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Antibiotic Susceptibility of *Staphylococcus sp.* in Diabetic Ulcer Patients at Dr. Mohammad Hoesin General Hospital, Palembang

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Abstract

The high prevalence of diabetic ulcers and the resistance of *Staphylococcus sp.* to antibiotics can complicate the treatment of diabetic ulcer patients with *Staphylococcus sp.* This study aimed to determine the pattern of susceptibility of *Staphylococcus sp.* in patients with diabetic ulcer at Dr. Mohammad Hoesin General Hospital Palembang in January 2019-August 2022. This research was an observational descriptive study using total sampling conducted in September-November 2022 at Dr. Mohammad Hoesin General Hospital Palembang. Data was obtained from patient medical records and laboratory results of patients with diabetic ulcer disease infected with *Staphylococcus sp*. from January 2019 to August 2022. Prevalence of *Staphylococcus sp.* infection in diabetic ulcer patients was low (15 of 77 diabetic ulcer patients, 19.5%). There were 19 bacterial isolates from 15 diabetic ulcer patients. A total of 8 coagulase-negative *Staphylococcus sp.* (CoNS) and 11 coagulase-positive *Staphylococcus sp.* (CoPS) isolates were tested for their susceptibility to antibiotics. Both CoPS and CoNS bacteria in diabetic ulcer patients were most susceptible to linezolid (90.9% for CoPS and 100% for CoNS) and most resistant to benzylpenicillin (81.8% for CoPS and 87.5% for CoNS).

Keywords: Diabetic Ulcer, Staphylococcus sp., Antibiotic Resistance

1. Introduction

Diabetes melitus (DM) is a chronic metabolic disease characterized by hyperglycemia, or an abnormal increase of blood glucose levels.[1] DM patients who do not maintain a healthy lifestyle are at risk of microvascular and macrovascular complications. One complication which may affect a DM patient's quality of life is diabetic ulcer.[1,2] Incidence of infection in diabetic ulcer patients has also been the reason behind increased hospitalizations, and the infection may be caused by Gram-positive or Gram-negative bacteria.[3–5]

Previous studies had reported that the main causative organisms of infection in diabetic ulcers are *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Escherichia coli*.[5] *Staphylococcus sp*. are commensal bacteria on human skin, so their infection may be passed on via contact with skin, open lesions, or respiratory tracts. Risk of contagion is heightened at hospitals. *Staphylococcus aureus* is a Gram-positive, coagulative and hemolytic species of the *Staphylococcus* genus, where this species is the most frequent causative agent of infections in humans. There also exist coagulase-negative *Staphylococcus* (CoNS) species.[6]

Severity of *Staphylococcus sp.* infections is influenced by host immunity and bacterial virulence.[7–9] Some virulence factors in *Staphylococcus sp.,* especially *S. aureus,* are cytolytic toxins, exfoliative toxin, enterotoxin and toxic shock syndrome toxin-1 (TSST-1). Enterotoxin and TSST-1 are otherwise known as pyrogenic toxin superantigens (PTSAgs). Weakened immunity and increased blood glucose level

in diabetic ulcer patients make the virulence of *Staphylococcus sp.* even harder to manage.[9]

Diabetic ulcers had been known to provide an ideal environment for *S. aureus* growth.[8] The immediate eradication of these bacteria is complicated by their ability to adapt and become resistant to various antibiotics, such as in cases of methicillin-resistant Staphylococcus aureus (MRSA). Some isolates of *S. aureus* have been reported to develop resistance towards vancomycin (vancomycin-resistant S. aureus, VRSA) after previous instances of vancomycin intermediate-resistant S. aureus (VISA).[10,11] Data from 8 main referral hospitals in Indonesia showed that the prevalence of MRSA infection was at 38%.[12] Methicillin resistance had also been reported on CoNS bacteria. An Ethiopian study in patients with infected wounds reported that out of the 12% of infections caused by CoNS, 52% were resistant to methicillin.[13]

Considering the high incidence of infection and antibiotic resistance by *Staphylococcus sp*., this study aimed to determine the susceptibility pattern of *Staphylococcus sp.* bacteria to antibiotics in patients with diabetic ulcers, therefore providing a scientific base for proper antimicrobial therapy.

