

## Original Article

**Evaluation of pathogens isolated from wound infections and their antibiotic susceptibility profiles in a wound care unit**Mehmet Yucens<sup>1</sup>, Tuğba Sari<sup>2</sup>, Nadir Aydemir<sup>1</sup>, Suna Seçil Öztürk<sup>2</sup>, Alp Akman<sup>1</sup>, Ozan Inal<sup>1</sup>, Fahir Demirkan<sup>1</sup><sup>1</sup> Pamukkale University School of Medicine Department of Orthopaedics and Traumatology, Denizli, Türkiye<sup>2</sup> Pamukkale University School of Medicine Department of Infectious Diseases, Denizli, Türkiye**Abstract**

**Introduction:** Wound infections, particularly diabetic foot infections (DFIs), are major clinical challenges, often exacerbated by polymicrobial colonization and rising antibiotic resistance. This study evaluates the pathogens isolated from DFIs in a Turkish hospital and their antibiotic susceptibility profiles to guide empirical treatment.

**Methodology:** A retrospective study was conducted in the orthopaedic wound care unit of Pamukkale University Hospital. Data from 478 wound cultures of 136 patients treated between 11/02/2021 and 02/03/2023 were analyzed. Pathogens were identified using microbiological methods and the VITEK 2 system, with antibiotic susceptibility testing performed via the Kirby-Bauer disc diffusion method, following Clinical and Laboratory Standards Institute (CLSI) guidelines.

**Results:** Among the 478 clinical samples, 66.9% displayed monomicrobial growth, and 32.1% were polymicrobial. Gram-negative bacteria were more prevalent, found in 85.7% of samples, with *Escherichia coli* (21.5%) and *Pseudomonas aeruginosa* (14%) being the dominant isolates. Gram-positive organisms were identified in 50.6% of cases, with *Staphylococcus aureus* (11.7%) as the most common, of which 8.9% were methicillin-resistant (MRSA). Multidrug-resistant (MDR) strains were detected in 320 Gram-negative isolates, including *E. coli* (89 isolates), *P. aeruginosa* (40), and *Proteus spp.* (50). Extended-spectrum beta-lactamase (ESBL) production was observed in 27.1% of the isolates, and carbapenem resistance in 2.3%. Notably, MDR pathogens were more frequent in patients with osteomyelitis, amputations, or HbA1c levels > 8%.

**Conclusions:** Gram-negative bacteria, particularly *E. coli* and *P. aeruginosa*, were the predominant pathogens in DFIs in Turkey. The increasing prevalence of MDR strains necessitates careful selection of empirical antibiotics, favoring coverage of *Enterobacteriaceae* and *P. aeruginosa* over MRSA until culture results are available.

**Key words:** Diabetic foot infections (DFIs); antibiotic susceptibility; Gram-negative bacteria; Gram-positive bacteria.

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**Introduction**

Diabetic foot infections (DFIs) represent one of the most severe complications of diabetes and remain a leading cause of morbidity and mortality globally. In individuals with diabetes, the development of foot ulcers and subsequent infections is facilitated by several contributing factors, including peripheral neuropathy, vascular insufficiency, and impaired immune response [1,2]. When not adequately treated, DFIs may result in life-threatening complications such as osteomyelitis, limb amputation, and sepsis [3].

The disruption of skin integrity fosters a moist, warm, and nutrient-rich environment, which in turn promotes bacterial colonization and growth. This often leads to polymicrobial wound infections [1]. The spectrum of microorganisms isolated from infected wounds can change over time and is frequently influenced by local hospital flora, particularly in

surgical wards, rather than solely by the patient's endogenous microbiota [2]. DFIs are typically polymicrobial, comprising both Gram-positive and Gram-negative bacteria, in addition to anaerobic organisms [3].

In recent years, the management of DFIs has become increasingly complicated due to the global rise in antibiotic resistance, particularly among multidrug-resistant (MDR) pathogens. Notably, methicillin-resistant *Staphylococcus aureus* (MRSA), extended-spectrum beta-lactamase (ESBL)-producing Gram-negative bacilli, and carbapenem-resistant *Enterobacteriaceae* (CRE) have emerged as prevalent threats in both hospital and community settings [4,5]. Successful treatment of DFIs requires the selection of appropriate antibiotics based on the antimicrobial susceptibility profiles of isolated pathogens [5]. These susceptibility patterns can vary significantly across

countries and even between different regions within the same country [4].

