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Isolation and diagnosis of multi-drug resistance antibiotics bacteria in diabetic foot infections of Mansoura University Hospitals

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Abstract: One of the most frequent consequences of diabetes is diabetic foot infection (DFI). The antibiotic susceptibility of the pathogens causing DFI varies depending on the region. The infections that are most likely to be present should therefore be the basis for empirical antibiotic therapy**. The objective** is to enable clinicians in our community select the most appropriate empirical antibiotic for DFI by identifying the common aerobic bacteria that cause DFI and determining their antibiotic susceptibility**. Methods:** 200 diabetic foot ulcers (DFUs) had swabs taken from males and females admitted to Mansoura University Hospitals. Testing for antibiotic susceptibility and bacterial identification by culturing, morphology and biochemical tests of all isolated bacterial cultures. **Results**: A total of 49 clinical isolates of the four different microorganisms were cultured and sensitivity tested towards 14 antibiotics [(Cefazolin, Ceftazidime, Cefotaxime, Ciprofloxacin, Meropenem, CEP, Ampicillin, Amoxicillin + clavulanic acid, Piperacillin, Trimethoprim, RTE, SAM and Amikacin)] to determine the extent of the bacteria's resistance to antibiotics. The results in the current study revealed that there were statistically significant difference between strain resistance to ceftazidime , As the results showed that there was a statistically significant difference between strain resistance to CEP, also registered a statistically significant difference between strain resistance to Amoxicillin + clavulanic acid, Likewise for the resistance to RTE, SAM and Amikacin, The study also showed that *Staphylococcus* bacteria had the highest rate of resistance to different tested antibiotics. **Conclusions**: *S. aureus* is the most common cause of diabetic foot infection at our study, at the same time, it is the most resistant to antibiotics.

keywords: Diabetic foot infection, *E. coli*, Staphylococcus aureus , *Streptococcus mutans* , Antibiotic resistance

1.Introduction

For the tertiary health sector, diabetic foot infections (DFI) pose a significant risk to morbidity, mortality, and economics. However, a lack of solid evidence supporting certain treatment plans for DFI patients could lead to uneven therapy **(1)**. It is anticipated that the number of diabetics will rise quickly, from 425 million in 2017 to 600 million by 2030. Over one-third of individuals with diabetes will at some point in their lives develop diabetic foot ulcers (DFUs), of which half will get infected and result in diabetic foot infections (DFIs). In 15% of cases, lower limb amputation is necessary to stop the infection from getting worse **(2)**.

The biological process of wound healing is dynamic and intricate, regain skin function following harm. However, this mechanism is hindered in diabetes circumstances. Many risk factors that hinder and delay the normal wound healing process are present in diabetics, including hyperglycemia, extended hypoxia, chronic inflammation, peripheral neuropathy, poor neovascularization, and problems combating infections. This leads to the development of persistently pro-inflammatory wounds that do not heal over time. Furthermore, bacterial colonies infect roughly

60% of DFUs, which adds to the chronic wound healing failure **(3).**

Direct eradication of microorganisms can be achieved by AMPs through mechanisms such as membrane disruption, interaction with intracellular targets, recruitment, and activation of immune cells. These molecules can also promote wound healing by re-epithelization, support of angiogenesis, and enhancement of extracellular matrix synthesis. Thus, AMP-based approaches may be a good solution to fight the emergence of antimicrobial resistance **(3).** The antibiotic susceptibility of bacterial cells is affected by their physiological states. One important consequence of this phenomenon is the occurrence of “per sister” cells. Thus, it was discovered early that even high concentrations of antibiotics do not kill all of the bacterial population, leaving behind a per sister population that is genetically identical to the susceptible cells **(4).**

