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# Isolation and identification of aerobic bacterial species isolated from diabetic foot infections and determination of their sensitivity to several antibiotics

Zainab Youssef Mohammed[1]* ، Assistant Professor Haifa Rajab Alwan[2]
Department of Biology, College of Education for Pure Sciences, University of Tikrit, Iraq
*Corresponding author zy2408pep@st.tu.edu.iq
Email (2nd Author) hyafaass@tu.edu.iq

**Abstract:**

This study aimed to isolate and identify bacterial species responsible for diabetic foot infections and to determine their antibiotic resistance patterns. A total of 100 clinical samples were collected from patients with diabetic foot infections at hospitals in Tikrit between July and December 2024. The samples included wound swabs and blood specimens. Bacterial culture revealed positive growth in all samples. Identification was conducted using morphological, microscopic, biochemical tests, and for some isolates, the VITEK-2 system. The findings revealed that Gram-negative bacteria constituted the majority (71%) of isolates, including *Klebsiella pneumoniae*, *E. coli*, Pseudomonas aeruginosa, and *Proteus mirabilis*. Gram-positive bacteria accounted for 29% of the isolates, with Staphylococcus aureus and S. epidermidis being the most prevalent. Antibiotic susceptibility tests indicated high resistance to many commonly used antibiotics, particularly penicillins and cephalosporins, reflecting a concerning trend of multidrug resistance.

**Keywords** : :*Klebsiella pneumonia, Pseudomonas aeruginosa* ، *Proteus mirabilis*، *E. coli*، , Diabetic foot infections, Bacterial pathogens .

# عزل وتشخيص الأنواع البكتيرية الهوائية المعزولة من اخماج القدم السكري وتحديد حساسيتها تجاه العديد من المضادات الحيوية

زينب يوسف محمد ، هيفاء رجب علوان

**مستخلص:**

يهدف هذا البحث إلى عزل وتشخيص الأنواع البكتيرية المسببة لعدوى القدم السكرية وتحديد أنماط مقاومتها للمضادات الحيوية. تم جمع 100 عينة سريرية من المرضى المصابين بعدوى القدم السكرية من مستشفيات مدينة تكريت خلال الفترة من تموز إلى كانون الأول 2024، وشملت مسحات من الجروح وعيّنات دم. أظهرت نتائج الزرع البكتيري نموًا في جميع العينات، وتم تحديد البكتيريا باستخدام الفحوصات المورفولوجية والمجهرية والكيميائية الحيوية، بالإضافة إلى نظام VITEK-2 لبعض العزلات. كشفت الدراسة أن البكتيريا السالبة لصبغة غرام شكّلت النسبة الأكبر (71٪)، وتضمنت أنواعًا مثل *Klebsiella pneumoniae*، *E. coli*، Pseudomonas aeruginosa، و *Proteus mirabilis*. أما البكتيريا الموجبة لصبغة غرام فمثّلت 29٪، وتضمنت أنواعًا مثل Staphylococcus aureus و S. epidermidis. أظهرت اختبارات الحساسية للمضادات الحيوية مقاومة مرتفعة تجاه العديد من المضادات، خاصة البنسلينات والسيفالوسبورينات، مما يعكس مشكلة متفاقمة في مقاومة الأدوية.

**الكلمات المفتاحية** : *Klebsiella pneumoniae* ، *Pseudomonas aeruginosa* ، *Proteus mirabilis*، *E. coli*، داء قدم السكري ، العدوى البكتيرية .

## Introduction:

Diabetes occurs when the pancreas fails to produce enough insulin, or when the body cannot effectively use the insulin it secretes. As a result of microcirculatory dysfunctions and blood vessel problems, diabetics face a range of complications. Common complications include neuropathy, nephropathy, obesity, high blood pressure, and increased susceptibility to several viral diseases. Additionally, Poor blood sugar management can lead to life-threatening health complications, such as heart disease, foot damage, hearing loss, fungal and bacterial infections, Alzheimer's disease, memory loss, and depression (Kim, 2019). In industrialized countries, Unhealthy eating habits and a lack of physical exercise are modifiable risk factors for overweight and obesity. Compared to individuals who engage in regular physical activity, The prevalence of type 2 diabetes is two to four times greater among those with lower levels of physical activity. (Luisi *et al*., 2019) Type 2 diabetes (T2DM) affects 422 million people worldwide, representing 85-90% of all diabetes patients. The number of individuals diagnosed with this disease has increased significantly over the past decade, due to changes in lifestyle. (Kyu *et al*., 2016)

Although it is difficult to define specific cases of diabetes, the majority of cases can be divided into two categories: T1DM and T2DM. Gestational diabetes is a type of glucose intolerance that develops or is first noticed during pregnancy. (Amed and Oram, 2016)

Type 1 diabetes mellitus (T1DM) is an autoimmune disorder resulting from a complex interplay of genetic, environmental, and immunological variables, culminating in the loss of pancreatic cells that produce insulin in the islets of Langerhans. causing severe insulin deficiency. Although cytotoxic CD8+ T cells and macrophages have been noted in abundance in T1DM, rendering them the most conspicuous cells entering the insulin-deficient islets, numerous studies have also emphasized the importance of other types of cells infiltrating the islets, such as leukocytes and macrophages (MCs). (Dong *et al*., 2020). (5) Type 2 diabetes mellitus (T2DM) is the predominant

kind of diabetes, sometimes referred to as non-insulin-dependent diabetic mellitus. Elevated blood glucose levels occur as a result of insulin deficiency and insulin resistance, both of which may be caused by obesity. Obesity correlates with inflammation in adipose tissue, marked by the infiltration of immune cells, including proinflammatory macrophages (M1), neutrophils, and natural killer cells (NKs). as well as T helper cells (Th1) and T helper cells (Th17), as well as B cells and mast cells (MCs). (Elieh *et al*., 2020). Gestational diabetes mellitus (GDM) is diabetes that develops during pregnancy, also known as gestational diabetes. It is identified during the second or third trimester of gestation. Post-delivery, blood glucose levels may normalise, or diabetes may manifest thereafter. Including foetal abnormalities, newborn obesity, cardiac anomalies, maternal hypertension, and obstetric problems. The fasting blood glucose level is 126 mg/dL, and the random blood glucose level is 199 mg/dL, blood glucose levels are above normal but below normal (Abbas, 2021)

Diabetic foot This condition is one of the most serious complications associated with diabetes and is the leading cause of hospitalization for patients with this disease. 1, 2 Patients with diabetes face a lifetime risk of up to 25% of developing foot ulcers. , Diabetic ulcers increase the risk of amputation by 15 to 46 times compared to foot ulcers resulting from other causes. Each year, more than one million patients with diabetes require care. Poor microcirculation in patients with diabetic foot impedes the flow of macrophages, contributing to an increased risk of infection. 2, The growing correlation between multidrug-resistant bacteria and diabetic foot ulcers exacerbates the issue. The difficulties encountered by physicians and surgeons in managing these ulcers without resorting to amputation. (Khanolkar *et al.,*2008).