Antibiotic resistance has emerged as a critical public health concern, particularly among immunocompromised populations such as patients with diabetes. The indiscriminate use of antimicrobial agents has been identified as a major contributor to the rise in resistance. Resistant pathogens not only elevate healthcare costs but also prolong hospitalization and worsen clinical outcomes [6]. To address this growing problem, clinicians must possess accurate, region-specific knowledge regarding the microbial spectrum and antibiotic susceptibility profiles of DFIs and adhere to principles of rational antibiotic use [7].

This study aims to evaluate the distribution of pathogens isolated from DFIs and their corresponding antibiotic susceptibility patterns in a tertiary care hospital in Türkiye. The findings are expected to contribute to a better understanding of local resistance trends and support the optimization of empirical antibiotic treatment strategies.

## Methodology

### *Study Design and Setting*

This retrospective cross-sectional study was conducted in a 6-bed wound care unit located within an 800-bed tertiary care hospital. Patients who presented to the wound care clinic with a preliminary diagnosis of diabetes and exhibited foot ulcers were included in the study. Inpatient and outpatient records of the wound care center were reviewed. Wound culture results, antibiograms, and antibiotic susceptibility data for patients meeting the inclusion criteria were retrieved from the hospital's electronic information system.

Samples collected at different time points during the follow-up period were individually analyzed. It was observed that samples taken from the same patient at different times frequently yielded distinct microorganisms and antibiotic susceptibility patterns. Therefore, the unit of analysis was defined as individual samples rather than patients. A total of 478 wound culture samples obtained from 136 patients between 2 November 2021 and 2 March 2023 were included. Ethical approval was granted by the Ethics Committee for Non-Interventional Clinical Research of Pamukkale University (approval number: E-60116787-020-590653).

### *Sample Collection*

Wound cultures were obtained after the wound surface had been cleansed with a sterile saline solution. Specimens were collected by scraping the base of the

ulcer, performing wound curettage, or aspirating necrotic tissue and bone fragments. All samples underwent Gram staining and were assessed using the Q-score to differentiate between colonisation and infection. The specimens were then incubated at 37 °C in ambient air on MacConkey and blood agar plates.

### *Microbiological Analysis*

Bacterial isolates were identified to the species level using standard microbiological procedures and the VITEK 2 automated system (BioMérieux, France), in accordance with the guidelines established by the Clinical and Laboratory Standards Institute (CLSI) [8]. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar, with Oxoid brand antibiotic discs. The antibiotic panel included gentamicin (10 µg), clindamycin (2 µg), linezolid (30 µg), erythromycin (15 µg), ampicillin (10 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), ciprofloxacin (5 µg), amoxicillin-clavulanic acid (30 µg), piperacillin-tazobactam (100/10 µg), imipenem (10 µg), meropenem (10 µg), amikacin (10 µg), doxycycline (30 µg), vancomycin (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), cefuroxime (30 µg), ceftazidime (30 µg), and colistin (10 µg). Minimum inhibitory concentrations (MICs) for vancomycin and oxacillin were determined using the E-test method (BioMérieux), again in line with CLSI guidelines [8].

### *Quality Control*

Quality control procedures were conducted using standard reference strains: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 29213. Antibiotic susceptibility results were interpreted as susceptible, intermediate, or resistant based on CLSI standards [8].

### *Definition of Multidrug Resistance (MDR)*

Multidrug resistance was defined as resistance to at least one antimicrobial agent in three or more distinct antimicrobial classes. MDR pathogens were further categorized as methicillin-resistant *Staphylococcus aureus* (MRSA), extended-spectrum beta-lactamase (ESBL)-producing Gram-negative bacteria, and carbapenem-resistant *Enterobacteriaceae* (CRE).

### *Testing for Methicillin Resistance*

*Staphylococcus* species were tested for methicillin resistance using a cefoxitin disc (30 µg). According to CLSI guidelines, isolates with an inhibition zone diameter of 21 mm were classified as methicillin-

resistant, whereas those with a diameter  $\geq 22$  mm were considered susceptible [8].

**Testing for ESBL Production**

Gram-negative bacilli were evaluated for ESBL production via the double-disc synergy test using ceftazidime (30  $\mu$ g) and ceftazidime/clavulanic acid (30/10  $\mu$ g). An increase  $\geq 5$  mm in the inhibition zone diameter in the presence of clavulanic acid was interpreted as indicative of ESBL production [9].

**Carbapenem and Colistin Resistance**

Resistance of Gram-negative *Enterobacteriaceae* isolates to carbapenems and colistin was classified, respectively, as CRE (carbapenem-resistant *Enterobacteriaceae*) and colistin-resistant organisms.

