CASE REPORT

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Streptococcus agalactiae is resistant to β-lactam antibiotics in a diabetic patient with foot infection: a case report

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ABSTRACT

Introduction: Diabetic foot infection is a complication that often occurs in people with diabetes mellitus. *Staphylococcus aureus* is the most common microorganism found in diabetic foot infections. In addition, coagulase-negative staphylococci, *Enterococcus faecalis*, *Streptococcus agalactiae,* and *Pseudomonas aeruginosa* can also be demonstrated. Diabetic foot infection treatment usually takes a long time which may increasing the risk of antibiotic resistance. This article will present a unique and interesting case about *Streptococcus agalactiae* resistant to β-lactam infection.

Case description: A 56-year-old man presented with a long history of diabetes mellitus but had not taken anti-diabetic drugs and had no history of previous use of antibiotics. Since 2016 his right foot had a recurring wound that he routinely treated. Microbiology culture of the wound swab obtained three bacteria namely *Streptococcus agalactiae, Proteus mirabilis* and *Klebsiella pneumoniae* which is resistant to β-lactam antibiotics.

Conclusion: The identification of Group B *Streptococcus* bacteria (*Streptococcus agalactiae*) which are resistant to β-lactam antibiotics (penicillin, third and fourth generation cephalosporins) which were found in this case, reminds all medical personnel to be more careful and prudent in the rational use of antibiotics.

Keywords: Streptococcus agalactiae, β-lactam antibiotics, diabetic foot infection
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INTRODUCTION

Diabetes mellitus affects over 422 million people worldwide.[1] The data from the International Diabetes Federation (IDF) indicated there are 537 million adults worldwide of which 90 million are from South East Asia.[2] In Indonesia alone, diabetes is ranked as the third most common cause of death based on a sample registration survey in 2014.[3]

One of the complications that often occur in diabetics is foot ulcers with a prevalence of about 25%. In foot ulcers, infection is common with a prevalence of 40%-80%.[4] Diabetic foot infection (DFI) is an infection that often occurs in the soft tissue or bone under the malleoli and the infection usually occurs in areas of skin trauma or ulceration.[5]

The most common pathogen found in diabetic foot infection is *Staphylococcus aureus*. In addition, methicillin-resistant *Staphylococcus aureus*, β-hemolytic streptococci, coagulase-negative staphylococci (CoNS), *Pseudomonas aeruginosa*, *Corynebacterium sp.*, *Enterococcus spp.*, and other anaerobic bacteria can also be found. According to a study conducted by Lebowitz et al., the five most common pathogens found in DFI include *Staphylococcus aureus*, coagulase-negative staphylococci, *Enterococcus faecalis*, *Streptococcus agalactiae*, and *Pseudomonas aeruginosa*.[4–8] This article reports a case of diabetic foot infection in a 56-year-old man with a microbiology culture of wound swab that indicated the presence of *Streptococcus agalactiae, Proteus mirabilis* and *Klebsiella pneumoniae*.

Streptococcus agalactiae (group B streptococcus) is an opportunistic gram-positive bacterium. *Streptococcus agalactiae* is a normal microbiota in the female genital tract, lower gastrointestinal tract and upper respiratory tract. Infections involving *Streptococcus agalactiae* in adults include bacteremia, pneumonia, endocarditis, osteomyelitis, skin, and soft tissue infections. Most skin and soft tissue infections caused by *Streptococcus agalactiae* manifest as cellulitis, abscesses, foot infections, or decubitus ulcers. The common underlying condition in patients with skin and soft tissue infections due to *Streptococcus agalactiae* is diabetes mellitus.[9,10]

Streptococcus agalactiae resistant to β-lactam antibiotics was found in patients with a long history of diabetes mellitus accompanied by foot infections and no history of antibiotic use. This is quite a

concern for the author because of the conflict between the absence of a history of antibiotic use and the discovery of beta-lactam antibiotic resistance.

Streptococcus agalactiae resistant to β-lactam antibiotics was first discovered in our laboratory, which, to the knowledge of the author, had never been found in our laboratory, so the authors decided to write this article.

CASE DESCRIPTION

A 56-year-old man came to the wound care clinic to treat wounds on his left leg (sole and ankle) accompanied by pain in the wound area, nausea but not vomiting, weakness, and dizziness. The results of the physical examination showed blood pressure of 158/70 mmHg, a temperature of 37.4°C, respiratory rate of 20x/minute, pulse 96x/minute, and current blood sugar of 388 mg/dL. The patient had a long history of diabetes mellitus and has had leg wounds since 2016. The patient routinely treated the wounds but was not willing to take anti-diabetic drugs and preferred herbal medicines. There was no history of using antibiotics. The patient did not want to go to the first level health service or hospital because he did not want his leg amputated. Patients do not have problems accessing health services and do not have financial problems.