## Material and methods

### Collection of Samples:

A total of 100 clinical samples were collected in this study, including swabs and blood samples, from patients with diabetic foot infections, both inpatients and outpatients, at Tikrit Teaching Hospital and its outpatient clinics. The pa-

tients represented various age groups and both sexes, during the period from July 21, 2024, to December 28, 2024. In addition, blood samples were collected from a control group consisting of 5 healthy individuals and 5 diabetic patients without foot ulcers.

**A. Swabs**

Swab samples were collected exclusively from infected foot areas and immediately placed in sterile tubes containing transport media to preserve the viability of the microorganisms until processing.

**B. Culture**

Swab specimens from diabetic foot ulcers were cultured on Blood Agar, MacConkey Agar, and Mannitol Salt Agar. The culture plates were cultured aerobically at 37°C for 24 hours to identify the responsible bacterial pathogens. After primary culture, the isolates were purified by subculturing onto appropriate selective and differential media to ensure the growth of pure colonies for further identification.

**Antibiotic Sensitivity Test**

Antibiotic susceptibility testing was conducted for all bacterial isolates against 12 commonly used antibiotics, as listed in Table 1 The Kirby-Bauer The disc diffusion technique was used using Mueller-Hinton Agar. following the guidelines described by (Lepp ,2010) The resistance profiles of the isolates were assessed by measuring the diameters of the inhibition zones using the Scan 4000 equipment. (Interscience) in line with the requirements set out by the Clinical and Laboratory requirements Institute (CLSI, 2025) To create the bacterial inoculum, 1–2 new bacterial colonies were put into a test tube containing 5 ml of normal saline, and the suspension was adjusted to achieve a turbidity corresponding to the 0.5 McFarland standard, roughly $1.5 \times 10^8$ CFU/ml. A sterilised cotton swab was immersed in the solution. The surplus fluid was extracted by applying pressure with the brush on the inner surface of the tube. The swab was then streaked uniformly in three directions over the surface of pre-prepared Mueller-Hinton Agar plates to guarantee equal dispersion. The plates were allowed to dry for 4 to 5 minutes. Antibiotic discs were then positioned on the surface using sterile metal forceps, applying mild pressure to achieve

adequate adhesion. Each plate had six antibiotic discs. The plates were incubated in aerobic conditions at 37°C for 24 hours. Following incubation, the diameters of the inhibitory zones were measured in millimetres using a ruler, and the findings were analysed in accordance with the worldwide criteria established by CLSI 2025.

## Results:

### Isolation and Identification of Bacterial Species:

The results showed that 100% of the collected samples were positive for bacterial growth on the utilized culture media. One hundred samples were obtained from inpatients and outpatients at Tikrit Teaching Hospital and its outpatient clinics, all diagnosed with diabetic foot infections. Samples were collected from male and female patients across diverse age ranges. during the period from July 21, 2024, to December 28, 2024. These swabs were collected using sterile cotton swabs and were specifically taken from infected foot wounds. These swabs were diagnosed based on the cultural characteristics of the morphological diagnosis, microscopic examination, biochemical tests, and some of them using the Vitek-2 technique. The results showed a percentage of 100%. The number of positive samples was distributed based on the type of bacteria, as 29 samples were positive bacteria, or 29%, while 71 samples were negative bacteria, or 71%, as shown in the table.1.

**Table (1) presents the distribution of swab samples according to bacterial growth:**

<table>
<tr><th colspan="2">Notes</th><th>Percentage</th><th>Number of isolates</th><th>Bacteria species</th></tr>
<tr><td rowspan="5">Gram p isolation</td><td rowspan="5">29<br>(29%)</td><td>20%</td><td>20</td><td>Staphylococcus aureus</td></tr>
<tr><td>4%</td><td>4</td><td>Staphylococcus epidermidis</td></tr>
<tr><td>3%</td><td>3</td><td>Staphylococcus saprophyticus</td></tr>
<tr><td>1%</td><td>1</td><td>Staphylococcus xylosus</td></tr>
<tr><td>1%</td><td>1</td><td>Staphylococcus pseudintermedius</td></tr>
<tr><td rowspan="10">Gram negative isolation</td><td rowspan="10">71<br>Isolation<br>(71%)</td><td>19%</td><td>19</td><td>Klebsiella pneumonia</td></tr>
<tr><td>15%</td><td>15</td><td>E. coli</td></tr>
<tr><td>11%</td><td>11</td><td>Pseudomonas aeruginosa</td></tr>
<tr><td>9%</td><td>9</td><td>Citrobacter freundii</td></tr>
<tr><td>9%</td><td>9</td><td>Proteus mirabilis</td></tr>
<tr><td>4%</td><td>4</td><td>Enterococcus faecalis</td></tr>
<tr><td>1%</td><td>1</td><td>Serratia marcescens</td></tr>
<tr><td>1%</td><td>1</td><td>Proteus vulgaris</td></tr>
<tr><td>1%</td><td>1</td><td>Enterobacter aerogenes</td></tr>
<tr><td>1%</td><td>1</td><td>Pseudomonas luteola</td></tr>
<tr><td colspan="3">100%</td><td>100</td><td>Total Isolation</td></tr>
</table>

**Laboratory Identification**

**Morphological Identification**

Isolates were initially identified based on their morphological characteristics, particularly when grown on culture media. Culture findings indicated that some isolates proliferated on mannitol salt agar, exhibiting golden yellow colonies. The colour of the medium was altered from pink to yellow due to mannitol fermentation. These characteristics are indicative of S. aureus bacteria. Meanwhile, the growth of creamy white colonies that did not ferment mannitol indicates the growth of S. epidermidis and S. saprophyticus bacteria. On blood agar, they were hemolytic, as shown in Figure 1