2. Methods

This study was a descriptive observational study, which was conducted from September to December 2022 at the Microbiology Laboratory of Dr. Mohammad Hoesin General Hospital, Palembang and using secondary data from medical records and laboratory exam reports. Samples were all medical records and laboratory result documents of diabetic ulcer patients at Dr. Mohammad Hoesin General Hospital, Palembang, from January 1, 2019 to August 31, 2022. Samples were obtained by using total sampling method. Damaged, incomplete, or defective documents were excluded from this study. Obtained data were processed univariately by using IBM SPSS Statistics 26 for Windows, and results were presented in tables and descriptive narration.

3. Results

Out of 77 patients with diabetic ulcer who underwent laboratory examination, 15 were infected by *Staphylococcus sp*. The prevalence of *Staphylococcus sp.* infection in diabetic ulcer patients was 19.5%. Five patients were infected from January 2019 to December 2020, and 10 were infected from January 2021 to August 2022. Nineteen (19) specimens were obtained from the patients for susceptibility testing. There were 11 cases of coagulase-positive *Staphylococcus* (CoPS) infections and 8 cases of CoNS infections. The species of CoPS bacteria identified in this study were *S. aureus* and *S*. *pseudintermedius,* and there were 4 identified species of CoNS bacteria (*S. epidermidis, S. haemolyticus, S. hominis* ssp *hominis,* and *S. warneri)* (Table 1).

Table 1. Distribution of *Staphylococcus sp.* infection in diabetic ulcer by bacterial species

Species	n; %
CoPS	**11; 57.9%**
Staphylococcus aureus	10; 52.6%
Staphylococcus pseudintermedius	1; 5.3%
CoNS	**8; 42.1%**
Staphylococcus epidermidis	1; 5.3%
Staphylococcus haemolyticus	4; 21,1%
Staphylococcus hominis ssp *hominis*	1; 5,3%
Staphylococcus warneri	2; 10,5%

Table 2. Antibiotic susceptibility pattern of CoPS in diabetic ulcer patients

No.	Antibiotic	Susceptibility Test Results						Total
		Resistant		Intermediate		Sensitive		
		n	%	n	%	n	%	
1.	Benzylpenicillin	9	81,8	0	0	2	18,2	11 (100%)
2.	Tetracycline	6	54,5	0	0	5	45,5	11 (100%)
3.	Oxacillin	5	45,5	0	0	6	54,5	11 (100%)
4.	Ciprofloxacin	5	45,5	1	9,1	5	45,5	11 (100%)
5.	Trimethoprim/sulfamethoxazole	4	36,4	0	0	7	63,6	11 (100%)
6.	Levofloxacin	4	36,4	1	9,1	6	54,5	11 (100%)
7.	Moxifloxacin	4	36,4	1	9,1	6	54,5	11 (100%)
8.	Gentamicin	3	27,3	1	9,1	7	63,6	11 (100%)
9.	Clindamycin	1	9,1	0	0	10	90,9	11 (100%)
10.	Linezolid	1	9,1	0	0	10	90,9	11 (100%)
11.	Nitrofurantoin	1	9,1	0	0	10	90,9	11 (100%)
12.	Quinupristin/dalfopristin	1	9,1	0	0	10	90,9	11 (100%)
13.	Rifampicin	1	9,1	0	0	10	90,9	11 (100%)
14.	Tigecycline	1	9,1	0	0	10	90,9	11 (100%)
15.	Vancomycin	1	9,1	0	0	10	90,9	11 (100%)
16.	Erythromycin	1	9,1	1	9,1	9	81,8	11 (100%)

Table 3. Antibiotic susceptibility pattern of CoNS in diabetic ulcer patients

No.	Antibiotic	Susceptibility Test Results						Total
		Resistant		Intermediate		Sensitive		
		n	%	n	%	n	%	
1.	Benzylpenicillin	7	87,5	0	0	1	12,5	8 (100%)
2.	Erythromycin	7	87,5	0	0	1	12,5	8 (100%)
3.	Oxacillin	7	87,5	0	0	1	12,5	8 (100%)
4.	Ciprofloxacin	6	75	0	0	2	25	8 (100%)
5.	Clindamycin	6	75	0	0	2	25	8 (100%)
6.	Levofloxacin	6	75	0	0	2	25	8 (100%)
7.	Tetracycline	5	62,5	0	0	3	37,5	8 (100%)
8.	Trimethoprim/sulfamethoxazole	5	62,5	0	0	3	37,5	8 (100%)
9.	Moxifloxacin	5	62,5	1	12,5	2	25	8 (100%)
10.	Gentamicin	4	50	0	0	4	50	8 (100%)
11.	Rifampicin	3	37,5	0	0	5	62,5	8 (100%)
12.	Quinupristin/dalfopristin	1	12,5	0	0	7	87,5	8 (100%)
13.	Tigecycline	1	12,5	0	0	7	87,5	8 (100%)
14.	Vancomycin	1	12,5	1	12,5	6	75	8 (100%)
15.	Linezolid	0	0	0	0	8	100	8 (100%)
16.	Nitrofurantoin	0	0	1	12,5	7	87,5	8 (100%)

