



DETECTION OF THE blaZ GENE AS A PENICILLIN RESISTANCE MARKER IN STAPHYLOCOCCUS AUREUS FROM DIABETIC ULCER SWABS

Salsabila Anggia Indrayani^a ; Pestariati^{b*} ; Sri Sulami Endah Astuti^c

^{a,b,c} Medical Laboratory Technology, Poltekkes Kemenkes Surabaya ; Pucang Jajar Tengah No. 56 ; Surabaya and 60282 ; Indonesia

Abstract

Diabetic ulcer is the most common complication in patients with diabetes mellitus, and *Staphylococcus aureus* is the predominant bacterial cause of infection. The aim of this study was to detect the presence of the blaZ gene as a penicillin-resistance marker in *S. aureus* isolated from diabetic ulcer swabs. This research was a descriptive quantitative study. The study population consisted of patients with diabetic ulcers treated at the Diabetes Wound Care Center Branch in Surabaya. Respondents (samples) were selected using purposive sampling, with inclusion criteria being patients diagnosed with diabetic ulcers and eligible for swab collection. A total of 30 ulcer swab samples were obtained between April and May 2025. Isolation and identification were conducted at the Bacteriology Laboratory of Poltekkes Surabaya, resulting in 16 isolates (54%) identified as *S. aureus*. Among these, 10 isolates (62.5%) were resistant to penicillin G. Detection of the blaZ gene using Real-Time PCR at the Molecular Biology Laboratory of Poltekkes Surabaya showed that 8 isolates (80%) were positive for the gene. These findings indicate that most penicillin-resistant *S. aureus* isolates carry the blaZ gene, suggesting high potential for β -lactamase production. Future studies are recommended to include larger sample sizes, analyze additional resistance genes, and investigate clinical factors influencing the emergence of antibiotic resistance in diabetic ulcers.

Keywords: blaZ gene, diabetic ulcer, staphylococcus aureus

1. Introduction

Diabetes mellitus is a metabolic disorder characterized by chronic elevated blood glucose levels (Ojo et al., 2023). High blood glucose levels in people with diabetes can trigger various types of complications, one of which is diabetic foot ulcers. Foot ulcers in diabetic patients are a complication of diabetes mellitus caused by nerve damage and peripheral vascular disorders in the lower extremities (Prasetyo, 2020).

Diabetic foot ulcers (DFUs) are a serious complication of diabetes, estimated to occur in 19–34% of patients during their lifetime. Globally, this complication affects approximately 15–25% of people with diabetes (Thotad et al., 2023).

*) Corresponding Author (Pestariati)
Email: pestariati@poltekkesdepkes-sby.ac.id

Hyperglycemia also creates an environment conducive to bacterial growth, particularly *Staphylococcus aureus*, an aerobic Gram-positive coccus (Ramirez-Acuña et al., 2019). *Staphylococcus aureus* is generally part of the normal flora, but under certain conditions, such as when the immune system is compromised, wounds are present, or the microbiota balance is disrupted, it can trigger various infections (Prila Nanggita et al., 2023). Chronic hyperglycemia can also interfere with leukocyte function, increasing the risk of bacterial infection in wounds (Prasetyo, 2020).

Staphylococcus aureus has various virulence factors, including surface proteins that play a role in the adhesion process to host cells, enzymes involved in protein degradation, and toxins that can damage cells in the host body (Umarudin et al., 2023).

In recent years, there has been a significant increase in antimicrobial resistance, with many strains now recognized as multidrug resistant. These resistance mechanisms are generally encoded by plasmids, such as the *blaZ* gene, which regulates the production of penicillinase enzymes (Andrzejczuk et al., 2023). The *blaZ* gene contributes to *Staphylococcus aureus* resistance to penicillin by encoding the β -lactamase enzyme, which destroys the β -lactam ring and inactivates the antibiotic. The activity of this enzyme enables the bacteria to survive despite antibiotic exposure, making treatment increasingly challenging (Okiki et al., 2020).