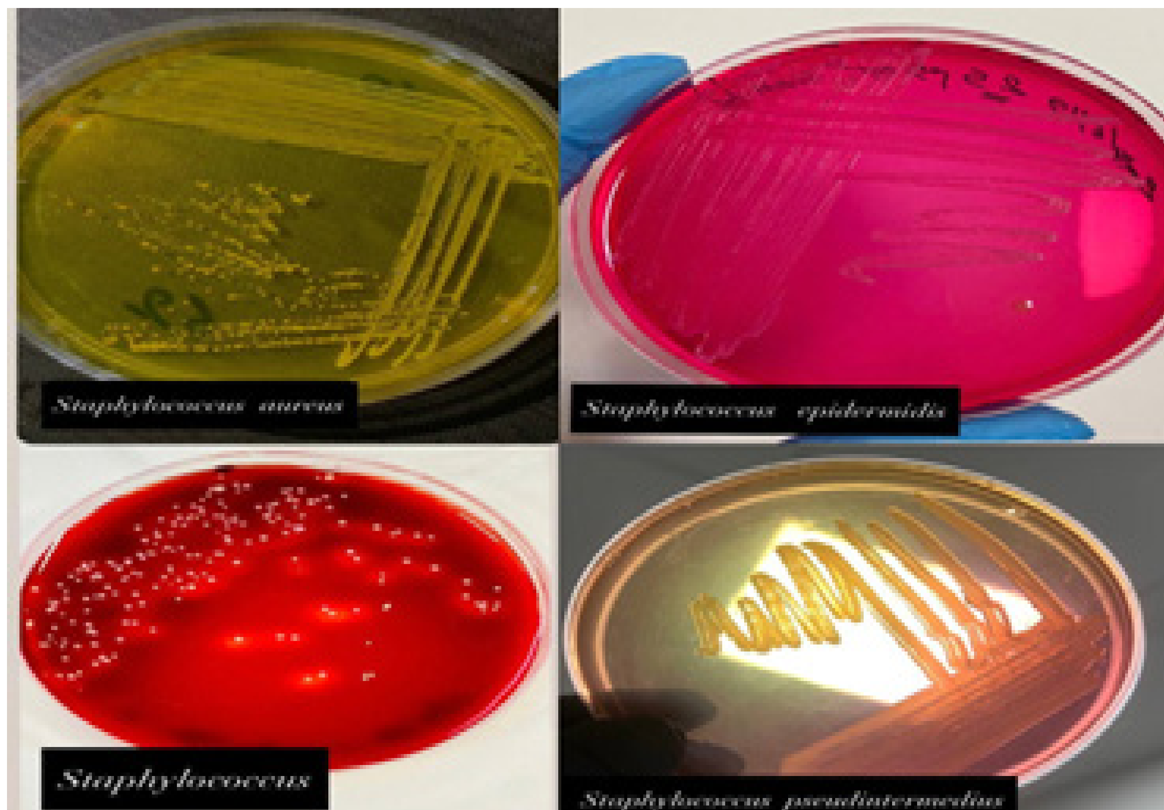



**Figure.1** Bacterial species growing on different media

The remaining set of isolates was cultivated on MacConkey Agar medium, where their colonies exhibited a light hue and had an odour reminiscent of fermented grapes, attributable to their failure to ferment lactose sugar. When they were grown on Nutrient Agar medium, the growing colonies appeared bluish-green as a result of their secretion of the pigment pyocyanin, which is considered one of their important diagnostic characteristics, as they appeared yellowish-green as a result of their secretion of the pigment pyoverdin. As a result, all these phenotypic characteristics are attributed to the bacteria Psedomonas spp. While other colonies that appeared pale pink fermenting lactose and appeared bright green on EMB agar medium indicate the growth of *E. coli* bacteria, while colonies that appeared pink mucous color due to the presence of the capsule indicate the growth of K. pneumonuae bacteria, while colonies that produce colorless (transparent) or pale color irregular borders or flattened with a moist surface and growing and spreading in

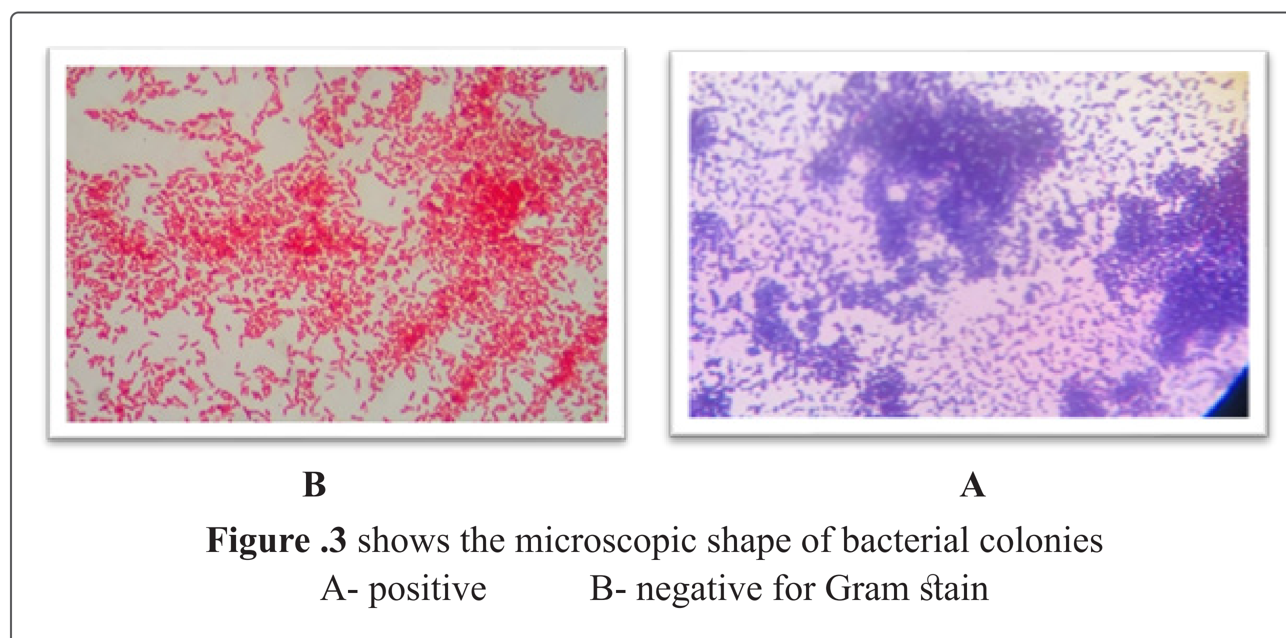
the medium in a swarming manner indicate the growth of Proteus bacteria. As for colonies that are mostly smooth, round, shiny, and non-fermenting lactose, they indicate the growth of Serratia, as shown in Figure .2