All 19 isolates were tested for antibiotic susceptibility to 16 different antibiotics. On examination, the CoPS bacterial isolates had a high resistance level towards benzylpenicillin (81.8%). CoPS isolates were susceptible to clindamycin, linezolid, nitrofurantoin, quinupristin/dalfopristin, rifampicin, tigecycline, and vancomycin with a susceptibility level of 90.9% (Table 2). On the other hand, CoNS isolates showed high resistance towards benzylpenicillin, erythromycin, and oxacillin (87.5% each, respectively). CoNS isolates were most sensitive to linezolid (100%), followed by nitrofurantoin, quinupristin/dalfopristin, and tigecycline (87.5% each, respectively) (Table 3).

4. Discussion

The prevalence of *Staphylococcus sp.* Infection in diabetic ulcer patients from January 2019 to August 2022 was 19.5%. This finding is similar to a study in diabetic ulcer patients of Justice K.S. Hegde Charitable Hospital, India, in September 2019 to February 2020, where the prevalence of *Staphylococcus sp.* Infection was 18.17%, and a study at Baqai Institute of Diabetology and Endocrinology (BIDE), Pakistan, where a prevalence rate of 21.4% was reported.[14,15] These findings were also in line with several studies across regions in Indonesia from 2015 to 2020, which stated that most diabetic ulcer infections in Indonesia were caused by Gram-negative bacteria, while *Staphylococcus sp.* Are Gram-positive. However, there had been no exact explanation why Gram-negative bacteria tends to be the dominant cause of infection in Asian/Eastern countries. [5,16,17]

In this study, there were 19 isolates of *Staphylococcus sp.* obtained from wound swabs and blood specimens of diabetic ulcer patients, 8 of which (42.1%) were CoNS and 11 (57.9%) were CoPS. This higher prevalence of CoPS infections aligned with the findings of a 2022 study by Chelkeba and Melaku, where *S. aureus* infections were reported at 36%, while CoNS infections were only reported at 12%.[14,16] The higher incidence of CoPS infections, especially *S. aureus*, is due to toxins produced by *S. aureus.* These toxins are able to avoid the host cell's macrophages and avoid elimination, activate proinflammatory cytokines, and lyse neutrophils, hence causing released lysozyme to damage surrounding tissues, break the desmoglein-1 bonds of the skin epidermis, and cause inflammation. *S. aureus* are also able to colonize, forming small colony variants (SCVs) which are more resistant towards antibiotics, may mutate, and are harder to eliminate in chronic wounds, such as diabetic ulcers.[7,18,19]

While there were only two species of CoPS identified in this study, there were 4 identified species of CoNS (*S. epidermidis*, *S. haemolyticus*, *S. hominis ssp. hominis*, and *S. warneri*). *Staphylococcus haemolyticus* are the dominating causative bacteria in CoNS infections of diabetic ulcer patients (4 cases, 21.1%). There was a gap between the number of isolates and the number of patients. This might have been caused by repeat examinations on the same patient, or an infection by more than one *Staphylococcus* species, or otherwise known as polymicrobial infections. Polymicrobial infections are frequent in diabetic ulcers, and an increased number of infecting bacterial species have a direct association with the severity of diabetic ulcers.[5,20] The ability of *Staphylococcus* sp. bacteria to colonize skin may contribute to polymicrobial infections in diabetic ulcer patients.[7,18,21]

This study presented the antibiotic susceptibility pattern of *Staphylococcus sp*.

by coagulase categories. Susceptibility testing was performed to 16 different antibiotics, and results were interpreted as resistant, intermediate, and sensitive. In this study, CoPS bacteria were susceptible to a number of antibiotics, such as clindamycin, linezolid, nitrofurantoin, quinupristin/dalfopristin, rifampicin, tigecycline, and vancomycin, with a susceptibility rate of 90.9%. Similar results were reported for CoNS, where CoNS bacteria were susceptible to linezolid (100%), nitrofurantoin, quinupristin/dalfopristin, and tigecycline (87.5% each). These findings align with previous studies reporting that in general, *Staphylococcus sp*. are susceptible to tigecycline (100%), nitrofurantoin (96,9%), linezolid (96,8%), rifampicin (87,5%), and vancomycin (75%).[5]