in poor nations, are infectious illnesses. Antibiotic resistance is also a major worldwide health concern that compromises patient prognoses, increases treatment costs and demands on the healthcare system by requiring the use of second or third lines of antibiotics and lengthening treatment durations. Bacteria will certainly continue to evolve strategies to modify pertinent characteristics in order to withstand exposure to novel antibiotics through mutations or DNA exchange (horizontal gene transfer), giving rise to what are known as "superbugs."This typically refers to situations in which the microbe is resistant to two or more distinct antibiotic classes **(5)**. *Staphylococcus aureus* is still the most frequently isolated bacteria in instances of osteomyelitis, although polymicrobial infections are more common (6).**).** It could comprise microorganisms belonging to the Enterobacteriaceae, *Pseudomonas, Streptococci*, and Enterococci families **(7).** Additionally, there has been evidence of a 15% – 30% frequency of methicillin-resistant *S. aureus* **(8,9).** in addition to being recognized as a re-hospitalization risk factor **(10).** Several studies on DFUs confirmed disorders with multidrug-resistant organisms (MDROs) (**11**) and they were found in as many as 53% of individuals (**12**).Recently, Dai *et al.* (**13**) conducted a meta-analysis and found that ischemic aetiology, greater ulcer size, more severe ulcer classification, osteomyelitis, prior history of antibiotic medication, and institutionalization are risk factors for the development of MDRO in DFUs. Regarding the clinical results resulting from MDRO in DFU, multiple studies have considered factors including the ulcer's evolution period (**14**). Death, prosthesis, and being hospitalized extension The impact of these characteristics on DFU remains unclear, as some studies have found no link between them and this type of infection, while others have found one **(15).**

2. Materials and methods

This study was performed on 49 *E. coli*, *Proteus* sp, *Pseudomonas* sp and *Staphylococcus* isolates that were randomly selected and isolated from cultured plates of different wound swap samples from patients who were admitted to different Mansoura University Hospitals from July 2021 to July 2022. The laboratory procedures were carried out at: (i) Microbiology Diagnostic and Infection Control Unit (MDICU) of the Medical Microbiology and Immunology department at the Faculty of Medicine, Mansoura University. (ii) Medical Microbiology and Immunology department at the Faculty of Medicine, Microbiology Department at the Faculty of Science.

Media used for isolation, purification, identification, maintenance and sensitivity of the bacterial isolate were: MacConkey agar medium, Blood agar medium, Chocolate Agar medium, Nutrient agar and Mueller Hinton agar medium for sensitivity test, and then the biochemical tests performed for cultures to identify the bacteria. After that direct microscopic examination (gram stain film) performed Culture**:** samples will be inoculated on blood agar, MacConkey agar and Chocolate agar plates and incubated aerobic and anaerobic at 37° C for 24-28hrs. **Identification:** positive growth will be identified by Gram stained film, colony morphology and biochemical reactions namely, catalase, oxidase, urease, Simmons citrate utilization and methyl red as per the standard method **(16).**

3. Results

200 swabs were obtained from Patients of both sexes (130 males and 70 females) attending the emergency and counseling units

of Mansoura University Hospitals. Each sample was cultured on a specific medium to investigate bacteria within the study, which is (*Escherichia coli*, *Pseudomonus* spp., *Proteus mirablas*, *Staphylococcus aureus*) The most dominant microorganisms were *E.coli* and *Staphylococcus aureus* respectively. It has been noted that the infection rate of DFU for males is approximately twice that of females, as shown in the **Figure (1)**.

When comparing the percentage of resistance of different strains of the four studied bacterial groups to antibiotics in this study. The results revealed that there was statistically significant difference between strain resistances to ceftzidime **(Table 1 and Figure 2)**. In this connection, as the results showed that there was statistically significant difference between strain resistance to CEP (table 4-6) also registered a statistically significant difference between strain resistance to Amoxicillin + clavulanic acid, Likewise for the resistance to RTE, SAM and Amikacin.

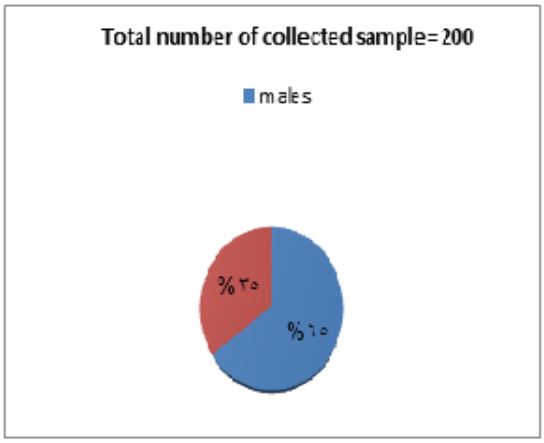

Figure (1); The percentage of infected mals and females of DEU (samples = 200)

Table (1): distribution of antibiotic resistance among different types of studied bacteria.