**Statistical Analysis**

Data were entered and analyzed using SPSS version 23 (IBM, Chicago, IL, USA). Descriptive statistics were used to summarize demographic and clinical data. Inferential statistical analyses were performed to assess the relationship between clinical variables and antibiotic resistance profiles. Categorical variables were compared using chi-square ( $\chi^2$ ) tests, with associations examined between amputation and variables such as MDR status, peripheral neuropathy, peripheral artery disease (PAD), osteomyelitis, and gender. A  $p < 0.05$  was considered statistically

**Table 2.** The characteristics of bacterial cultures and bacterial species isolated from clinical samples.

|                                   | n   | %    |
|-----------------------------------|-----|------|
| Number of clinical samples        | 478 |      |
| Number of isolates                | 657 |      |
| Gram positives isolated           | 242 | 50.6 |
| <i>S. aureus</i>                  | 56  | 11.7 |
| CNS                               | 31  | 6.4  |
| <i>Streptococcus spp</i>          | 15  | 3.1  |
| <i>Enterococcus spp</i>           | 93  | 19.4 |
| Gram negatives isolated           | 410 | 85.7 |
| <i>P. aeruginosa</i>              | 61  | 14   |
| <i>E. coli</i>                    | 103 | 21.5 |
| <i>K. pneumoniae</i>              | 9   | 1.8  |
| <i>Proteus spp.</i>               | 59  | 12.3 |
| <i>Citrobacter spp.</i>           | 8   | 1.6  |
| <i>Enterobacter spp.</i>          | 39  | 8.1  |
| <i>A. baumannii</i>               | 26  | 5.4  |
| <i>M. organii</i>                 | 19  | 3.9  |
| MDR pathogens                     |     |      |
| MRSA                              | 5   | 8.9  |
| ESBL                              | 130 | 27.1 |
| CRE                               | 11  | 2.3  |
| Colistin resistant gram negatives | 67  | 14   |

CNS: Coagulase negative *staphylococcus*; MDR: Multidrug-resistant; *S. aureus*: *Staphylococcus aureus*; *P. aeruginosa*: *Pseudomonas aeruginosa*; *E. coli*: *Escherichia coli*; *K. pneumoniae*: *Klebsiella pneumoniae*; *A. baumannii*: *Acinetobacter baumannii*; *M. organii*: *Morganella organii*; ESBL: Extended spectrum beta - lactamase; CRE: Carbapenem-resistant *Enterobacteriaceae*; MRSA: Methicillin resistant *S. aureus*.

**Table 1.** Patients followed up with the diagnosis of diabetic foot infection Sociodemographic characteristics.

| Characteristics                     | n (%)             |
|-------------------------------------|-------------------|
| Age (average $\pm$ SD)              | 60.4 $\pm$ 11.705 |
| <b>Gender</b>                       |                   |
| Female                              | 27 (19%)          |
| Male                                | 109 (81%)         |
| <b>Characteristics of the wound</b> |                   |
| Ulcer                               | 115 (84%)         |
| Gangrene                            | 66 (48%)          |
| Abscess                             | 48 (35%)          |
| <b>Comorbidities</b>                |                   |
| Peripheral vascular disease         | 95 (69%)          |
| Cardiac disease                     | 97 (71%)          |
| Peripheral neuropathy               | 60 (44%)          |
| Retinopathy                         | 50 (36%)          |
| Osteomyelitis                       | 102 (75%)         |
| Amputation                          | 78 (57%)          |

significant.

**Results**

**Sociodemographic and Clinical Characteristics**

A total of 478 clinical samples were obtained from 136 patients diagnosed with diabetic foot infections (DFI). Of these patients, 27 (19%) were female and 109 (81%) were male. The mean age was 59.1 years for men and 65.7 years for women. Osteomyelitis was present in 75% of patients, and 57% had undergone at least one amputation. The most prevalent comorbidities were peripheral artery disease (PAD) (69%), hypertension (63%), peripheral neuropathy (44%), nephropathy (71%), retinopathy (36%), and heart disease (71%). A detailed summary of these characteristics is presented in Table 1.