**Figure 2**. Gram-negative bacterial species growing on different media

**Microscopic Identification**

Bacterial cells were identified microscopically by staining with Gram stain. This was done by taking a single young colony of each bacterial species and smearing it on microscopic slides with a drop of physiological solution. The samples were mixed well and left to dry at room temperature. They were then flame-fixed, stained with Gram stain, and examined under a light microscope using an oil-based lens at 100x magnification. The shape, regularity, and method of grouping and interaction with the stain were observed. Some of these cells appeared under the microscope as small, purple clusters, indicating Gram staining (positive). These characteristics are consistent with the genus Staphylococcus,

according to (Atlas etal., 1995.)The other type of cells appeared under the microscope as single or double rods, or short, pink chains, due to their lack of Gram staining and the persistence of the safranin stain, making them negative. For Gram negative staining, these characteristics are attributed to Gram negative bacteria, including the genera Pseudomonas, *K.pneumoniae*, E.coli, Proteus, and Serratia. (Brooks *et al*., 2013) as shown in Figure.3 .



B A

**Figure .3** shows the microscopic shape of bacterial colonies
A- positive B- negative for Gram stain

**Biochemical Identification**

Biochemical analysis was performed on the bacterial isolates to determine their biological characteristics and differentiate between them. A set of standard tests was used, each with a specific objective that reveals a particular enzymatic or metabolic property, as shown in Table 2. An oxidase test was conducted to identify the presence of the enzyme cytochrome oxidase.which contributes to the electron transport chain in aerobic bacteria. It was positive in Pseudomonas aeruginosa and *Proteus mirabilis*, indicating aerobic activity. *E. coli* and *Klebsiella pneumoniae* were negative, indicating that they do not rely on oxygen as a final receptor. The catalase test was used to detect the ability of bacteria to decompose hydrogen peroxide into water and oxygen. All species tested positive, indicating that all isolates were aerobic or facultative aerobes. The

urease test was used to determine the ability of bacteria to decompose urea to produce ammonia and carbon dioxide. Proteus spp. and Klebsiella tested positive, indicating the presence of the urease enzyme. *E. coli* tested negative, indicating the absence of this enzyme (Leboffe, Pierce 2020). The indole test was used to detect the ability of bacteria to decompose tryptophan to produce indole. *E. coli* and *Proteus vulgaris* gave positive results, indicating tryptophanase activity, while *Klebsiella pneumoniae* was negative. The methyl red (MR) test was performed to detect the production of strong acids resulting from glucose fermentation. *E. coli* and *Citrobacter freundii* were positive, indicating their ability to produce strong acids, while Klebsiella pneumonia was negative (Forbes *et al*., 2007). The Voges-Proskauer (VP) test was performed to detect the production of acetoin resulting from an alternative fermentation pathway to glucose. It was positive in *Enterobacter aerogenes* and *Klebsiella pneumoniae*, indicating their use of the butylene glycol pathway. The H2S production test was positive in Proteus spp. and *Citrobacter freundii*, reflecting the activity of sulfur-containing enzymes, while they were negative in *E. coli* (Willey *et al*., 2017). In carbohydrate fermentation tests (glucose, lactose, sucrose), the ability of bacteria to metabolize sugars to produce acid or gas was determined. For *E. coli*, the ratio was A/A, indicating complete fermentation, while for Pseudomonas aeruginosa, the ratio K/K indicated the absence of fermentation. In the citrate utilization test, the result was positive for Klebsiella, Enterobacter, and Proteus, indicating the presence of the citrate permease enzyme, and negative for *E. coli*. It is used to assess bacteria's capacity to utilise citrate as the only carbon source. The nitrate reduction test produced a positive result in Pseudomonas aeruginosa and Proteus spp., showing the existence of an anaerobic respiration mechanism, but a negative result in the other species. This test revealed bacteria's capacity to degrade nitrate to nitrite, or gaseous nitrogen. (Tortora *et al*., 2019**).**

**Table . 2** Biochemical Tests of Gram-Negative Bacterial Species

| Bacterial Species | Oxidase Test | Catalase Test | Indole Test | Methyl Red (MR) Test | Voges-Proskauer (VP) Test | Citrate Utilization | Glucose/Lactose/Sucrose Fermentation | Urease Test | Lactose Fermentation on MacConkey Agar | H2S Production | Motility Test |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *E. coli* | - | + | + | + | - | - | + / - / A/A | - | + | - | + |
| *Klebsiella pneumoniae* | - | + | - | + | - | + | + / - / A/A | + | + | - | - |
| *Citrobacter freundii* | - | + | - | + | - | + | - / - / A/A | + | + | + | + |
| *Pseudomonas aeruginosa* | + | + | - | - | - | + | - / - / K/K | - | - | - | + |
| *Pseudomonas luteola* | + | + | - | - | - | + | - / - / K/K | - | - | - | + |
| *Proteus mirabilis* | + | + | - | + | + | + | + / + / K/A | + | - | + | + |
| *Proteus vulgaris* | + | + | + | + | - | + | + / - / K/A | + | - | + | + |
| *Enterococcus faecalis* | - | + | - | + | - | - | - / - / A/A | - | - | - | + |
| *Serratia marcescens* | - | + | - | - | + | + | - / - / K/A | - | - | - | + |
| *Enterobacter aerogenes* | - | + | - | + | + | + | + / - / A/A | + | + | - | + |