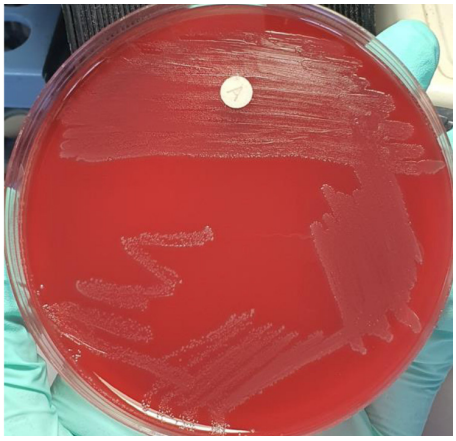

Figure 1. Bacitracin test is used to differentiate β-hemolytic group A streptococci (*Streptococcus pyogenes*) from other β-hemolytic streptococci. In this case, the result is no zone of inhibition/resistant, which means that the colony being tested is considered as non-group A streptococcus, which includes *Streptococcus agalactiae.*

Figure 2. CAMP test is used to differentiate group B streptococci (*Streptococcus agalactiae*) from other streptococcal species. In this case report, the result is negative (no enhancement of hemolysis), which means that the colony being tested is *Streptococcus pyogenes.*

Table 1. The results of the antibiotic sensitivity tests in this case report

Antibiotic	*Streptococcus agalactiae*	*Proteus mirabilis*	*Klebsiella pneumoniae*
Ampicillin	R	R	R
Cefazolin	-	I	S
Gentamycin	-	I	S
Tobramycin	-	R	S
Amikacin	-	S	S
Amoxycillin clavulanic-acid	-	I	S
Piperacillin tazobactam	-	S	S
Cefuroxime	-	S	S
Cefepime	R	S	S
Ceftriaxone	R	S	S
Ciprofloxacin	-	R	S
Levofloxacin	S	R	S
Meropenem	-	S	S
Cotrimoxazole	-	R	S
Penicillin	R	-	-
Cefotaxime	R	-	-
Erythromycin	S	-	-
Clindamycin	S	-	-
Vancomycin	S	-	-
Chloramphenicol	S	-	-

Note: (S) sensitive; (I) intermediate; (R) resistant; (-) not tested

At first, the patient was hospitalized for 13 days (treatment history and hospital examination unknown). He went home with insulin medication, diarrhea medication, nausea and vomiting pills, pain medication, and vitamins. The patient began to routinely use anti-diabetic drugs and underwent routine treatment at the outpatient clinic.

When he came to the wound care clinic after being treated at the hospital, the patient complained of being weak, nauseated, and diarrhea. The results of the physical examination showed blood pressure 117/68 mmHg, the temperature of 36.8°C, breath 18x/minute, pulse 86x/minute, blood sugar at 226 mg/dL, and pain scale of 3. During wound care, bone fragments were found. Because the condition did not improve, he was referred back to the hospital. The patient was then hospitalized a second time (treatment history and hospital examination unknown), where his condition worsened and 12 days later the patient passed away. The patient gives permission for the use and publication of the data before the patient passed away.

Samples in the form of wound swabs were sent to the microbiology laboratory for culture and antibiotic sensitivity tests. Samples were obtained when he went to the wound care clinic and before he was hospitalized. The culture results obtained three bacteria namely *Streptococcus agalactiae, Proteus mirabilis,* and *Klebsiella pneumoniae*. *Proteus mirabilis* and *Klebsiella pneumoniae* were identified with the API 20E identification system by Biomérieux (results of 99.9% and 97.3%). At the same time, *Streptococcus agalactiae* was identified with BBL™ Crystal™ identification system by BD (the result of 99.9%) with resistant status in

the bacitracin test [Figure 1] and CAMP test negative [Figure 2]. The antibiotic sensitivity test was done by the Kirby-Bauer method [Table 1] and interpreted according to CLSI 2021.

DISCUSSION

The incidence of diabetic foot infection (DFI) caused by *Streptococcus agalactiae* has been increasing. Usually, the patient presents with severe and extensive soft tissue inflammation.[11] Treatment of DFI should be based on the extent and severity of the infection. In mild infections, antibiotics can be given orally, while some moderate infections and all severe infections require hospitalization for parenteral antibiotics, additional evaluation and consideration for surgery. Patients with DFI, especially in recurrent cases, require long-term treatment with broad-spectrum antibiotics. The duration of antibiotics for mild infections is 1-2 weeks and antibiotics for moderate to severe infections are 2-3 weeks. This can increase the risk of developing antibiotic resistance.[5,8]

The sample in this case report comes from a patient with a long history of diabetes mellitus and for approximately 5 years (2016-2021), he had sores in the leg area that often recurred. There was no history of using antibiotics or anti-diabetic drugs and patient was treating the wounds until finally hospitalized. The patient had not previously been tested for microbiological culture and antibiotic sensitivity. There were three bacteria found during culture examination in our microbiology laboratory, namely *Streptococcus agalactiae*, *Proteus mirabilis* and *Klebsiella pneumoniae*. In this discussion, the emphasis will be on *Streptococcus agalactiae*.