In this study, both CoPS and CoNS bacteria showed a high resistance level to benzylpenicillin (81.8% and 87.5%, respectively). These findings are in line with an Indian study reporting high benzylpenicillin resistance levels of *S. aureus* (80%), *S. epidermidis* (88%), *S. haemolyticus* (100%), *S. hominis* (69%), and *S. warneri* (72%).[22] A previous study in Banjarmasin, Indonesia, reported a 100% resistance level of *Staphylococcus sp.* to benzylpenicillin.[5] The resistance of *Staphylococcus sp*. might have been due to the adaptation of bacteria to *β*-lactam antibiotics, such as benzylpenicillin, by producing *β*-lactamase enzyme that may bind to *β*-lactam and degrade it into penicilloic acid.[23,24]

CoNS bacteria also showed resistance to erythromycin (87.5%) and oxacillin (87.5%). These findings are similar to a study at Government Medical College & Hospital, India, to 120 CoNS isolates, which reported 97.06% of the isolates were resistant to penicillin and 64.71% were resistant to erythromycin and oxacillin.[25] Oxacillin is a *β*-lactamase-resistant penicillin, hence its frequent use in patients with known resistance to *β*-lactam antibiotics, while erythromycin is a macrolide antibiotic. Resistance to oxacillin, as with methicillin, is caused by the presence of penicillin binding protein-2a (PBP-2a), which is homologous to PBP-2. PBP-2a is encoded by *mec-A* gene, with its active site facing into the cell, causing it to avoid targeting by antibiotics. Macrolide resistance happens due to methylation of rRNA, preventing antibiotic attachment, or the expulsion of the antibiotic through active efflux pumps.[18,23,26]

The gold standards in diabetic ulcer management currently comprise of glycemic control, wound debridement, infection management, offloading, revascularization, and reconstruction as indicated.[27] Infection management consists of antibiotics administration as indicated via suitable route to maximize its effectiveness. In the case of diabetic foot ulcer infections by Gram-positive bacteria, antibiotics may be administered via topical, oral, and parenteral routes. Topical preparations are advised due to its minimal side effects to host cells, hence reducing the cost of treatment. One of the topical antibiotics that may be used is 0.1% gentamicin.[28] In this study, 63.6% of CoPS bacteria and 50% of CoNS bacteria are susceptible to gentamicin.

Antibiotics may also be administered orally or parenterally, depending on the severity of the ulcer. Patients with mild diabetic ulcer may be treated with oral broad-spectrum antibiotics, while those with moderate or severe ulcers may require oral or parenteral narrow-spectrum antibiotics. Available oral and parenteral antibiotics include vancomycin, linezolid, and rifampicin.[28] In this study, CoPS showed

susceptibility to these antibiotics (90.9%), while CoNS showed susceptibility rates of 75% to vancomycin, 100% to linezolid, and 62.5% to rifampicin. Despite the moderate level of CoNS susceptibility to rifampicin, a Turkish study in 2018 reported that rifampicin was more effective to manage bacterial infections with biofilm formation in comparison to other antibiotics.[28] On the other hand, the high susceptibility of CoPS and CoNS to linezolid indicates that linezolid is still effective for use in Dr. Mohammad Hoesin General Hospital. Linezolid is one of the 3 antibiotics recommended by FDA for *Staphylococcus sp.* infections, the others being ertapenem and piperacillin-tazobactam.[29]

It should be noted that this study was limited to investigation of one bacterial genus in a single healthcare centre, and it might not have given a more comprehensive picture of antibiotic susceptibility in diabetic ulcer patients. Further studies may also need to note the severity of the patients' diabetic ulcers in order to more specifically depict the causative organisms and, eventually, the efficacy of antibiotic therapy in varying degrees of diabetic ulcer severity.

5. Conclusion

Fifteen (19.5%) patients with diabetic ulcers were infected by *Staphylococcus sp*. There were 19 bacterial isolates, 11 of which were CoPS (57.9%) and 8 others were CoNS 8 (42.1%) isolates. CoPS and CoNS were most susceptible to linezolid (90.9% and 100%, respectively) and most resistant to benzylpenicillin (81.8% and 87.5%, respectively).

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