**Table 3.** Distribution of monomicrobial isolates.

|                                  | n   | %     |
|----------------------------------|-----|-------|
| Monomicrobial Sample             | 320 |       |
| Isolated Gram-Positive Bacteria  | 127 | 39.6  |
| <i>S. aureus</i>                 | 32  | 25.1  |
| CNS                              | 27  | 21    |
| <i>Streptococcus spp</i>         | 6   | 4     |
| <i>Enterococcus spp</i>          | 52  | 40.9  |
| Isolated Gram-Negative Bacteria  | 193 | 60.4  |
| <i>P. aeruginosa</i>             | 33  | 17.09 |
| <i>E. coli</i>                   | 62  | 32.1  |
| <i>K. pneumoniae</i>             | 5   | 2.5   |
| <i>Proteus spp.</i>              | 29  | 15    |
| <i>Citrobacter spp.</i>          | 2   | 1     |
| <i>Enterobacter spp.</i>         | 19  | 9.8   |
| <i>A. baumannii</i>              | 11  | 5.6   |
| <i>M. organii</i>                | 8   | 4.1   |
| MDR Pathogens                    |     |       |
| MRSA                             | 2   | 0.6   |
| ESBL                             | 50  | 15    |
| CRE                              | 7   | 2.1   |
| Colistin-resistant Gram-negative | 28  | 8.7   |

CNS: Coagulase negative staphylococci; MDR: Multidrug-resistant; *S. aureus*: *Staphylococcus aureus*; *P. aeruginosa*: *Pseudomonas aeruginosa*; *E. coli*: *Escherichia coli*; *K. pneumoniae*: *Klebsiella pneumoniae*; *A. baumannii*: *Acinetobacter baumannii*; *M. organii*: *Morganella organii*; ESBL: Extended-spectrum beta-lactamase; CRE: Carbapenem-resistant *Enterobacteriaceae*; MRSA: Methicillin resistant *S. aureus*.

**Table 4.** Comparison of microbial diversity and antimicrobial susceptibility patterns in different regions.

| Study                          | Gram-Negative (%) | Gram-Positive (%) | Most Common Pathogen                                 | MRSA (%) | ESBL (%) | Polymicrobial (%) |
|--------------------------------|-------------------|-------------------|--|----------|----------|-------------------|
| Jouhar et al. (Lebanon)        | -                 | -                 | <i>E. coli</i> (15%), <i>P. aeruginosa</i> (11%)     | 50       | 25       | 54                |
| Taki et al. (Tehran, Iran)     | 47.8              | 52.2              | <i>Staphylococcus spp.</i> (52.2%)                   | 58.9     | 40       | 69.6              |
| Carro et al. (Latin America)   | 43.8              | 56.2              | <i>S. aureus</i> (19.9%)                             | -        | -        | -                 |
| Dawaiwala et al. (India)       | 40                | 60                | <i>S. aureus</i> (24.5%)                             | 70.3     | 14.5     | -                 |
| Du et al. (China)              | 52.4              | 43.4              | <i>S. aureus</i> (17.7%), <i>E. coli</i> (10.9%)     | -        | 19.1     | 22.8              |
| Coşkun et al. (Ankara, Turkey) | 57.6              | 41.2              | <i>E. coli</i> (11.1%), <i>P. aeruginosa</i> (11.1%) | 51.3     | 66.7     | 40.5              |
| <b>This Study (Turkey)</b>     | <b>60.4</b>       | <b>39.6</b>       | <i>E. coli</i> (21.5%), <i>P. aeruginosa</i> (14%)   | 8.9      | 27.1     | 32.1              |

Of the 478 clinical samples, 320 (66.9%) demonstrated monomicrobial growth, while 158 (32.1%) exhibited polymicrobial growth involving two or more microorganisms. Gram-negative bacteria were isolated in 410 samples (85.7%), with *Escherichia coli* (21.5%) and *Pseudomonas aeruginosa* (14%) being the most frequently identified pathogens. Gram-positive organisms were detected in 242 samples (50.6%), with *Staphylococcus aureus* representing the most frequent isolate (11.7%). Among the *S. aureus* isolates, 58.9% were methicillin-resistant (MRSA). Additional Gram-positive isolates included *Streptococcus spp.* (3.1%), *Staphylococcus epidermidis* (3.7%), and *Enterococcus faecalis* (14.8%). The complete distribution of bacterial species is shown in Table 2.

Of the 320 monomicrobial isolates, 127 (39.6%) were Gram-positive and 193 (60.3%) were Gram-negative. The detailed characteristics of the monomicrobial isolates are provided in Table 3. Furthermore, Table 4 presents a comparative overview of antimicrobial susceptibility patterns across different regions.