The culture examination found gram-negative coccus bacteria with a negative catalase test and can be interpreted as *Streptococcus sp*. To find out more about the species, bacitracin and CAMP tests were conducted. The bacitracin test [Figure 1] showed resistance results which indicated that the bacterial colonies tested were *Streptococcus agalactiae*. On the other hand, the CAMP test [Figure 2] was negative, which means that the bacterial colony tested was *Streptococcus pyogenes*. The results of the two tests are certainly very contradictory. Therefore, an additional examination was done with the BBL™ Crystal™ identification system by BD and the results were *Streptococcus agalactiae* (99.9%).

The CAMP test is the standard test for the identification of *Streptococcus agalactiae*. The CAMP test was conducted by scratching *Staphylococcus aureus* (ATCC25923) on blood agar vertically, then colonies suspected of *Streptococcus agalactiae* were streaked vertically without touching the *Staphylococcus aureus* scratches. A positive result (*Streptococcus agalactiae*) will form arrowheads (the lysis of red blood cells as a result of the synergy between beta-lysin *Staphylococcus aureus* and CAMP factor produced by *Streptococcus agalactiae*). If the result is negative (*Streptococcus pyogenes*) then no arrowhead will be formed.[10,12] Group A *Streptococcus* (GAS; *Streptococcus pyogenes*) has long been considered CAMP negative and this is what is used to differentiate it from *Streptococcus agalactiae*. However, research conducted by Gase et al. showed that GAS has a gene (*cfa*) to activate the protein factor CAMP so that when the CAMP test is done an arrowhead will be formed.[13] Temporarily based on the results of the study, the presence of *Streptococcus agalactiae* was found with a negative CAMP test. A negative CAMP test result can be influenced by various factors such as the conditions, time and temperature of the culture, the quality of the blood agar medium and the bacteria itself. Group B Streptococcus (GBS; *Streptococcus agalactiae*) may not have the *cfb* gene or vice versa but the strain may have a transcriptional defect, low gene expression or low CAMP factor expression activity.[12,14,15] Therefore, the authors concluded that we should conduct other tests besides the CAMP test in identifying *Streptococcus agalactiae*.

Antibiotic sensitivity test on *Streptococcus agalactiae* using the Kirby-Bauer method [Table 1] showed that five antibiotics were still sensitive, including levofloxacin (zone diameter result 24), erythromycin (zone diameter result 34), clindamycin (zone diameter result 26), vancomycin (zone diameter result 20) and chloramphenicol (zone diameter result 30). Additionally, the another five antibiotics that gave resistance results were penicillin (zone diameter result 0), ampicillin (zone diameter result 11), cefepime (zone diameter result 0), ceftriaxone (zone diameter result 0), and cefotaxime (zone diameter result 0). The interpretation result and zone diameter breakpoint ware read according to CLSI 2021, where the zone diameter results obtained from disks containing levofloxacin, erythromycin, clindamycin, vancomycin and chloramphenicol are more than the zone diameter sensitive breakpoint criteria, while the diameter zone results obtained from disk containing penicillin, ampicillin, cefepime, ceftriaxone and cefotaxime did not match the criteria-sensitive breakpoint diameter zone.[16]

Beta-lactam (β-lactam) antibiotics are the most frequently prescribed antibiotics for various clinical indications. The β-lactam antibiotics from a biochemical point of view are characterized by the presence of a β-lactam ring (3-carbon and 1-nitrogen ring). The penicillin group (penicillin, ampicillin) and the cephalosporin group (cefotaxime, ceftriaxone, cefepime) are included in the category of β-lactam antibiotics.[17]

Penicillin is one of the most widely used antibiotics globally. Penicillin itself is effective against various types of infections caused by gram-positive cocci, gram-positive rods, anaerobes, and gram-negative cocci. Ampicillin is second-generation penicillin that is effective against *Proteus mirabilis*, *Shigella*, *H. influenzae*, *Salmonella* and *E. coli*.[18]