The expression of the *blaZ* gene is regulated by two regulatory genes, *blaR1* and *blaI*. When *S. aureus* is exposed to β -lactam antibiotics, the *blaR1* gene acts as a sensor and undergoes autocatalytic cleavage, triggering the degradation of the repressor *blaI*. This process allows the transcription of the *blaZ* gene and subsequent β -lactamase production (Juwita et al., 2024).

However, previous studies have reported inconsistent findings regarding the prevalence of the *blaZ* gene in *S. aureus* isolates from different clinical settings, indicating that the role and distribution of this gene remain debatable across populations and geographical regions. Such variability suggests a gap in current knowledge, particularly in diabetic ulcer cases in Indonesia, where molecular data remain limited. Therefore, the aim of this study was to detect the presence of the *blaZ* gene as a marker of penicillin resistance in *Staphylococcus aureus* isolated from diabetic ulcer swabs.

2. Method

This quantitative descriptive study involved 30 diabetic foot ulcer (DFU) patients selected through purposive sampling from the Diabetes Wound Care Center in Surabaya. The study population consisted of all DFU patients treated at the center, with inclusion criteria being DFU patients showing clinical signs of infection, not receiving systemic antibiotics within 48 hours, and providing informed consent for swab collection. Wound swabs were taken using sterile cotton swabs from the cleaned base of the ulcer and transported in sterile media to the laboratory. *Staphylococcus aureus* was isolated using Mannitol Salt Agar and Blood Agar, followed by Gram staining, catalase and coagulase tests, and penicillin G susceptibility testing using the Kirby-Bauer method. Genomic DNA was extracted from confirmed isolates using the Geneaid Bacterial Genomic DNA Minikit. The *blaZ* gene was amplified using specific primers (Forward: ACTTCAACACCTGCTGCT; Reverse: TGACCACTTTTATCAGCAACC) and detected through Real-Time PCR using the Bio-Rad CFX96 Touch™ system with SYBR Green chemistry. Amplicons were verified using 1.5% agarose gel electrophoresis. No plasmid isolation, sequencing, or 16S rRNA analysis was performed. Ethical clearance was obtained from the Health Research Ethics Committee of Poltekkes Kemenkes Surabaya.

3. Result and Discussion

A total of 30 diabetic ulcer swab samples were cultured on Blood Agar Plate (BAP) and Mannitol Salt Agar (MSA), followed by biochemical identification using catalase and coagulase tests. Of these samples, 16 isolates (53.33%) were identified as *Staphylococcus aureus*. This proportion aligns with earlier studies reporting that *S. aureus* is one of the dominant pathogens in diabetic foot ulcers due to its strong virulence factors and adaptability in chronic wound environments (Susanti et al., 2023). The presence of *S. aureus* in more than half of the samples reinforces the fact that diabetic ulcers are highly susceptible to colonization by this bacterium, which worsens tissue damage and delays healing. Macroscopic observations on BAP showed that positive isolates exhibited round, yellow-gold colonies with clear hemolysis zones, consistent with the characteristics of *S. aureus*. Hemolysis results from hemolysin activity, a major virulence factor capable of lysing erythrocytes. This finding supports previous reports that hemolysin plays a major role in bacterial survival by destroying host tissues and aiding in nutrient acquisition. The presence of hemolysis in all positive isolates suggests that the strains found in DFU patients possess strong pathogenic potential.

On MSA media, 16 samples (53.33%) demonstrated mannitol fermentation, indicated by the yellow coloration of the media due to decreased pH detected by the phenol red indicator. This result reflects the classical behavior of *S. aureus*, which is capable of fermenting mannitol, unlike many other *Staphylococcus* species. The fact that not all isolates fermented mannitol highlights that DFU infections may also involve coagulase-negative staphylococci (CoNS), which often resemble *S. aureus* morphologically but lack certain virulence traits. This reinforces the need for biochemical confirmation when dealing with clinical isolates. The catalase test further supported the identification, as all isolates suspected to be *S. aureus* exhibited vigorous bubble formation, consistent with their known catalase-positive nature. Catalase activity is essential for bacterial survival in infected tissues because it neutralizes hydrogen peroxide produced by host immune cells (Pulungan & Tumangger, 2018). This ability may explain why *S. aureus* thrives in chronic diabetic wounds, which are characterized by prolonged inflammatory responses and oxidative stress.