**Antibiotic sensitivity and resistance of Gram-positive bacterial species:**

Table .3 shows the results of sensitivity and resistance tests studied on Gram-positive bacterial species isolated from diabetic foot, namely *E. coli*, *K. pneumoniae*, *Citrobacter freundii*, *Pseudomonas auroginosa*, *P. luteola*, *Proteus mirabilis*, *Proteus vulgaris*, *Enterococcus faecalis*, *Seratia marcescens*, and *Enterobacter arogenes*. For 12 antibiotics, according to the Kirby-Bauer method (1966), sensitivity and resistance were determined based on measuring the diameter of the inhibition zone of the antibiotic discs used in the study and compared with those specified in CLSI 2025. The isolates showed moderate resistance to Kanamycin, with 73.3%, 63.2%, 77.8%, 100%, 100%, 66.7%, 100%, 100%, and 100% for *E. coli*, *K. pneumoniae*, *Citrobacter freundii*, *Pseudomonas auroginosa*, P. luteola, *Proteus mirabilis*, Proteus vulgaris, *Enterococcus faecalis*, and *Enterobacter arogenes*, respectively. The sensitivity rate was 100% for *Seratia marcescens*. This antibiotic may work by binding to the 30S ribosome subunit of the bacterial cell, disrupting protein synthesis. Kanamycin binds to the 30S subunit of the bacterial ribosome, leading to misreading of mRNA. This results in the production of distorted and non-functional proteins, impeding bacterial growth. Resistance may occur as a result of the antibiotic being expelled from the cell by excretory pumps, or it may reduce antibiotic penetration by altering the composition of the outer membrane (Punetha *et al.,*2021). For Gentamicin, the isolates were 100% resistant to P.luteola and *Enterobacter arogenes*, respectively, and with a sensitivity rate of (26.7, 21, 44, 54.5, 11.1, 50%) 100% for E.coli, *K.pneumoniae*, *Citrobacter freundii*, *Pseudomonas auroginosa*, *Proteus mirabilis*, *Proteus vulgaris*, *Enterococcus faecalis*, *Seratia marcescens*, respectively. Gentamicin specifically binds to the 30S subunit of the bacterial ribosome, which leads to the inhibition of accurate translation of mRNA. and causing errors in reading the genetic code (misreading). Resistance to this antibiotic may be due to the degradation of the drug by enzymes or the absence of receptor sites

on the ribosome (Wang et al., 2022). Meanwhile, azithromycin showed resistance rates of 80, 84, 55.6, 54.5, 66.7, 100, 75, and 100% for all Gram-negative bacterial species except *Seratia marcescens*, where the sensitivity rate was 100%. Macrolide antibiotics may bind to the 50S subunit of the ribosome, thereby inhibiting polypeptide chain synthesis and preventing protein production (Wang *et al*.,2024). Resistance occurs as a result of modifications to the 50S subunit of the ribosome (Berbel *et al.,*2022). For the antibiotic chloramphenicol, the resistance rates were 66.7%, 90.9%, 100%, 77.8%, and 50% for each of *Citrobacter freundii*, *Pseudomonas auroginosa*, P. luteola, *Proteus mirabilis*, and *Enterococcus faecalis*, respectively. The sensitivity rates were 53.4%, 47.3%, and 100% for each of *E. coli*, *Seratia marcescens*, and K. pneumonae, respectively. Resistance may occur as a result of chloramphenicol-acetyltransferases. Enzymes known as chloramphenicol-acetyltransferases modify the antibiotic chloramphenicol by acetylating its hydroxyl group, resulting in an altered form of the antibiotic that is unable to bind to its ribosome target (Varela *et al*., 2021). The resistance rate to cefepime was 10%. (60%, 89.5, 77.8, 81.8, 100, 55.6, 100, 100, 100, 100%) respectively, while the resistance rate to Cefrtiaxone was (80, 79, 77.8, 63.6, 100, 44.4, 75, 100%), and for Cefexime the resistance rate was (86.7, 84.2, 100, 90.9, 100, 100, 100, 50, 100, 100%) for all types. This means that the antibiotic is destroyed by bacteria as a result of the production of beta-lactamase enzymes (β-lactamases). These enzymes destroy the beta-lactam ring in the cephalosporin molecule, which makes the antibiotic lose its effectiveness. Bush, Bradford, (2020) and for Amoxycillin the resistance rate was (100,100,77.8,100,81.8,100,66.7,100 ,100,100%, while the sensitivity rate was (100%) for *Seratia marcescens*, and for Piperacillin the resistance rate was (80,73.7,55.6,72.7,100,66.7,100 ,100,100%) and 100% sensitivity for *Seratia marcescens*. The reason for the high resistance to penicillin antibiotics may be attributed to the bacteria possessing beta-lactamase enzymes that degrade the penicillin group, whose genes are either chromosomal

or plasmid in origin. These bacteria also produce penicillin-binding proteins (PBPs) located in the cytoplasmic membrane that are linked to the cell wall. These proteins are a target for both penicillin and cephalosporin antibiotics, as they alter the target site of beta-lactam antibiotics, resulting in bacterial resistance to them (Drwaz, Bonomo 2010). This means preventing the antibiotic from forming the cell wall in the early stages of bacterial growth (Drwaz, Bonomo 2010). While the resistance rate to Aztreonam was (80, 68.5, 100, 63.6, 100, 66.7, 100, 100, 100%), and 100% sensitivity for *Proteus vulgaris*. The resistance to the antibiotic may be due to the production of resistant beta-lactamases (β-lactamases) (Bush & Bradford, 2016). As for Augmentin, the sensitivity rate was (26.3, 22.2, 33.4, 100, 100, 25%) for *K. pneumoniae*, *Citrobacter freundii*, *Proteus mirabilis*, *Proteus vulgaris*, *Enterococcus faecalis*, *Seratia marcescens*, as it works to inhibit the synthesis of the bacterial cell wall by binding to penicillin-binding proteins (PBPs). While the resistance rate was 100% for P.auroginosa, P.vulgaris, and *Enterobacter arugenes*, which means the antibiotic is destroyed by the production of the beta-lactamase enzyme (Drawz, &. 2010). As for the Imipenem antibiotic, the sensitivity rate was (93.3, 86.4, 55.6, 45.5, 100, 50%) for E.coli, *K.pneumoniae*, *Citrobacter freundii*, *Pseudomonas auroginosa* , *Proteus mirabilis*, *Proteus vulgaris*, *Enterococcus faecalis*, *Seratia marcescens*, and 100% for *Enterobacter arugenes*. This antibiotic works by inhibiting the synthesis of the bacterial cell wall by binding to penicillin-binding proteins (PBPs) and preventing the production of Peptidoglycan, leading to cell death (bactericidal). It is resistant to degradation by most β-lactamase enzymes. (Queenan, Bush 2007).