Cephalosporins are antibiotics used to treat infections caused by gram-positive and gram-negative bacteria. Cefotaxime and ceftriaxone are third-generation cephalosporins that are commonly used when bacteria are resistant to first and second-generation cephalosporins or other β-lactam antimicrobials. Cefepime is a fourth-generation cephalosporin and it can fight *Streptococcus pneumoniae*, methicillin-sensitive *Staphylococcus aureus* (MMSA), *Pseudomonas aeruginosa* and other gram-positive and gram-negative bacteria.[19]

Penicillin and ampicillin are the drugs of choice to treat infections caused by

β-hemolytic streptococci. The presence of β-hemolytic streptococci that are non-susceptible to penicillin and ampicillin is extremely rare.[16] Infections due to *Streptococcus agalactiae* can be treated with penicillin, ceftriaxone, cefotaxime and vancomycin (if penicillin-allergic) and there is no known resistance to penicillin, cephalosporins or vancomycin.[10] However, this case report found *Streptococcus agalactiae* which is resistant to penicillin and cephalosporin groups.

Group B *Streptococcus* (GBS; *Streptococcus agalactiae*) is a major cause of neonatal sepsis and meningitis. GBS can also infect the elderly, pregnant women, and people who have medical conditions such as diabetes. GBS is generally considered to be sensitive to β-lactams including penicillin which is the first-line drug for the prevention and treatment of GBS infection.[20] However, GBS appears with decreased sensitivity to a penicillin (PRGBS). The first report are from Japan.[20–22] In addition, PRGBS has also been identified and reported in Canada[23,24] and the USA.[25]

In Japan, there was an increase in PRGBS isolates from 2.3% (2005-2006) to 14.7% (2012-2013). The studies conducted on isolates of the PRGBS strain found amino acid substitutions in penicillin-binding proteins (PBPs) that affect insensitivity to β-lactam antibiotics. The presence of amino acid substitutions in PBP2X, PBP1A, and PBP2b cause penicillin resistance and cephalosporin resistance in GBS. In addition, there are reports that PRGBS exhibits multidrug resistance to fluoroquinolones and macrolides. The drug options for the prevention and treatment of infections due to PRGBS will be limited and are increasingly limited for infections due to multidrug-resistant PRGBS (MDR-PRGBS). The presence of this pathogen is certainly a serious health problem, especially since there are still few reports on PRGBS, so further research is highly recommended.[20,23,25–29]

The presence of bacteria that are resistant to antimicrobial agents is a worldwide problem because, with the increase in antimicrobial resistance, there will be a decrease in the choice of therapy for patients and an increase in morbidity and mortality. This is due to the unwise use of antimicrobials. Increased consumption of antimicrobial drugs (both in humans and animals) and inappropriate antimicrobial prescribing are factors that contribute to the development of resistance. Bacteria can have resistance traits naturally (intrinsically resistant) and acquired (horizontal gene transfer). In general, the mechanism of antimicrobial resistance in bacteria can be divided into 1) limiting drug uptake; 2) modifying the drug target; 3) deactivating the drug, and 4) drug efflux.[30]

β-lactam antibiotic resistance can occur through modification of the drug target and inactivation of the drug. Modification of drug targets is a mechanism of resistance to β-lactam antibiotics by changing the number and or structure of PBPs (penicillin-binding proteins). changes in the number of PBPs have an impact on the amount of drug that can bind to the target, while changes in structure cause a decrease in the drug's ability or even be unable to bind to the target. Another mechanism is to inactivate the drug, where this can be done by transfer of chemical groups to the drug and degradation of the drug (β-lactamases). β-lactamases can inactivate β-lactam antibiotics by hydrolyzing a specific part of the β-lactam ring structure which makes the ring open so that the drug cannot bind to the target protein PBP.[30,31] In this case, it is possible that the mechanism described above can cause resistance to β-lactam antibiotics.

Limitation of this study, according to CLSI, the finding of penicillin-resistant streptococcus must be retested in another referral laboratory, which could not be done due to the unavailability of this kind of laboratory.

CONCLUSION

We found *Streptococcus agalactiae* that is shown resistance to β-lactam antibiotics (penicillins and cephalosporins) originating from patients with diabetic foot infections. This case is the first found in our microbiology laboratory. Accordingly, this is a special concern for all of our medical personnel and an important reminder to be wiser in the rational use of antibiotics for prevention and treatment.

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Ethical Approval
This article has obtained patient consent for publication.

Conflict of Interest
There is no conflict of interest in this study.

Author Contributions
Conceptualization, methodology and writing original draft preparation: Nuryastuti T; Formal analysis: Kusumadewi YP; Data curation: Kusumadewi YP, Triyana K, Puspitasari I, Allatief GR, Tazkiya I, Astuti CW, Febiyanti AMG, Somaningtyas A; Validation: Wibawa T; Writing, review and editing: Kusumadewi YP; Approval of final manuscript: all authors.

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