The coagulase test confirmed pathogenic *S. aureus* in 16 samples, consistent with coagulase being a hallmark enzyme that differentiates *S. aureus* from non-pathogenic *Staphylococci*. Coagulase contributes to abscess formation, allowing the bacteria to evade immune responses (Ni Wayan Desi Bintari et al., 2023). The detection of coagulase-positive isolates in more than half of the samples indicates that diabetic ulcers in the study area are predominantly infected by virulent strains capable of causing severe tissue infection. This finding is clinically significant because coagulase-positive *S. aureus* strains are often associated with higher antibiotic resistance and more aggressive clinical outcomes. Overall, the combination of cultural characteristics, biochemical profiles, and virulence factor expression demonstrates that *S. aureus* is a major pathogen in DFU cases in this population. This supports existing literature while also highlighting the need for molecular confirmation to understand resistance patterns, especially regarding the *blaZ* gene responsible for penicillin resistance.

The growth of *Staphylococcus aureus* bacteria was followed by antibiotic sensitivity testing using the Kirby-Bauer disk diffusion method on Mueller Hinton Agar (MHA) medium with penicillin G antibiotic disks.

The sensitivity test results on 16 confirmed *Staphylococcus aureus* isolates showed that 10 isolates (62.5%) were resistant to penicillin G, indicating a high level of β -lactam resistance among *S. aureus* from diabetic ulcer infections. This finding aligns with the well-documented global trend of increasing penicillin resistance in *S. aureus*, which is primarily driven by the widespread presence of the *blaZ* gene encoding β -lactamase. However, the remaining 6 isolates (37.5%) were still sensitive to penicillin G. This proportion of susceptibility is higher than what many earlier studies have reported. For example, McNeil et al. (2024) found that only 9% of *S. aureus* isolates from skin and soft-tissue infections in a pediatric hospital remained susceptible to penicillin. The higher susceptibility in this study may reflect differences in patient population, infection severity, or antibiotic exposure history. It is also possible that environmental or clinical management factors in diabetic wound care settings influence the selective pressure for resistance development.

Ten isolates that showed resistance were subsequently processed for genomic DNA extraction using the Promega Wizard Genomic DNA Purification Kit. This extraction method effectively separates pure DNA from other cellular components—including RNA, proteins, lipids, and carbohydrates—thus ensuring that the final DNA preparation is suitable for downstream molecular analysis using Real-Time PCR. The extracted DNA was then quantified with a NanoDrop spectrophotometer by measuring absorbance at 260 nm and 280 nm to determine DNA concentration and purity. Purity assessment through A260/A280 ratios is essential because protein contamination or leftover extraction reagents can inhibit PCR amplification, and previous studies have emphasized that optimal DNA purity significantly enhances the reliability of molecular detection of resistance genes such as **blaZ**.

Table 1. Quantification test results on the NanoDrop spectrophotometer

Sampel Code	Consentration (ng/ul)	Purity (A260/280)
Control +	54.35	1.95
001	41.34	1.86
002	35.15	1.91
004	43.83	1.96
009	9.53	1.93
010	45.12	1.92
012	54.34	1.95
016	16.72	1.89
017	46.13	1.83
020	17.51	1.93
022	6.84	1.81

The data in Table 1. show that the DNA quantification results using the Nanodrop spectrophotometer have met the standards, with a purity level between 1.8–2.0 as indicators of good DNA quality (Nuraeni et al., 2024). DNA that has a good concentration and purity level and meets the standards can be used further in the DNA amplification stage.

