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Antibiotic Resistance Profiling and *mecA* Gene Detection in Diabetic Foot Infections: A Study from Ardabil City, Iran

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ABSTRACT

Background and Aim: Diabetic foot infections (DFIs) are common complications for individuals suffering from diabetes. The aim of this study was to monitor the presence of the *mecA* gene in diabetic foot infections in diabetic patients in Ardabil city, northwest Iran.

Materials and Methods: A total of eighty samples were collected from Imam Khomeini Hospital in Ardabil, Iran, between March and February 2023. Bacterial isolates were identified using conventional microbiological tests, and their antibiotic susceptibility was tested. The presence of the *mecA* gene was determined by PCR assay.

Results & Conclusion: A total of 119 bacterial strains were isolated from 80 diabetic foot ulcer samples. The most common aerobic Gram-positive bacteria were *Staphylococcus aureus* (n=34) and coagulase-negative *Staphylococcus* spp. (n=21). Enterobacteriaceae included *Escherichia coli* (n=18), *Citrobacter* spp. (n=5), and *Enterobacter* spp. (n=5). *Enterococcus* spp. were found in 18 isolates. Gram-negative aerobic bacteria included *Pseudomonas aeruginosa* (n=8) and *Acinetobacter* spp. (n=5). The main anaerobic isolate was *Bacteroides fragilis* (n=5). Thirteen MRSA strains were detected, 12 of which carried the *mecA* gene; one strain was MRSA by cefoxitin testing but lacked *mecA*, suggesting alternative resistance mechanisms. All Gram-positive isolates were susceptible to linezolid, and all Enterobacteriaceae were sensitive to imipenem. *S. aureus* and *B. fragilis* were the predominant aerobic and anaerobic bacteria, respectively. These findings support the early use of combined antimtabicrobial therapy for diabetic foot infections.

Keywords: Diabetes, Infection, MRSA, Polymerase Chain Reaction (PCR), *Staphylococcus aureus*

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1. Introduction

Diabetes mellitus (DM) is classified as one of the four key non-communicable diseases and is increasingly recognized as a significant global health issue that plays a substantial role in premature mortality and disability. This chronic condition has emerged as a major contributor to the overall burden of disease worldwide, leading to severe health complications **(1)**. The continuing rise in diabetes cases positions it as a critical public health concern, necessitating urgent attention and action to mitigate its effects on global health systems and populations **(2)**.

Diabetes, as a serious chronic condition, is linked to numerous complications, among which diabetic foot ulcers (DFUs) stand out. These ulcers frequently become infected, with estimates suggesting that infection occurs in 40% to 80% of cases. The

development of infections often necessitates hospitalization for the affected patients **(3)**.

Methicillin-resistant *Staphylococcus aureus* (MRSA) strains represent a major threat in diabetic foot infections (DFIs) and have become a pressing public health issue due to their enhanced virulence and increasing resistance to a wide range of antibiotics. The heightened presence of MRSA in DFI highlights the challenges faced in effectively managing these infections and raises concerns regarding treatment options available. Furthermore, the rising prevalence of MRSA not only affects patients' outcomes but also contributes to the elevated healthcare costs and complicates existing therapeutic strategies **(4)**. DFIs are a frequent complication faced by those with diabetes mellitus. Approximately 15% of individuals with this condition will develop a foot ulcer during their lifetime. These ulcers are particularly vulnerable to infections and can deteriorate rapidly, leading to considerable tissue damage and possibly necessitating amputation **(5)**.

Diabetic individuals suffering from foot ulcers are at heightened risk of infection from multidrug-resistant (MDR) organisms, including MRSA. This vulnerability is attributed to several factors, including inadequate antibiotic treatment, extended healing times, and frequent hospital admissions **(6)**. Research indicates that *S. aureus* is not only the most frequently isolated organism but also contributes significantly to the complexities seen in diabetic foot infections, which are typically polymicrobial in nature **(7)**. Different classes of antibiotic medications have been employed in the management of infections **(8)**. The management of *S. aureus* infections presents difficulties because of its resistance to most antimicrobial agents **(9)**. It is well established that organisms quickly acquire resistance following the first use of new antibiotics **(9)**. The *mecA* gene is a crucial determinant of antibiotic resistance in certain strains of *S. aureus*, particularly MRSA. The *mecA* gene encodes PBP2a, a modified penicillin-binding protein that confers resistance by reducing the effectiveness of β-lactam antibiotics like penicillins and cephalosporins. Normally, these antibiotics inhibit cell wall synthesis by binding to PBPs, but PBP2a allows bacteria to bypass this inhibition **(10, 11)**.