Resistance of bacteria to antibiotics	***Staphylococcus aureus***	***E-coli***	***Proteus mirabilis***	***Pseudomonas spp.***
	N(%)	**N(%)**	**N(%)**	**N(%)**
Cefazolin (CZ)	49(100)	40(81.6)	49(100)	47(95.9)
Ceftazidime (CIP)	49(100)	44(89.8)	40(81.6)	49(100)
Mikacin (MEM)	44(89.8)	46(93.9)	49(100)	47(95.9)
Ampicillin (AM)	43(87.8)	49(100)	49(100)	45(91.8)
Ciproflin (CEP)	3(6.1)	49(100)	32(65.3)	0(0)
Cefotaxime (CTX)	49(100)	46(93.9)	41(83.7)	47(95.9)
Trimethoprim (TR)	49(100)	41(83.7)	45(91.8)	49(100)
Amikacin (AM)	30(61.2)	38(77.6)	49(100)	39(79.6)
Piperacillin (PRL)	32(65.3)	46(93.9)	43(87.8)	47(95.9)
Amiklin (AK)	49(100)	49(100)	6(12.2)	49(100)
Ciprofloxacin (CAZ)	49(100)	8(16.3)	41(83.7)	49(100)
Amoxicillin (AMC)	43(87.8)	7(14.3)	43(87.8)	47(95.9)
Rlavulane (RTE)	37(75.5)	49(100)	42(85.7)	41(83.7)

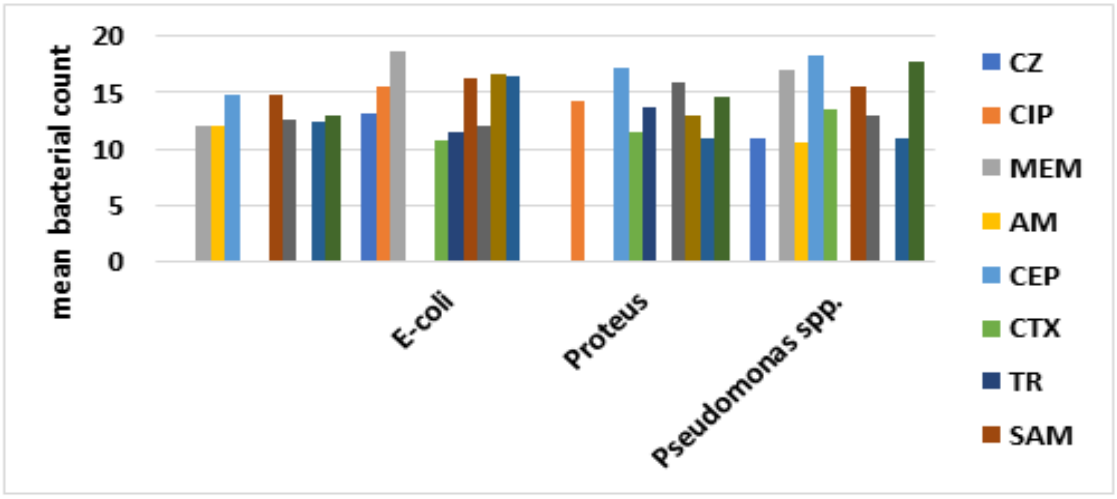

Figure (2): effect of antibiotics on bacterial count

Minimum inhibitory concentrations (MIC) of antibiotic resistance among different types of studied clinical isolates bacteria

Table (2) and Figure (3) lists the widths of the inhibition zones of different antibiotics against studied clinical isolates. that there was statistically significant difference between strain resistance to CEP, also registered a statistically significant difference between strain resistance to Amoxicillin + clavulanic acid, Likewise for the resistance to RTE, SAM and Amikacin, The study also showed that *Staphylococcus* bacteria had the highest rate of resistance to different tested antibiotics.

Table (2): Minimum inhibitory concentrations (MIC) of antibiotic resistance among different types of clinical studied bacteria.