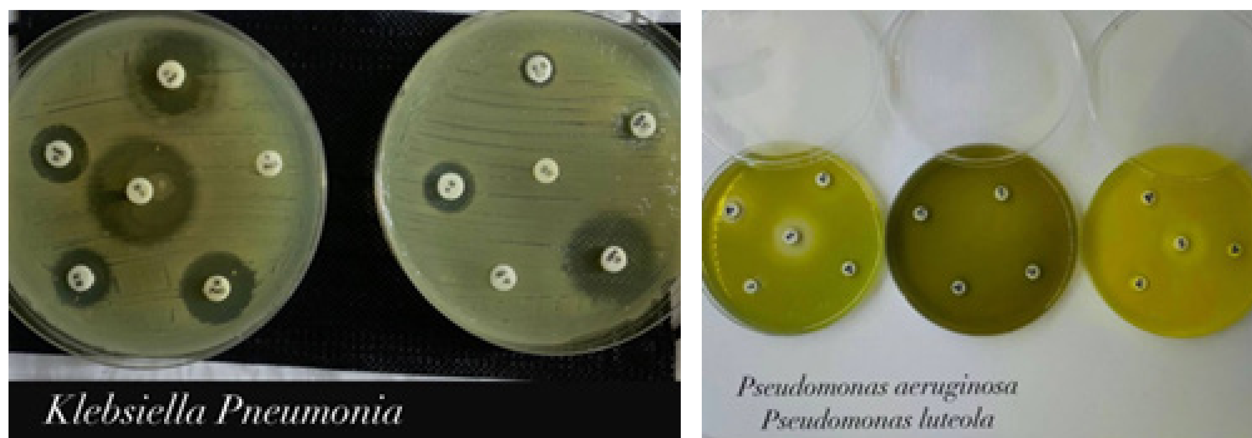



**Figure .5 Inhibitory diameters of antibiotics for Gram-negative bacterial isolates**

**Table 3.Antibiotic sensitivity and resistance of Gram-negative bacterial species**

| المضادات / الانواع | *E. coli* | | | *Klebsiella Pneumonia* | | | *Citrobacter freundii* | | | *Pseudomonas aeruginosa* | | | *Pseudomonas luteola* | | | *Proteus mirabilis* | | | *Proteus vulgaris* | | | *Enterococcus faecalis* | | | *Serratia marcescens* | | | *Enterobacter aerogenes* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | R % | I % | S % | R % | I % | S % | R % | I % | S % | R % | I % | S % | R % | I % | S % | R % | I % | S % | R % | I % | S % | R % | I % | S % | R % | I % | S % | R % | I % | S % |
| Kanamycin (K) | 73.3 | 0 | 26.7 | 63.2 | 10.5 | 26.3 | 77.8 | 0 | 22.2 | 100 | 0 | 0 | 100 | 0 | 0 | 66.7 | 0 | 33.3 | 100 | 0 | 0 | 100 | 0 | 0 | 0 | 0 | 100 | 100 | 0 | 0 |
| Azithromycin (AZM) | 80 | 13.3 | 6.7 | 84.2 | 5.3 | 10.5 | 55.6 | 0 | 44.4 | 45.5 | 9 | 45.5 | 0 | 0 | 100 | 66.7 | 11.1 | 22.2 | 100 | 0 | 0 | 75 | 25 | 0 | 0 | 0 | 100 | 100 | 0 | 0 |
| Gentamicin (GEN) | 26.7 | 6.7 | 66.6 | 21 | 10.5 | 68.5 | 44.4 | 11.1 | 44.5 | 54.5 | 0 | 45.5 | 100 | 0 | 0 | 11.1 | 11.1 | 77.8 | 0 | 0 | 100 | 50 | 0 | 50 | 0 | 0 | 100 | 100 | 0 | 0 |
| Amoxycillin (AX) | 100 | 0 | 0 | 100 | 0 | 0 | 77.8 | 0 | 22.2 | 100 | 0 | 0 | 100 | 0 | 0 | 88.9 | 0 | 11.1 | 100 | 0 | 0 | 100 | 0 | 0 | 0 | 0 | 100 | 100 | 0 | 0 |
| Cefepime (CPM) | 60 | 33.3 | 6.7 | 89.5 | 5.3 | 5.2 | 77.8 | 11.1 | 11.1 | 81.8 | 18.2 | 0 | 100 | 0 | 0 | 55.6 | 22.2 | 22.2 | 0 | 100 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 |
| Aztreonam (AT) | 80 | 0 | 20 | 68.5 | 15.8 | 15.7 | 100 | 0 | 0 | 63.6 | 36.4 | 0 | 100 | 0 | 0 | 66.7 | 11.1 | 22.2 | 0 | 0 | 100 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 |
| Chloramphenicol (C) | 33.3 | 13.3 | 53.4 | 47.4 | 5.3 | 47.3 | 66.7 | 0 | 33.3 | 90.9 | 0 | 9.1 | 100 | 0 | 0 | 77.8 | 0 | 22.2 | 0 | 100 | 0 | 50 | 50 | 0 | 0 | 0 | 100 | 100 | 0 | 0 |
| Piperacillin (PI) | 80 | 6.7 | 13.3 | 73.7 | 10.5 | 15.8 | 55.6 | 0 | 44.4 | 72.7 | 18.2 | 9.1 | 100 | 0 | 0 | 66.7 | 11.1 | 22.2 | 100 | 0 | 0 | 100 | 0 | 0 | 0 | 0 | 100 | 100 | 0 | 0 |
| Cefixime (CFM) | 86.7 | 0 | 13.3 | 84.2 | 0 | 15.8 | 100 | 0 | 0 | 90.9 | 0 | 9.1 | 100 | 0 | 0 | 100 | 0 | 0 | 0 | 100 | 0 | 50 | 50 | 0 | 100 | 0 | 0 | 100 | 0 | 0 |
| Augmentin (AMC) | 33.3 | 13.3 | 53.4 | 52.7 | 21 | 26.3 | 66.7 | 11.1 | 22.2 | 100 | 0 | 0 | 100 | 0 | 0 | 33.3 | 33.3 | 33.4 | 0 | 0 | 100 | 50 | 25 | 25 | 0 | 0 | 100 | 100 | 0 | 0 |
| Imipenem (IPM) | 0 | 6.7 | 93.3 | 26.3 | 5.3 | 68.4 | 33.3 | 11.1 | 55.6 | 45.4 | 9.1 | 45.5 | 100 | 0 | 0 | 0 | 0 | 100 | 0 | 0 | 100 | 50 | 0 | 50 | 0 | 0 | 100 | 100 | 0 | 0 |
| Ceftriaxone (CTR) | 80 | 20 | 0 | 79 | 15.7 | 5.3 | 77.8 | 11.1 | 11.1 | 63.6 | 27.3 | 9.1 | 100 | 0 | 0 | 44.4 | 55.6 | 0 | 0 | 100 | 0 | 75 | 25 | 0 | 0 | 100 | 0 | 100 | 0 | 0 |