*Antibiotic Susceptibility Patterns*

The antimicrobial susceptibility profiles of the isolated pathogens are summarized in Table 5. Among the Gram-positive isolates, both MRSA and *Enterococcus spp.* exhibited high susceptibility to vancomycin and linezolid, accounting for 74.6% of the relevant cases.

Regarding Gram-negative bacteria, susceptibility rates were as follows:

- Carbapenems: 466 of 770 isolates were susceptible; 120 of 770 were intermediate.
- Piperacillin-tazobactam: 254 of 385 isolates were susceptible; 44 of 385 were intermediate.
- Aminoglycosides: 644 of 810 isolates were susceptible; 2 of 810 were intermediate.

Susceptibility to other antibiotic classes was also examined:

- Cephalosporins: 537 of 1,405 isolates were susceptible; 263 of 1,405 were intermediate.
- Amoxicillin-clavulanic acid: 100 of 310 isolates were susceptible; 300 of 310 were intermediate.
- Ampicillin: 187 of 334 isolates were

**Table 5.** Antibiotic susceptibility patterns of Gram-negative and Gram-positive microorganisms.

|         | <i>S. aureus</i> | MRSA | CNS     | <i>Streptococcus spp</i> | <i>Enterococcus spp</i> | <i>P. aeruginosa</i> | <i>A. baumannii</i> | <i>E. coli</i> | <i>K. pne</i> | <i>Prot</i> | <i>Citro</i> | <i>Enterobacter</i> | <i>M. morgani</i> |
|---------|------------------|------|---------|--------------------------|-------------------------|----------------------|---------------------|----------------|---------------|-------------|--------------|---------------------|-------------------|
| AMC     |                  |      |         |                          | 67/75                   |                      |                     | 51/3/170       | 28/48         | 43/57       | 3/7          | 2/38                | 0/19              |
| CIP     | 18/33/56         | 1/5  | 4/5/31  |                          | 8/1/19                  | 11/34/67             | Md 3/26             | 41/103         | 21/1/49       | 33/1/57     | 8/8          | 31/5/38             | 4/19              |
| AMP     | 15/21            |      | 0/1     |                          | 78/93                   |                      |                     | 11/104         |               | 115/59      | 0/8          | 3/39                | 0/19              |
| TMP_SXT | 54/56            | 4/5  | 21/1/30 | 2/2                      |                         |                      | 3/4/26              | 55/102         | 26/46         |             | 8/8          | 33/38               | 6/19              |
| ERT     | 47/56            | 2/5  | 5/31    | 7/11                     |                         |                      |                     |                |               |             |              |                     |                   |
| CLIN    | 47/56            | 1/5  | 6/31    | 7/12                     |                         |                      |                     |                |               |             |              |                     |                   |
| AMIK    | 32/34            | 1/2  | 17/21   |                          |                         | 66/67                |                     | 104/104        | 47/49         | 57/58       | 8/8          | 38/38               | 19/19             |
| GENT    | 41/47            | 2/5  | 8/28    | 3/3                      | 74/93                   | 12/13                | 3/25                | 76/1/104       | 35/49         | 33/58       | 8/8          | 38/39               | 9/19              |
| DOXC    |                  |      |         |                          |                         |                      |                     |                |               |             |              |                     |                   |
| VAN     | 31/31            | 5/5  | 27/27   | 5/5                      |                         |                      |                     |                |               |             |              | 1/1                 |                   |
| LINZ    | 15/15            | 5/5  | 23/24   | 4/4                      | 48/48                   |                      |                     |                |               |             |              | 1/1                 |                   |
| CFTX    |                  |      |         | 9/9                      |                         | 0/13                 | 0/2                 | 6/6            | 3/7           | ½           |              |                     |                   |
| CTAZ    |                  |      |         |                          |                         | 3/41/67              | 0/2                 | 55/5/103       | 23/49         | 52/57       | 7/7          | 19/1/38             | 17/1/19           |
| CFRKM   |                  |      |         |                          |                         |                      | 0/2                 | 6/3/10         |               | ½           |              |                     |                   |
| TAZP    |                  |      |         |                          |                         | 6/37/67              | 0/2                 | 82/3/103       | 31/49         | 57/59       | 8/8          | 21/38               | 18/19             |
| IMIP    |                  |      |         |                          |                         | 10/34/66             | 3/25                | 74/1/81        | 22/5/34       | 1/44/54     | 5/5          | 23/25               | Md 8/19           |
| MERO    |                  |      |         |                          |                         | 42/2/66              | 3/25                | 63/3/72        | 24/21/33      | 40/1/42     | 5/5          | 18/21               | 15/2/18           |
| CL      |                  |      |         |                          |                         | 37/38                | 23/23               | 31/42          | 21/21         | 0/51        | ½            | 8/18                | 0/18              |