Bacteria count	Staphylococcus aureus	E-coli	Proteus mirabilis	Pseudomonas spp.
	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD
CZ Cefazolin	NA	13.22±2.5	NA	11.0±1.41
CIP Ceftazidime	NA	15.60±3.85	14.22±3.80	NA
MEM Mikacin	12.0±3.74	18.67±3.06	NA	17.0±1.41
AM Ampicillin	12.0±2.19	NA	NA	10.50±2.52
CEP Ciproflin	14.74±3.25	NA	17.23±2.91	18.24±1.74
CTX Cefotaxime	NA	10.67±2.31	11.50±1.69	13.50±4.95
TR Trimethoprim	NA	11.50±2.07	13.75±3.86	NA
AM Amikacin	14.84±2.95	16.27±1.10	NA	15.60±1.65
PRL Piperacillin	12.58±1.91	12.0±0.0	15.83±4.62	13.0±1.41
CAZ Ciprofloxacin	NA	16.70±2.3	13.0±2.82	NA
AMC Amoxicillin	12.33±1.97	16.36±2.63	11±1.67	11±1.41
RTE Rlavulane	13.0±4.43	NA	14.57±2.51	17.75±3.77

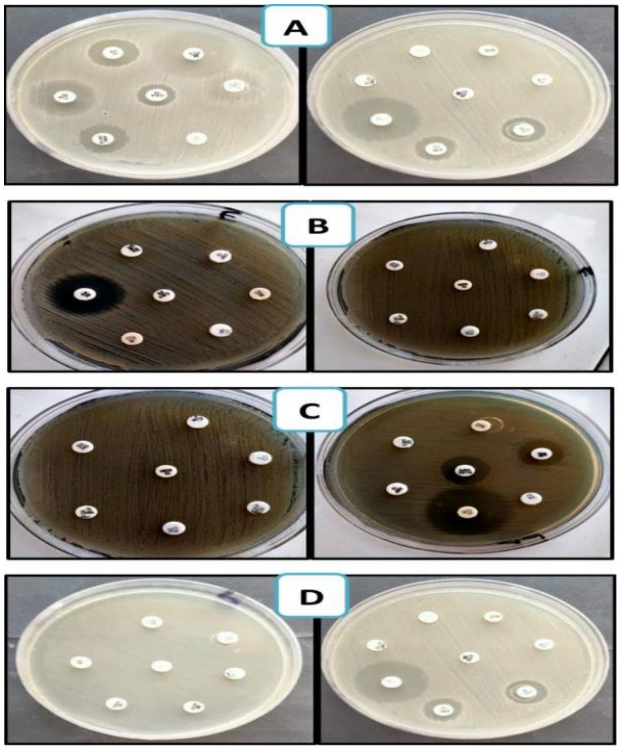

Figure (3): Inhibition zones of (A): *Staphylococcus aureus* (B): *E.coli* (C): *Proteus mirabilis* (D): *Pseudomonas* spp resistance to different antibiotics.

Discussion:

Isolating microbial strains from individuals with diabetic foot illness and assessing their antibiotic sensitivity is a crucial step in the diagnosis and treatment of infections **(17).** Samples of tissue or secretions associated with diabetic foot need to be collected to identify the bacteria or fungi that may be causing the infection **(18)**. These microbial strains undergo a series of assays to ascertain their antibiotic sensitivity after being isolated, and they are cultivated in a suitable lab medium **(19)**. This test's objective is to determine whether antibiotics could be useful against the isolated strains **(20)**.

In our investigation, *Staphylococcus aureus* exhibited 100% resistance to Cefazolin, Ceftazidime, Cefotaxime, Ciprofloxacin, Trimethoprim, and Amikacin; 89.7% resistance to Meropenem and Amoxicillin + clavulanic acid, 87.7% resistance to Ampicillin, 75.5% resistance to RTE, 67.3% resistance to Piperacillin, and 63.2% resistance to CEP. The dangerous bacteria Staphylococcus aureus, also referred to as the "staph" bacterium, can cause ulcers on the feet in diabetic patients **(21)**. The emergence of antibiotic resistance in Staphylococcus aureus is a major worry in the medical community because it reduces the efficacy of antibiotic therapy **(22)**.