**Antibiotic sensitivity and resistance of Gram-positive bacterial species:**

Table . 4 shows the results of sensitivity and resistance tests studied on Gram-positive bacterial species isolated from diabetic foot, namely *S.aureus, S.epidermidis, S.saprophyticus, S.xylosus, and S.psedointermedius,* to 12 antibiotics according to the method of Kirby Bauer (1966). Sensitivity and resistance were determined based on measuring the diameter of the inhibition zone of the antibiotic discs used in the study and compared with what is stated in CLSI 2025.

The isolates showed moderate resistance to Kanamycin at 40%, 25%, 33.33%, and 66.67% for *S.aureus, S.epidermidis, and S.psedointermedius*, respectively. These results are comparable to those of Chieffi *et al*. al., 2023)) and Correia, 2020). While the sensitivity rate was 100% for both *S. xylosis* and *S. sedointermedius*. This antibiotic may function by attaching to the bacterial cell's 30S ribosome component, which disrupts protein synthesis. Kanamycin binds to the 30S component of the bacterial ribosome, causing misreading of mRNA. This leads to the synthesis of deformed and non-functional proteins, which inhibits bacterial growth. Resistance may arise as a consequence of the antibiotic being evacuated from the cell by excretory pumps, or it may impede antibiotic penetration by changing the makeup of the outer membrane. (Punetha *et al*.,2021). Gentamicin resistance was 20%, 25%, and 33.33% for the isolates, with a high sensitivity ranging between 65, 75, 66, and 57%. This is consistent with the results of (Thuraya *et al*., 2022) and close to (Asmirah *et al*., 2022). Sensitivity was 100% for both S. xylosis and S. sedointermedius. Gentamicin selectively interacts to the bacterial ribosome's 30S subunit. inhibiting accurate mRNA translation and causing errors in reading the genetic code (misreading). This resistance to the antibiotic may be due to the degradation of the treatment by enzymes or the absence of receptor sites on the ribosome. (Wang *et al*., 2022)

While Azithromycin showed a resistance rate of 60.25% for *S.aureus and S.epidermidis*, which is close to what was found by the researcher (Al-Jubori,

Dahham, 2023), while the resistance was (33.33, 0%) and the sensitivity rate was 100% for *S.saprophyticus, S.xylosus,* and *S.psedointermedius*. Macrolide antibiotics may attach to the 50S component of the ribosome, which prevents the synthesis of polypeptide chains and protein biosynthesis. (Wang *et al*., 2024). Resistance occurs as a result of modification of the 50S subunit of the ribosome, specifically the ribosomal RNA (23S rRNA). This is usually done through methylation by enzymes encoded by the erm (erythromycin ribosomal methylase) genes, such as erm(A), erm(B), and erm(C). This modification prevents azithromycin from binding to its target site (Berbel *et al*.,2022). For the antibiotic chloramphenicol, the resistance rate was 20, 50, 100, and 100% for *S. aureus, S. epidermidis, S. saprophyticus,* and S. *psedointermedius,* respectively. This result is close to what was found by the researcher (Shariati *et al*., 2020). Resistance may develop as a consequence of chloramphenicol-acetyltransferase enzymes. Enzymes known as chloramphenicol-acetyltransferases change the antibiotic chloramphenicol by acetylating its hydroxyl group, resulting in an altered version of the antibiotic that is incapable of binding to its ribosome target. (Varela *et al*., 2021). The sensitivity rate was 100% for S. xylosus, which means that the antibiotic successfully reached the target site. The resistance rate to Cefepime was (60%, 100%, 66.67, 100%) for S. *aureus, S. epidermidis, S. saprophyticus, S. xylosus* respectively, and the sensitivity rate was 100% for *S. psedointermedius*, while the resistance rate to Cefexime was (95, 75, 100, 100, 100), and for Ceftiaxone the resistance rate was (50, 50, 33, 100%) for all species. This indicates that the antibiotic is rendered ineffective by bacteria due to the synthesis of beta-lactamase enzymes (β-lactamases). These enzymes destroy the beta-lactam ring in the cephalosporin molecule, which makes the antibiotic lose its effectiveness. Bush, Bradford, 2020) ) and for Amoxycillin the percentage was Resistance (60, 33.33%) to both *S.aureus and S.saprophyticus*. This aligns with the findings of the researcher. (Thuraya *et al*., 2022). While the sensitivity rate was (75%, 100%) for *both S.epidermidis*

*and S.xylosis*, and for Piperacillin, the resistance rate was (90, 25, 33.33, 100, 100%). The increased resistance to penicillin antibiotics may be ascribed to the presence of beta-lactamase enzymes in bacteria that breakdown the penicillin class. whose genes are either chromosomal or plasmid in origin. These bacteria manufacture penicillin-binding proteins (PBPs) that are situated in the cytoplasmic membrane and are affixed to the cell wall. These proteins are a target for both penicillin and cephalosporin antibiotics, as they alter the target site of beta-lactam antibiotics, resulting in bacterial resistance to them (Drwaz & Bonomo, 2010). This means preventing the antibiotic from forming a cell wall during the early stages of bacterial growth (Drwaz & Bonomo, 2010). While the resistance rate to aztreonam was (100, 75, 66.67, 100, 100) for all bacterial species, Antibiotic resistance may result from the synthesis of resistant beta-lactamase enzymes (β-lactamases). (Bush & Bradford, 2016). For Augmentin, the sensitivity rate was (50, 75, 66.67, 100%). For S. aureus, S. epidermidis, S. saprophyticus, S. xylosus, It obstructs bacterial cell wall formation by attaching to penicillin-binding proteins (PBPs). While the resistance rate was 100% for S. psedointermedius, which means that the antibiotic is destroyed by the production of the beta-lactamase enzyme (Drawz, &. 2010). As for the antibiotic Imipenem, the sensitivity rate was (90, 75, 100, 100%) for each of S. aureus, S. epidermidis, S. saprophyticus, and S. xylosus, and the resistance rate was 100% for S. psedointermedius. This antibiotic functions by obstructing the creation of the bacterial cell wall by its binding to penicillin-binding proteins (PBPs), hence hindering peptidoglycan production, which results in cell death (bactericidal). It is distinguished by its capacity to withstand degradation by the majority of beta-lactamase enzymes. (Queenan, Bush 2007).