DNA amplification is a technique for rapidly and specifically replicating DNA fragments through a repeated thermal cycle consisting of three main stages: denaturation, annealing, and extension. This process is generally carried out for up to 40 cycles, with each cycle resulting in an exponential multiplication of the target DNA, thereby significantly increasing the total concentration of DNA formed (Ningsih et al., 2021).

Table 2. blaZ gene primer specificity

Target Gene	Primer	DNA Sequence (5'-3')
blaZ	Forward	ACTTCAACACCTGCTGCTTTC
	Reverse	TGACCACTTTTATCAGCAACC
Thermocycling		
Pre Denaturation		95°C for 5 minutes
Denaturation		95°C for 15 seconds
Annealing		58.2°C for 15 seconds
Cycle		40 cycles

Table 2. presents the amplification reaction conditions or thermocycling settings that have been adjusted to the specificity of the blaZ gene primer.

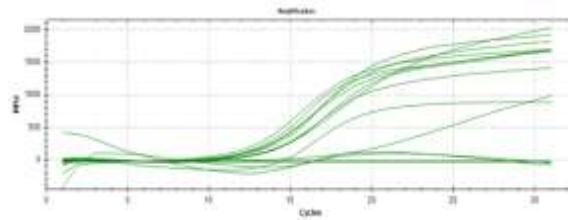


Figure 1. *blaZ* gene detection curve in Real-time PCR

After the amplification process is complete, the Real-Time PCR results are displayed in the form of an amplification curve and Cq (cycle quantification) values. Figure 1 shows the detection curve for the *blaZ* gene in 10 *Staphylococcus aureus* isolates. Two types of curve patterns are observed: sigmoid and non-sigmoid curves. The sigmoid curve appears in the positive control and eight of the ten samples, indicating the presence of the target gene. Meanwhile, the other two samples and the negative control show a non-sigmoid curve, indicating that the *blaZ* gene was not detected in those samples.

Table 3. *blaZ* Cq Value in Real-Time PCR

Sample	Cq Value	Description
Positive control	36.27	Positive
Negative control	N/A	Negative
001	3.65	Positive
002	35.49	Positive
004	4.42	Positive
009	33.57	Positive
010	5.09	Positive
012	N/A	Negative
016	N/A	Negative
017	17.30	Positive
020	2.78	Positive
022	2.31	Positive

In addition to the amplification curve shape, positive samples can also be determined by looking at the Cq (Cycle quantification) value. Table 3 shows that the Cq value was detected in the positive control and eight clinical samples (001, 002, 004, 009, 010, 017, 020, and 022), indicating successful amplification of the *blaZ* gene. Meanwhile, samples 012 and 016, along with the negative control, showed N/A (Not Available), which means no amplification signal was detected. The variation in Cq values among the positive samples from very low (2.31 in sample 022 and 2.78 in sample 020) to relatively high (35.49 in sample 002) reflects differences in the initial concentration of target DNA. Lower Cq values indicate higher amounts of *blaZ* gene copies, while higher Cq values suggest a smaller initial quantity of the gene.

The detection of the *blaZ* gene in these eight isolates has important biological and clinical implications. The *blaZ* gene encodes β -lactamase, an enzyme capable of hydrolyzing the β -lactam ring of penicillin, rendering the antibiotic ineffective. Therefore, isolates carrying this gene possess a well-established genetic mechanism for penicillin resistance. This molecular finding is consistent with the phenotypic sensitivity test results, in which several *Staphylococcus aureus* isolates demonstrated resistance to penicillin G. The presence of *blaZ* provides a clear explanation for this resistance, strengthening the reliability of the laboratory findings.

From a microbiological standpoint, the presence of *blaZ* enhances bacterial survival in environments with repeated or prolonged antibiotic exposure. DFU patients commonly receive antibiotics over long periods, creating selective pressure that encourages the persistence of resistant strains. Previous studies

also reported the widespread distribution of blaZ-positive *S. aureus* in chronic wound infections (Smith et al., 2022). The similar pattern observed in this study suggests that antibiotic selection pressure in DFU cases contributes to the establishment of resistant microorganisms.