It is concerning that *Staphylococcus aureus* is becoming more resistant to antibiotics

because these infections are associated with a higher risk of disease and mortality **(23).** First-generation cephalosporins include cefazolin, while third-generation cephalosporins include ceftazidime and cefotaxime **(24).** When treating *Staphylococcus aureus* infections, the existence of antibiotic resistance suggests the need for other therapeutic alternatives **(22).** The ability of bacteria to resist the effects of ciprofloxacin, a type of fluoroquinolone antibiotic frequently used for a variety of bacterial illnesses, is referred to as ciprofloxacin resistance **(25).** With the frequency with which fluoroquinolones are used to treat a variety of ailments, the ciprofloxacin resistance is worrying. Due to the significant level of resistance, this antibiotic must be used more sparingly and carefully **(26).**

E. Coli was resistant to CEP, Ampicillin and Amikacin by 100%, Piperacillin (95.9%), Cefotaxime and Meropenem (93.8%), Ciprofloxacin (89.7%), Trimethoprim (83.6%), SAM (79.5%) but it was resistant to Amoxicillin + clavulanic acid and RTE only by 18.3%. According to **(27)**, the *Escherichia coli* bacteria that were examined showed resistance to cefadroxil but sensitivity to levofloxacin, amikacin, ceftriaxone, cefotaxime, imipenem, gentamicin, meropenem, and other drugs. According to another study, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, and *Staphylococcus aureus* were the most common bacteria recovered from people with diabetic foot ulcers. More isolated Gram-positive bacteria than isolated Gram-negative bacteria were found. *Staphylococcus aureus* and *Streptococcus pyogenes* both showed notable resistance to oxacillin and benzopenicillin. On the other hand, many *Pseudomonas aeruginosa* strains showed resistance to piperacillin/tazobactam, meropenem, and imipenem. *Escherichia coli* has demonstrated resistance to cefepime, ceftazidime, ticarcillin, and aztreonam.

There is a noticeable level of antibiotic resistance in *Klebsiella pneumoniae*. According to Ali and Kamil (2022), *Proteus mirabilis* is resistant to trimethoprim/sulfamethoxazole, cefepime, ceftazidime, gentamicin, meropenem, piperacillin/tazobactam, and tobramycin. *Proteus mirabilis* was resistant to Ampicillin, and SAM by 100%, Meropenem (97.9%), Cefazolin (95.9%), Trimethoprim (91.8%), Amoxicillin + clavulanic acid (89.7%), Piperacillin (87.7%), RTE (85.7%), and Ceftazidime, Cefotaxime and Ciprofloxacin by (83.6%) but it was less resistant to Amikacin only by 16.3%.

Proteus mirabilis is the name for gram-negative bacteria that belong to the Enterobacteriaceae family and have gram-negative cell walls. Numerous illnesses are caused by it, including over 3% of infections acquired in healthcare settings and up to 44% of urinary tract infections associated with catheter use **(29)**. An extremely important bacterium that was found in 18% of patients is frequently to blame for diabetic foot ulcer infections **(30)**. The virulence factors of *P. mirabilis* are encoded by genes that are either integrated into the chromosome or imported from outside of it **(31)**. Ceftazidime, Ciprofloxacin, RTE, and Amikacin were the antibiotics that Pseudomonas was resistant to 100% of the time; Cefazolin, Meropenem, and Cefotaxime were the antibiotics that Pseudomonas was resistant to (95.9%), Ampicillin and Trimethoprim (91.8%), SAM (79.5%), and Amoxicillin + clavulanic acid

The significant levels of antibiotic resistance found in *Pseudomonas*, particularly to many commonly used antibiotics, pose a serious threat to the effective treatment of bacterial illnesses **(32).** Given that these antibiotics are frequently used to treat various bacterial illnesses, the fact that they are 100% ineffective against Ceftazidime, Ciprofloxacin, RTE, and Amikacin is alarming. Insufficient options for treating Pseudomonas infections can lead to treatment failures and worsening health issues **(33).**

Conclusions:

Diabetes sufferers frequently develop foot ulcers. They commonly contract the infection and have a lifetime cumulative incidence of up to 25%. One of the main causes of lower limb amputation is the spread of infection to soft tissue and bone. This study underscores the need to prioritize patient-centered care for persons suffering from diabetic foot ulcers. The findings provide opportunities for tailored treatment choices that take into account the distinctive attributes of each patient's disease.

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