Studies indicate that patients hospitalized with MRSA infections have a fivefold higher risk of death during their hospital stay compared to those without MRSA infections **(12)**. The rise of MDR organisms in diabetic foot ulcers has severely limited treatment options, especially in low- and middle-income countries. This leads to longer hospital stays, higher costs, and increased morbidity and mortality **(13)**. Extended spectrum β-lactamases (ESBLs) are mainly encoded by TEM, SHV, and CTX-M genes in *Enterobacteriaceae* **(14)**. Cefotaxime can hydrolyze them but have limited activity against ceftazidime **(15)**. Molecular methods often require significant time and financial resources, leading to a continuous demand for a faster, cost-effective, and reliable approach to identify different bacteria involved in DFI. This necessity highlights the importance of exploring alternative technologies that can streamline the identification process while maintaining accuracy and efficiency in clinical settings. Conventional methods have been critiqued for their labor-intensive nature, suggesting a shift toward innovative strategies that could meet the urgent needs of practitioners in managing infections effectively **(16)**.

This study aimed to monitor the presence of the *mecA* gene in diabetic foot infections in Ardabil, Iran.

2. Materials and Methods

2.1 Specimen Collection

In this descriptive and cross-sectional study, samples were randomly collected from 80 diabetic foot ulcers from Imam Khomeini Hospital in Ardabil, North West Iran, between April and March 2023. Initially, the skin or mucous membrane was disinfected using 70% alcohol. Then, the surgeon collected purulent secretions from deep areas of the wound with a sterile syringe and the secretions were sterilely inoculated into thioglycolate broth and incubated at 37°C for overnight. To prevent air from entering the syringe, the thioglycolate broth medium was bent with needle head forceps immediately after inoculation. The samples, consisting of both the broth medium and the syringe containing the secretions, were promptly transported to the microbiology laboratory. The samples with delay transfer, possibility of air exposure and history of antimicrobial therapy excluded from the study.

2.2 Bacterial Isolation and Identification

To isolate and identify *S. aureus*, samples transported in thioglycolate broth were cultured on mannitol salt agar (Merck, Germany) and incubated aerobically overnight at 35–37°C. Colonies were examined for the phenotypic traits, followed by Gram staining to observe cellular characteristics. Further differentiation from other Gram-positive cocci was done using biochemical tests, including catalase and tube coagulase tests **(17)**.

Enterobacterales were identified using biochemical tests. Selected bacteria were cultured on Trypticase Soy Agar and screened with oxidase, OF glucose, and nitrate reduction tests. Oxidase-negative, nitrate-positive, glucose-fermenting strains were further characterized by biochemical assays for carbohydrate

fermentation, enzyme activity, and amino acid decarboxylation. Identification was validated with reference strains, and duplicates with similar antimicrobial profiles were excluded. Isolates were stored in Tryptic Soy Broth with glycerol at -70°C. Anaerobic bacteria were identified using the API 20A kit (bioMérieux).

2.3 Antimicrobial Assay

Antimicrobial susceptibility was assessed using disk diffusion method on Mueller-Hinton agar (Merck, Germany) following CLSI 2023 guidelines. Disks from Padtan Teb, Iran were used, with *S. aureus* ATCC 25923 (Pasteur Institute of Iran) as a quality control strain. Zones of inhibition were measured and categorized as resistant, intermediate, or susceptible according to CLSI standards. MRSA was identified using cefoxitin disk diffusion per CLSI M100 guidelines. The vancomycin MIC was determined by E-test (Mast, UK).

2.4 Phenotypic Screening for ESBL-PE

Enterobacterales isolates were tested for susceptibility using cefotaxime (CTX, 30 µg) and ceftazidime (CAZ, 30 µg) disks. Isolates with zone diameters ≤27 mm for CTX or ≤22 mm for CAZ were considered suspected ESBL producers. Confirmatory testing was done with the double-disk synergy test (DDST) per CLSI 2023 guidelines. Disks of CAZ and CTX, with and without clavulanic acid (30 µg/10 µg), were placed 30 mm apart on Mueller–Hinton agar inoculated with the isolates. After overnight incubation at 37°C, isolates were confirmed as ESBL producers if the zone around either combination disk was at least 5 mm larger than that around the cephalosporin-only disk. *Klebsiella pneumoniae* ATCC 700603 was the positive control, and *E. coli* ATCC 25922 was the negative control.