For patients, especially those with diabetes, the presence of blaZ-positive *S. aureus* has significant clinical consequences. Resistant strains are associated with delayed wound healing, greater risk of cellulitis, increased likelihood of treatment failure, and more complicated infection control. DFU patients already have impaired immunity and poor peripheral circulation, making them more vulnerable to persistent or recurrent infections. When infections are caused by blaZ-harboring bacteria, clinicians may be forced to use alternative antibiotics that are stronger, more expensive, or potentially more toxic.

In the broader context of DFU management, these findings highlight the need for early molecular detection of resistance genes to aid clinicians in choosing the most effective antibiotic therapy. The presence of blaZ in half of the tested isolates also underscores the importance of antibiotic stewardship, regular monitoring of resistance patterns, and strengthening wound care protocols to prevent the spread of resistant *S. aureus* strains.

Overall, the detection of the blaZ gene in eight samples confirms the molecular basis of penicillin resistance, provides insight into microbial adaptation in chronic diabetic wounds, and carries serious implications for clinical decision-making and patient outcomes.

4. Conclusion and Suggestion

This study aimed to detect the presence of the blaZ gene in *Staphylococcus aureus* isolates obtained from diabetic ulcer swabs. The findings confirmed that eight isolates carried the blaZ gene, indicating the presence of a genetic mechanism responsible for penicillin resistance in a portion of the bacterial population. These results show that blaZ-mediated resistance is present in DFU cases and highlight the importance of molecular screening to support more accurate antibiotic selection in clinical practice.

This study was limited by the relatively small sample size, the use of a single health-care facility as the sampling location, and the absence of additional molecular analyses such as sequencing, which restricted the ability to identify specific gene variants or mutations. The study also did not correlate clinical outcomes (healing rate, severity of infection) with the presence of the blaZ gene.

Future studies should involve larger and more diverse sample populations, include sequencing to examine blaZ gene variants, and explore other resistance genes that may coexist with blaZ. Research linking molecular findings with patient clinical profiles, treatment responses, and wound-healing outcomes is also recommended to provide a more comprehensive understanding of antibiotic resistance in diabetic foot ulcers.

5. Acknowledgments

-

6. References

- Andrzejczuk, S., Cygan, M., Dłuski, D., Stępień-Pyśniak, D., & Kosikowska, U. (2023). Staphylococcal Resistance Patterns, blaZ and SCCmec Cassette Genes in the Nasopharyngeal Microbiota of Pregnant Women. *International Journal of Molecular Sciences*, 24(9). <https://doi.org/10.3390/ijms24097980>
- Juwita, S., Indrawati, A., Damajanti, R., Safika, Mayasari, N. L. P., Purwanto, E., Halik, H., Ramadhan, M., & Suhartila. (2024). Pendekatan One Health: Deteksi Gen blaZ dari Isolat *Staphylococcus aureus*

