruginosa* resistant to IPM and susceptible to antibiotics AK, CN. Our result shows *Staphylococcus aureus* was susceptible to antibiotics AK, IPM with 100%. The *E. coli* was susceptible to antibiotics IPM with 100%.

Contrary to earlier research, a tertiary hospital in So Paulo, Brazil, proved in 2022 that 89% of cases were ampicillin-susceptible Enterococci and 47% were oxacillin-susceptible Enterococci. *Staphylococcus aureus*, however no coagulase was detected Oxacillin was only effective against staphylococci in 20% of Gram-negative bacteria with excellent susceptibility traits, and *Pseudomonas aeruginosa* was 76% sensitive to ceftazidime and meropenem. Other major Enterobacteriaceae are highly susceptible to ceftazidime, piperacizobactam, and 100% susceptible to meropenem, with the exception of *Klebsiella pneumoniae*, which is 75% susceptible to meropenem [16].

Another study at 2021 in Mansoura University Hospital, Egypt the most active antibiotics were amikacin, tigecycline and meropenem for GNB, and linezolid and vancomycin for staphylococci [17].

4. Conclusion

Gram-positive and Gram-negative bacteria accounted for 52.8% and 47.2%, respectively, of the isolates used to assess the bacteriological profile and susceptibility pattern of DFU. The pathogen with the highest prevalence was *Staphylococcus aureus*, followed by *Escherichia coli*. All bacterial strains, with the exception of *Pseudomonas aureginosa*, were confirmed to be susceptible to IPM by the susceptibility profile. IPM was the only antibiotic that was effective against all Gram positive bacteria. All *P. aeruginosa* isolates are sensitive to antibiotics AK and CN but resistant to IPM. Our findings indicate that *S. aureus* has a 100% susceptibility to the antibiotics AK and IPM. Antibiotics IPM had a 100% success rate against *E. coli*. Evidence-based clinical therapy benefits from knowing a patient's microbiological profile and antibiotic susceptibility.

Table 1: Antibiotics susceptibility of clinical bacterial strains

Pathogens	**Antibiotics**									
	AMC (30ug)	**SAM (20ug)**	**CEP (75ug)**	**CFR (30ug)**	**AZM (15ug)**	**CRO (30ug)**	**IPM (10ug)**	**AK (30ug)**	**GAT (5ug)**	**CN (10ug)**
S. aureus (25)	7(28%)	14(56%)	12(48%)	5(20%)	14(56%)	8(32%)	25(100%)	25(100%)	13(52%)	13(52%)
Streptococcus (7)	2(28%)	4(57%)	3(42%)	2(28%)	4(57%)	2(28%)	7(100%)	3(42%)	2(28%)	7(100%)

S. epidermidis (5)	1(20%)	4(80%)	3(60%)	1(20%)	4(80%)	3(60%)	5(100%)	3(60%)	3(60%)	4(80%)
E. coli (14)	5(35%)	7(50%)	4(28%)	4(28%)	5(35%)	3(21%)	14(100%)	8(57%)	10(71%)	9(64%)
K. pneumonia(6)	1(16%)	6(100%)	2(33%)	2(33%)	2(33%)	1(16%)	6(100%)	2(33%)	0	2(33%)
P. aeruginosa (4)	1(25%)	1(25%)	1(25%)	1(25%)	1(25%)	1(25%)	0	2(50%)	1(25%)	2(50%)
P. vulgaris(4)	1(25%)	3(75%)	2(50%)	1(25%)	3(75%)	1(25%)	4(100%)	2(50%)	4(100%)	3(75%)
Enterococci (3)	1(33%)	3(100%)	2(66%)	2(66%)	1(33%)	0	3(100%)	0	3(100%)	3(100%)
Acinetobacter(2)	0	2(100%)	2(100%)	0	2(100%)	0	2(100%)	0	2(100%)	2(100%)

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