2.5 Detection of MRSA

Methicillin resistance was identified using a cefoxitin disk (30 µg, Padtan Teb, Iran) following CLSI 2023 guidelines. Isolates with a zone diameter of less than 21 mm were classified as MRSA. The *mecA* gene responsible for methicillin resistance was detected via PCR (Bio-Rad Laboratories, USA).

2.6 Molecular Detection

DNA was extracted from clinical specimens using a Cinna-Gen Co kit (Iran) following the manufacturer's instructions. The purity of the extracted DNA was assessed using NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA), and the samples were stored at −70°C for further analysis. For PCR, reaction mixture was prepared at 25 µL; containing 12.5 µL of 2x Taq Premix Master Mix (Ampliqon UK), 7.5 µL of sterile double-distilled water, 1 µL each of forward and reverse primers (80 pmol/µL), and 3 µL of DNA (200–600 µg/mL). The thermocycling conditions included an initial denaturation at 94°C for 5 min, followed by 30 cycles of 94°C for 45 sec, annealing at 60°C for 45 sec, and extension at 72°C for 90 sec, with a final extension at 72°C for 6 min **(17)**. PCR products were electrophoresed on 1.5% agarose gel containing Safe Dye (Ferments, Denmark), and the gels were visualized under UV light to identify the expected band sizes. The primers used in this study are detailed in Table 1.

2.7 Statistical Analysis

Data were analyzed using SPSS version 23.0 (SPSS Inc., Chicago, IL, USA). The Chi-square test was employed for comparisons of categorical data. P-value less than 0.05 was considered statistically significant.

Table 1. Oligonucleotide primers for *mecA*.

Genes	Oligonucleotide primer(s)	Amplicon size (bp)	References
mecA Forward	3-5-GTAGAAATGACTGAACGTCCGATAA	310	Rasheed and Hussein **(17)**
mecA Reverse	5-CCAATTCCACATTGTTTCGGTCTAA-3		

3. Results & Discussion

3.1 Clinical samples

In this study, 80 samples from foot wound infections were collected from 18 female and 62 male patients diagnosed with diabetic foot ulcer infections based on the surgeon's assessment. The Patients' age ranged from a minimum of 37 years to a maximum of 70 years (Table 2).

3.2 Microbial Examination Results

From 80 samples 8 showed no microbial growth, indicating no bacterial isolation. Among the positive samples, 28 and 34 samples contained single and double bacterial species. Additionally, 10 samples yielded three distinct organisms. Overall, these results demonstrated that wounds are multi-microbial in 61.11% of cases, with

mixed aerobic-anaerobic flora present in 4% of instances. A total of 119 bacterial isolates were identified, of which 114 (95.8%) were aerobic and 5 (4.2%) were anaerobic, highlighting the dominance of aerobic bacteria in these wound cultures.

3.3 Organisms Isolated in Aerobic Conditions

The predominant aerobic organism identified was *S. aureus*, accounting for 28.57% of all isolates. Coagulase-negative staphylococci were the second most common organisms after *S. aureus*, accounting for 17.64% of all isolates (Table 3).

In this study, organisms from the *Enterobacteriaceae* family accounted for 28 isolates, representing 23.52% of the total organisms analyzed. This included 18 isolates of *E. coli* (15.12%), 5 isolates of *Enterobacter* spp. (4.2%), and 5 isolates of *Citrobacter* spp. (4.2%). Following the *Enterobacteriaceae*, the most prevalent organisms were *Enterococci*, with 18 isolates (15.12%). Other aerobic microorganisms identified included *Pseudomonas aeruginosa*, with 8 isolates (6.72%), and *Acinetobacter* spp., with 5 isolates (4.2%). This distribution is detailed in Table 4. There was a statistically significant association between the type of antibiotic treatment and the bacterial species observed (P<0.01, χ^2=16.25). In this study, *Bacteroides fragilis* was the only anaerobic organism identified API 20A (bioMérieux), with a prevalence of 4.2%.