- Asal Peternakan Sapi Perah di Sulawesi Selatan. *Jurnal Veteriner*, 25(1), 122–131. <https://doi.org/10.19087/jveteriner.2024.25.1.122>
- McNeil, J. C., Sommer, L. M., Joseph, M., Hulten, K. G., & Kaplan, S. L. (2024). Penicillin Susceptibility among *Staphylococcus aureus* Skin and Soft Tissue Infections at a Children's Hospital. *Microbiol Spectr*.
- Ni Wayan Desi Bintari, Ms., Noor Andryan Ilsa, Ms., Istyanto, F., Rochmanah Suhartati, M., Ratih Kartika Dewi, Ms., Biomed Zuraida, M., Herlina, M., Maulin Inggraini, Mk., Seftiwan Pratami, Ms., Jumriah Nur, Ms., Doni Setiawan, Ms., Biotek Dian Rachma Wijayanti, M., & Fitriani Safari, W. (2023). BAKTERIOLOGI UNTUK MAHASISWA KESEHATAN.
- Ningsih, H., Purnama Ramdan, E., Nurul Septariani, D., & Ferwita Sari, M. (2021). Pengantar Bioteknologi. <https://www.researchgate.net/publication/357680188>
- Nuraeni, U., Apriori, D., Susmiarni, R. D., Ain, M. M., & Damacena, R. (2024). PEDOMAN EKSTRAKSI DNA DALAM MATRIKS PANGAN DAN PAKAN. Direktorat Standar Nasional Ukuran Mekanika Radiasi, dan Biologi.
- Ojo, O. A., Ibrahim, H. S., Rotimi, D. E., Ogunlakin, A. D., & Ojo, A. B. (2023). Diabetes mellitus: From molecular mechanism to pathophysiology and pharmacology. In *Medicine in Novel Technology and Devices* (Vol. 19). Elsevier B.V. <https://doi.org/10.1016/j.medntd.2023.100247>
- Okiki, P. A., Eromosele, E. S., Ade-Ojo, P., Sobajo, O. A., Idris, O. O., & Agbana, R. D. (2020). Occurrence of *mecA* and *blaZ* genes in methicillin-resistant *Staphylococcus aureus* associated with vaginitis among pregnant women in Ado-Ekiti, Nigeria. *New Microbes and New Infections*, 38. <https://doi.org/10.1016/j.nmni.2020.100772>
- Prasetyo, B. (2020). TERAPI HIPERBARIK ULKUS KAKI DIABETIK. *Journal of Nutrition and Health*, 8, 42–45.
- Prila Nanggita, P., Mu'arofah, B., Imasari, T., Santoso, K., Kediri, B. W., Surya Prima, K., & Kunjang, H. (2023). Deteksi Bakteri *Staphylococcus* sp. pada Swab Rongga Mulut Mahasiswa D3 TLM IIK Bhakti Wiyata Kediri yang Memakai Kawat Gigi Detection of *Staphylococcus* sp. in Oral Cavity Swabs for D3 TLM Students IIK Bhakti Wiyata Kediri Who Wears Tooth Braces. In *J. Sintesis Submitted* : 19 Mei (Vol. 4, Issue 1).
- Pulungan, A. S. S., & Tumangger, D. E. (2018). ISOLASI DAN KARAKTERISASI BAKTERI ENDOFIT PENGHASIL ENZIM KATALASE DARI DAUN BUASBUAS (*PREMNA PUBESCENS* BLUME). *BIOLINK (Jurnal Biologi Lingkungan Industri Kesehatan)*, 5(1), 71–80. <https://doi.org/10.31289/biolink.v5i1.1665>
- Ramirez-Acuña, J. M., Cardenas-Cadena, S. A., Marquez-Salas, P. A., Garza-Veloz, I., Perez-Favila, A., Cid-Baez, M. A., Flores-Morales, V., & Martinez-Fierro, M. L. (2019). Diabetic foot ulcers: Current advances in antimicrobial therapies and emerging treatments. In *Antibiotics* (Vol. 8, Issue 4). MDPI AG. <https://doi.org/10.3390/antibiotics8040193>
- Susanti, M., & Gumilar, A. G. (2023). BUKU AJAR ISOLASI & IDENTIFIKASI BAKTERI KLINIK (M. Susanti, Ed.; 1st ed., Vol. 1). Yayasan Drestanta Pelita Indonesia.
- Thotad, P. N., Bharamagoudar, G. R., & Anami, B. S. (2023). Diabetic foot ulcer detection using deep learning approaches. *Sensors International*, 4. <https://doi.org/10.1016/j.sintl.2022.100210>
- Umarudin, Adnyana, I. G. A., Rohayati, Slamet, N. S., Sembiring, F., Rakanita, Y., Sari, N. K. Y., Sumariangen, A. B., Kurniati, I., Yuliawati, Permatasari, A. A. A. P., Merdekawati, F., & Dermawan, A. (2023). BAKTERIOLOGI 2 (H. Akbar, Ed.). MEDIA SAINS INDONESIA.