AMC: Amoxicillin-Clavulanic acid; CIP: Ciprofloxacin; AMP: Ampicillin; ERT: Erythromycin; CLIN: Clindamicin ; GENT: Gentamicin ; DOXC: Doxycycline ; VAN: Vancomycin; LINZ: Linezolid; CFTX: Cefotaxime; CTAZ: Ceftazidime; TAZP: Piperacillin-tazobactam; IMIP: Imipenem; MERO: Meropenem; CL: Colistin; AMIK: Amikacin; CFRKM: cefuroxime; TMP-SXT: Trimethoprim-sulfamethoxazole; CNS: Coagulase negative staphylococci; *S. aureus* : *Staphylococcus aureus*; *P. aeruginosa*: *Pseudomonas aeruginosa*; *E. coli*: *Escherichia coli*; *K. pne*: *Klebsiella pneumoniae*; *A. baumannii*: *Acinetobacter baumannii*; *M. morgani*: *Morganella morgani*; MRSA: Methicillin resistant *S. aureus* (susceptible/mild susceptible/ total isolates).

susceptible; 4 of 334 were intermediate.

- Trimethoprim-sulfamethoxazole (TMP-SXT): 187 of 334 isolates were susceptible; 4 of 334 were intermediate.

These findings highlight substantial variability in resistance patterns among different antibiotic classes, with significant resistance observed particularly in  $\beta$ -lactam combinations and cephalosporins.

*Multidrug-Resistant (MDR) Pathogens*

A total of 320 multidrug-resistant (MDR) Gram-negative bacterial isolates were identified. Among these, 102 were ESBL-producing, 10 were carbapenem-resistant *Enterobacteriaceae* (CRE), and 79 were colistin-resistant. The most frequently encountered MDR Gram-negative isolates included *E. coli* (89 isolates), *P. aeruginosa* (40 isolates), *Proteus* spp. (50 isolates), *Acinetobacter baumannii* (23 isolates), *Citrobacter* spp. (3 isolates), and *Enterobacter* spp. (36 isolates). Details of MDR organism distribution are provided in Table 6. Notably, MDR pathogens were more frequently observed in patients with HbA1c levels exceeding 8%.

*Statistical Analysis*

Descriptive statistics were used to summarize the data, and inferential analyses were performed to explore associations between clinical variables and antibiotic resistance. Logistic regression analysis revealed that higher HbA1c levels (> %) and the presence of osteomyelitis were significantly associated with isolation of MDR pathogens ( $p < 0.05$ ). Pearson’s chi-square test demonstrated a significant association between peripheral neuropathy and amputation ( $p = 0.001$ ). A strong statistical relationship was also observed between PAD and amputation ( $p = 0.000$ ).

However, no statistically significant association was found between MDR pathogen presence and amputation ( $p > 0.05$ ), suggesting that amputation rates did not differ significantly between patients with and without MDR infections. Similarly, no significant

relationship was found between gender and amputation ( $p > 0.05$ )

**Discussion**

Diabetic foot infections (DFIs) are a major complication of diabetes and a substantial cause of morbidity and mortality worldwide [3]. This study identified a statistically significant association between peripheral neuropathy and amputation ( $p = 0.001$ ), indicating that patients with neuropathy are at significantly higher risk of amputation than those without ( $p < 0.05$ ). Similarly, a strong association was observed between peripheral artery disease (PAD) and amputation ( $p = 0.000$ ), with significantly higher amputation rates observed in patients diagnosed with PAD than in those without ( $p < 0.05$ ). These findings emphasise the important role of vascular and neurological comorbidities in the prognosis of DFIs. Furthermore, the widespread use of antibiotics in the management of DFIs continues to contribute to the growing problem of antimicrobial resistance [3].