3.4 Phenotypic Investigation ESBL

The study found that 38.8% of the isolates, specifically *E. coli*, were Gram-negative bacilli producing broad-spectrum beta-lactamases.

3.5 *MecA* Investigation

Resistance to MRSA isolates was noted with 23.38% demonstrating resistance according to the cefoxitin 30 µg disc method, which accounted for 13 resistant isolates. The PCR method further revealed that 12 of these isolates possessed the *MecA* gene, confirming their methicillin-resistant characteristics significantly (χ^2=14.3, P<0.01) (Figure 1).

3.6 Discussion

Diabetic foot ulcer infections (DFUIs) represent a significant clinical challenge due to their propensity for rapid progression and the complex interplay of microbial pathogens involved **(7)**. Among diverse microorganisms implicated, *S. aureus* remains a predominant pathogen **(17)**, with the current study identifying 28.57% of isolates as *S. aureus*, aligning closely with earlier studies by Kandemir et al **(18)** (30%) and Ghoreyshizadeh et al **(11)** (28%). These findings underscore the persistent prevalence of *S. aureus* in DFUIs across different populations **(19)**. However, the observed incidence differs from reports by Ako-Nai et al **(2)**, which documented notably lower rates (14% and 13%, respectively), potentially reflecting geographic, methodological, or demographic variations **(20)**.

The rise in methicillin-resistant *S. aureus* (MRSA) represents a significant concern, as it complicates treatment options and elevates healthcare challenges **(18)**. In Iran, the choice of antimicrobial therapy for DFIs is primarily dependent on the ulcer grade and the infection severity, adhering to the global Empiric Antibiotic Regimens for DFUIs **(21)**. In our study, MRSA accounted for 23.38% of *S. aureus* isolates, slightly higher than 37.4% reported by Shanmugam and Jeya **(22)** and Al Benwan et al **(23)**, emphasizing the ongoing challenge of resistance development. The MRSA infections are also associated with prolonged bacteremia and heightened complication rates, which can further compromise patient outcomes.

Conversely, the present study found that *S. aureus* isolates were MRSA. The MRSA isolates are associated with prolonged bacteremia and increased complications related to infections, which can result in treatment failures **(24)**.

Sampling methodology remains a critical factor influencing microbiological diagnosis. While tissue biopsies and fluid aspirations are traditionally considered more reliable for identifying true pathogens, some evidence suggests that wound swabbing post-debridement can approximate the accuracy, especially when performed properly. Nevertheless, superficial swabs may sometimes reflect colonization rather than causative pathogens; thus, adopting standardized sampling techniques is essential for the accurate microbiological assessment **(25)**.

Genotypic detection of resistance genes complements phenotypic antimicrobial susceptibility testing. Detection of the *mecA* gene in our isolates confirmed phenotypic methicillin resistance, with 12 out of 13 MRSA isolates testing positive. This aligns with previous research by Palazzo et al **(26)**, who reported that 41.6% of MRSA cases carried the *mecA* gene. Further investigation into the presence of *mecC* and other resistance genes is necessary to gain a comprehensive understanding of the resistance mechanisms.

Despite its valuable insights, this study has limitations. The reliance on superficial swab samples may underestimate the detection of deeper pathogens, such as osteomyelitis. Additionally, the influence of prior antibiotic therapy within the hospital setting was not evaluated, which could impact the resistance patterns. The sample size and demographic scope limit the generalizability of these findings, highlighting the necessity for larger, multicenter studies encompassing various stages of skin lesions and broader patient populations. Future research should also focus on virulence factors and their role in infection severity.

The high prevalence of MRSA among *S. aureus* isolates, coupled with significant resistance to vancomycin highlights the critical need for ongoing antimicrobial monitoring and the development of personalized treatment approaches. Understanding the microbiology and resistance mechanisms in DFUIs is essential for optimizing the management and mitigating the threat of antimicrobial resistance in diabetic patients.

Table 2. Clinical characteristics of patients with DFUIs.

Variables	mean
age (years)	58.29±13.61
Man (%)	77.5
Female (%)	22.5
Type 1 diabetes (%)	22.5
Type 2 diabetes (%)	77.5
Duration of diabetes (years)	19
Wound duration (days)	29
Previous antibiotic use (%)	40
Current antibiotic use (%)	60

Table 3. Antibiotic resistance profile for Gram-positive organisms.