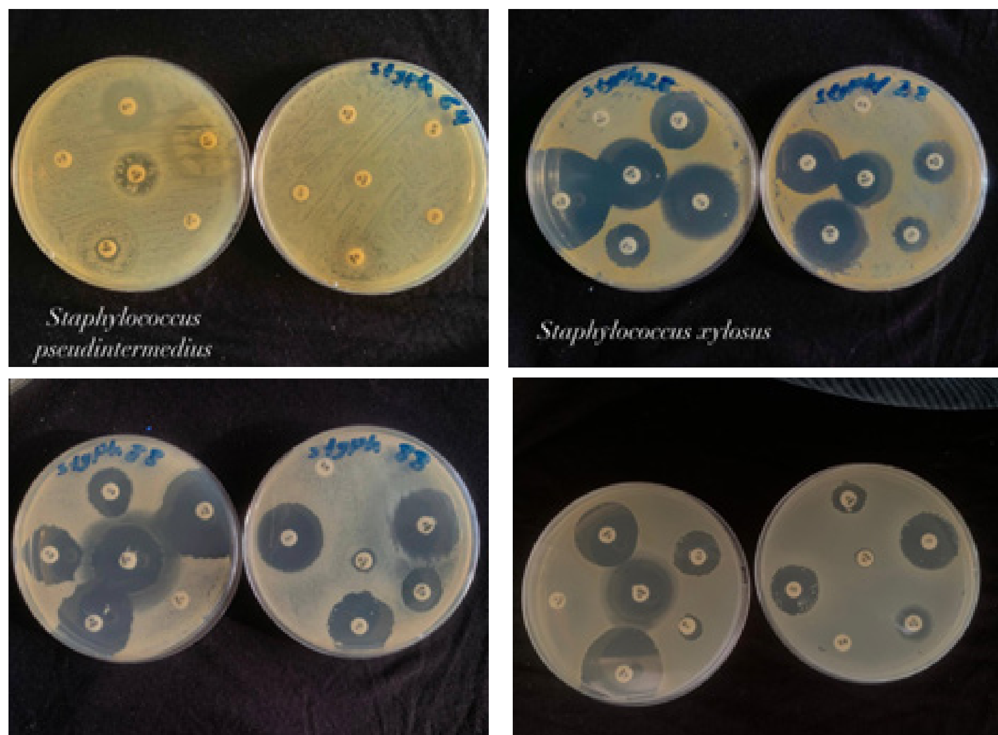



**Figure 6. Diameters of inhibition of antibiotics for Gram-positive bacterial isolates**

## Antibiotic sensitivity and resistance of *Staphylococcus* spp

## Table 4 .

| *Staphylococcus pseudintermedius* | | | *Staphylococcus xylosus* | | | *Staphylococcus saprophyticus* | | | *Staphylococcus epidermidis* | | | *Staphylococcus aureus* | | | الانواع / المضادات |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S% | I% | R% | S% | I% | R% | S% | I% | R% | S% | I% | R% | S% | I% | R% | |
| 0 | 100 | 0 | 100 | 0 | 0 | 66.67 | 0 | 33.33 | 75 | 0 | 25 | 45 | 15 | 40 | Kanamycin (K) |
| 0 | 0 | 100 | 100 | 0 | 0 | 33.34 | 33.33 | 33.33 | 75 | 0 | 25 | 35 | 5 | 60 | Azithromycin (AZM) |
| 100 | 0 | 0 | 100 | 0 | 0 | 66.67 | 0 | 33.33 | 75 | 0 | 25 | 65 | 15 | 20 | Gentamicin (GEN) |
| 100 | 0 | 0 | 0 | 100 | 0 | 66.67 | 0 | 33.33 | 75 | 0 | 25 | 30 | 10 | 60 | Amoxycillin (AX) |
| 0 | 0 | 100 | 100 | 0 | 0 | 33.33 | 0 | 66.67 | 0 | 0 | 100 | 35 | 5 | 60 | Cefepime (CPM) |
| 0 | 0 | 100 | 0 | 0 | 100 | 33.33 | 0 | 66.67 | 25 | 0 | 75 | 0 | 0 | 100 | Aztreonam (AT) |
| 0 | 0 | 100 | 100 | 0 | 0 | 0 | 0 | 100 | 50 | 0 | 50 | 75 | 5 | 20 | Chloramphenicol (C) |
| 0 | 0 | 100 | 0 | 0 | 100 | 0 | 66.67 | 33.33 | 25 | 50 | 25 | 10 | 0 | 90 | Piperacillin (PI) |
| 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 25 | 0 | 75 | 5 | 0 | 95 | Cefixime (CFM) |
| 0 | 0 | 100 | 100 | 0 | 0 | 66.67 | 0 | 33.33 | 75 | 0 | 25 | 50 | 25 | 25 | Augmentin (AMC) |
| 0 | 0 | 100 | 100 | 0 | 0 | 100 | 0 | 0 | 75 | 0 | 25 | 90 | 0 | 10 | Imipenem (IPM) |
| 0 | 0 | 100 | 0 | 100 | 0 | 33.34 | 33.33 | 33.33 | 0 | 50 | 50 | 5 | 45 | 50 | Ceftriaxone (CTR) |

## Conclusions:

1. Gram-negative bacteria, especially *K. pneumoniae*, *E. coli*, and *P. aeruginosa*, were the most common pathogens isolated from diabetic foot infections.

2. High levels of resistance were observed against conventional antibiotics, particularly penicillins and cephalosporins, suggesting the presence of multiple resistance mechanisms, including β-lactamase production.

3. Imipenem demonstrated the highest efficacy against most bacterial isolates, highlighting its potential as a treatment option for resistant infections.

4. Routine antibiotic susceptibility testing is essential for diabetic foot patients to avoid ineffective treatments and reduce the risk of resistance development.

5. The study recommends reinforcing antibiotic stewardship programs and implementing strict infection control measures in healthcare settings.

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