In the present study population, Gram-negative and Gram-positive organisms were identified in 85.7% and 50.6% of samples, respectively. Of the 320 samples showing monomicrobial growth, 193 (60.3%) were Gram-negative and 127 (39.6%) were Gram-positive. These results are consistent with previous reports indicating that Gram-negative organisms are more frequently encountered in subtropical regions, including Turkey, as well as parts of Africa and Asia, whereas Gram-positive pathogens are more prevalent in Western Europe [10–15]. The rate of polymicrobial growth observed in this study was 32.1%. Polymicrobial infections are typically associated with deep tissue involvement, such as that of tendons, joint capsules, fascia, or bone, and with features such as necrosis, foul odour, gangrene, or chronicity. Such infections often involve *Enterococci*, *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and anaerobic species, in addition to those found in superficial infections [16]. Given this complexity, the

**Table 6.** MDR strains Comparison of infected and uninfected patients.

|                               | Patients infected with MDR strains | non -MDR strains infected patients | OR (95%)                  |
|-------------------------------|------------------------------------|------------------------------------|---------------------------|
| Age                           |                                    |                                    |                           |
| < 50                          | 25/116                             | 3/19                               |                           |
| 51-60                         | 26/116                             | 6/19                               |                           |
| > 60                          | 65/116                             | 10/19                              |                           |
| HbA1c > 8                     | 106                                | 17                                 | MDR:10.599, NON-MDR:8.50  |
| HbA1c < 8                     | 10                                 | 2                                  |                           |
| With osteomyelitis            | 87                                 | 15                                 | MDR:3, NON MDR: 3.75      |
| Amputation                    | 67                                 | 11                                 | MDR:1.367, NON MDR: 1.375 |
| With additional comorbidities | 93                                 | 15                                 | MDR:4.0434, NON MDR: 3.75 |

HbA1c: Hemoglobin A1c; OR: Odds ratio; MDR: Multidrug-resistant microorganism.

selection of antibiotics should be tailored to the individual based on their risk factors and the regional microbiological profile [17]. Furthermore, it is recommended that treatment planning considers data from both monomicrobial and polymicrobial growth, given that the prevalence of Gram-negative organisms increases from 60.3% in monomicrobial samples to 85.7% when all samples are considered.

In this study, the most frequently isolated Gram-negative organisms were *Escherichia coli* (21.5%) and *P. aeruginosa* (14%). *P. aeruginosa* is commonly isolated in DFIs in warm climates [15]. Similarly, Jouhar *et al.* reported *E. coli* (15%) and *P. aeruginosa* (11%) as the dominant pathogens. However, they observed a higher polymicrobial infection rate (54%) than in the present study (32.1%), which may reflect the more complex clinical presentations of their patient population [18]. A systematic review of DFIs in China by Du *et al.* found that Gram-negative pathogens (52.4%) were more prevalent than Gram-positive organisms (43.4%). The pathogens they most commonly isolated - *Staphylococcus aureus* (17.7%), *E. coli* (10.9%), and *P. aeruginosa* (10.5%) - are consistent with the findings of the present study [19].

Another investigation of 434 patients with infected DFUs in northern India found that *P. aeruginosa* was the predominant isolate, detected in 20% of cultures [20]. Maceration, foot soaking, and exposure to moist environments are known risk factors for *P. aeruginosa* infection. Conversely, in temperate climates and the absence of such exposures, *P. aeruginosa* is less frequently isolated [21]. A study involving 292 hospitalised patients with DFIs and no osteomyelitis in the United States found a prevalence of *P. aeruginosa* of 9%, with risk factors including immunosuppression and recent antibiotic use [22].

DFIs caused by multidrug-resistant (MDR) bacteria reduce the effectiveness of treatment, complicate clinical management, and increase the risk of amputation and death [23,24]. In line with the present study, previous literature has identified elevated HbA1c levels (> 7.5%) as a risk factor for ulcer recurrence and MDR pathogen colonisation [25,26]. The presence of osteomyelitis has also been associated with higher rates of MDR pathogens, likely due to prolonged and high-dose antibiotic exposure [26].

In the current study, *S. aureus* was detected in 11.7% of isolates, 8.9% of which were methicillin-resistant (*MRSA*). *MRSA* is a globally recognised leading cause of DFIs, particularly in the United States. Patients undergoing haemodialysis or residing in long-term care facilities are at increased risk of *MRSA*

colonisation [27]. Dawaiwala *et al.* reported an MDR prevalence of 47.2% and an XDR rate of 5.4%. Their *MRSA* rate of 70.3% significantly exceeded that found in the present study (8.9%), indicating a potential need for routine empirical *MRSA* coverage in certain geographic regions [28]. They also reported an ESBL prevalence of 14.5%, which is lower than in the present study (27.1%), further highlighting regional variability in resistance patterns.