Organism / Antibiotics	*Enterococcus faecalis* (%)	*coagulase-negative staphylococci* (%)	*S. aureus* (%)
Vancomycin	44.44	0	20
Linezolid	0	0	0
Clindamycin	---------	38.09	23.52
Ciprofloxacin	---------	47.61	52.94
Erythromycin	72.22	23.80	61.76
Gentamicin	100	23.80	47.05
Rifampin	100	38.09	29.41
Ampicillin	83.33	----------	----------

Table 4. Antibiotic resistance profile for Gram-negative organisms.

Organism / Antibiotics	*Acinetobacter spp* (%)	*P. aeruginosa* (%)	*Citrobacter spp* (%)	*Enterobacter spp* (%)	*E. coli* (%)
Ciprofloxacin	40	62.5	100	40	83.33
Tetracycline	---	-------	100	40	72.22
Gentamicin	40	--	100	--	27.77
Ampicillin	--------	--------	100	100	55.55
Piperacillin-tazobactam	40	62.5	100	0	16.66
Amoxicillin clavulanic acid	--------	-------	40	0	23.33
Piperacillin	-------	50	-------	--------	--------
Imipenem	40	12.5	0	0	0
cefepime	100	--------	0	40	55.55
ceftriaxone	100	--------	40	40	72.22

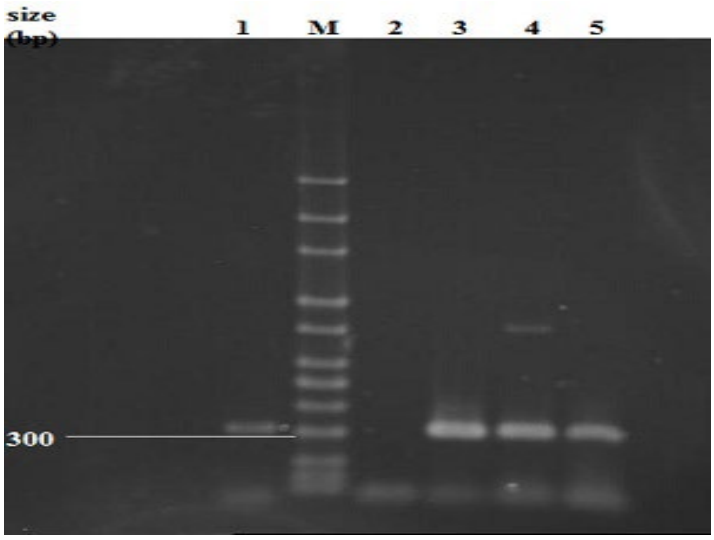

Figure 1. 1: *mecA* gene expression; **M:** DNA Ladder. **2:** Negative control, **3:** Positive control *mecA*, **4, 5:** are strains of *S. aureus* isolated from diabetic foot infection with *mecA* gene **(Prepared by Authors, 2025)**.

4. Conclusion

The high prevalence of MRSA in diabetic foot ulcers underscores the escalating issue of antibiotic resistance among diabetic patients, necessitating prudent antimicrobial use and vigilant monitoring. The predominance of *S. aureus*, particularly resistant strains, highlights the importance of the tailored treatment approaches, potentially involving combination antimicrobial therapy to improve infection control and reduce complications. Variations in local antimicrobial susceptibility patterns and microbiological profiles further emphasize the need for region-specific data to optimize the management strategies. To overcome these challenges, additional research is crucial to deepen our understanding of resistance mechanisms and to establish more effective, evidence-based treatment protocols for diabetic foot infections.

5. Declarations

5.1 Acknowledgment

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5.2 Ethical Considerations

The present study was ethically approved by the Science and Research Branch, Islamic Azad University, Ardabil, Iran, Institutional Review Board (IR.IAU.ARDABIL.REC1400.064).

5.3 Authors' Contributions

MTA design the study. MM, MTA and MF conducted the experiments. MM, MTA analyzed the data. MM, MTA drafted the article. All authors read and approved the final manuscript.

5.4 Conflict of Interests

The authors have no conflicts of interest to declare.

5.5 Financial Support and Sponsorship

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5.6 Using Artificial Intelligence Tools (AI Tools)

All authors declare that there is no use of AI Tools in this study, including the writing of this manuscript.

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