Gür *et al.* evaluated *S. aureus* resistance in wound infections in orthopaedic and trauma patients in Turkey and found that 21.5% of samples yielded *S. aureus*, 29.7% of which were *MRSA*. This rate was also higher than that in the present study. Their data indicated resistance to penicillin, rifampicin, tetracycline, and erythromycin, while vancomycin, teicoplanin, linezolid, tigecycline, and daptomycin remained effective [29].

By contrast, Du *et al.* reported a lower rate of polymicrobial infection (22.8%) than in this study (32.1%), and found that 20% of their isolates were multidrug-resistant (MDR), with *S. aureus* accounting for 30.4% of these cases. Their findings support the use of empirical regimens targeting MDR bacteria in chronic or severe cases [19]. The MDR pathogens identified in the current study included ESBL-producing organisms (27.1%), carbapenem-resistant *Enterobacteriaceae* (2.3%), and colistin-resistant Gram-negative bacteria (14%). These findings are consistent with the global trend of increasing rates of ESBL and carbapenem resistance among Gram-negative bacilli in DFIs [30].

While the present study revealed a predominance of Gram-negative pathogens overall (85.7%) and in monomicrobial cultures (60.7%), other studies have reported different trends. For instance, Taki *et al.* observed a higher prevalence of Gram-positive bacteria, particularly *Staphylococcus* spp. (52.2%), and reported a carbapenem resistance rate of 47.6% in Tehran — significantly higher than the rate observed in the present study (2.3%) — suggesting notable regional differences in resistance patterns [31,32].

Similarly, Coşkun *et al.* found Gram-negative bacteria to be predominant in DFIs in Turkey and reported an *MRSA* prevalence of 51.3% [33]. The prevalence of Gram-negative resistance varies significantly by country and hospital, being influenced by factors such as length of hospital stay, catheter use, prior antibiotic exposure, and long-term care facility residency.

Carro *et al.* reported an almost equal distribution of Gram-negative (43.8%) and Gram-positive bacteria

across multiple Latin American centres. *S. aureus* (19.9%) was the species most frequently isolated. Notably, *Enterococcus faecalis* was the most prevalent isolate in bone infections (16.8%), a finding not emphasised in the current dataset. The study also reported high levels of fluoroquinolone resistance among Gram-negative isolates, which is consistent with our observations and supports the limited use of fluoroquinolones in empirical therapy [34].

As in the present study, the role of anaerobic organisms in DFIs remains unclear, largely due to the lack of standardisation in previous research with regard to sampling, transport, and culture methodologies. One limitation of this study is the lack of information on whether patients had received antibiotic therapy prior to admission to the tertiary care centre. In cases involving necrosis, deep tissue involvement, foul-smelling discharge, gangrene, or severe infection, empirical antibiotic regimens should include anaerobic coverage [35,36].

## Conclusions

In conclusion, this study demonstrated that Gram-negative organisms were more frequently isolated than Gram-positive organisms in patients with diabetic foot infections (DFIs) in Turkey. *Pseudomonas aeruginosa* and other *Enterobacteriaceae* were predominant, rather than *Staphylococcus aureus*. In light of the rising levels of antimicrobial resistance among *Escherichia coli* and *P. aeruginosa*, it is crucial to consider individual risk factors for drug-resistant Gram-negative bacilli when initiating empirical antimicrobial therapy.

Furthermore, multidrug-resistant (MDR) strains were more frequently identified in patients with HbA1c levels > 8%, osteomyelitis, comorbidities, and prior amputations, compared to patients without these risk factors. While international guidelines offer a framework for empirical antimicrobial therapy in DFIs, the choice of antimicrobial agents must be tailored to the individual patient and informed by local antimicrobial resistance patterns.

Given the high prevalence of MDR organisms observed in this study, clinicians should prioritise early microbiological assessment and adopt de-escalation strategies based on culture and susceptibility results. They should also consider combination antibiotic regimens for severe infections, with the aim of enhancing treatment efficacy and reducing the risk of further resistance development. Until culture and susceptibility data are available to guide definitive therapy in clinical practice in Turkey, empirical treatment should generally target *Enterobacteriaceae*

and *P. aeruginosa*, whereas routine coverage for *methicillin-resistant Staphylococcus aureus* (MRSA) may not be necessary.

## Corresponding author

Ass. Prof. Dr. Mehmet Yucens  
Pamukkale University School of Medicine Department of Orthopaedics and Traumatology,  
Denizli, Türkiye  
Tel: +905337677506  
Fax: +902582961603  
Email: aflyucens@yahoo.com

## Conflict of interests

No conflict of interests is declared.

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