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ARTICLE

ANTIBIOGRAM OF PATIENTS WITH DIABETIC FOOT AT DR. SOEBANDI REGIONAL HOSPITAL OF JEMBER, INDONESIA

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ABSTRACT

Diabetic foot is a complication of diabetes mellitus that is still using antibiotic to control the infection as the main therapy. This study aimed to determine the type of bacteria and antimicrobial sensitivity patterns in the diabetic foot. The samples were taken from the medical records of the patients with diabetic foot, who performed the swab culture and antimicrobial susceptibility testing in the inpatient and outpatient care in dr. Soebandi Regional Hospital, Jember, Indonesia. The samples used were the patients diagnosed with diabetic foot from January 1, 2014 to December 31, 2018. The data in this study were univariately analyzed. Forty-three pathogens were isolated from 40 patients with 12 Extended Spectrum Beta-lactamase isolates. The most common bacteria found were Gram negative (90.7%), including *Escherichia coli* (33.33%), *Klebsiella pneumoniae* (12.82%) *Pseudomonas aeruginosa* (12.82%), *Enterobacter cloacae* (7.69%) and *Proteus mirabilis* (7.69%). Then, there were also 9.3% Gram-positive bacteria of all isolates, with *Staphylococcus aureus* as the most dominant species (50%). The antibiotic antimicrobial susceptibility testing also showed that Imipenem, amikacin, fosfomycin, cefoxitin, and netilmicin were the most sensitive antibiotics. The most common type of bacteria found was *Escherichia coli*, while the antibiotic that was still sensitive in most bacteria was imipenem.

Keywords: Diabetic foot; Antibiogram; Resistance of antibiotics

АБСТРАКТ

Диабетическая стопа является осложнением сахарного диабета, при котором в качестве основной терапии все еще используется антибиотик для борьбы с инфекцией. Целью данного исследования было определение типа бактерий и структуры чувствительности к антимикробным препаратам в диабетической стопе. Образцы были взяты из медицинских карт пациентов с диабетической стопой, у которых проводилось исследование культуры мазка и определение чувствительности к антимикробным препаратам в стационаре и амбулаторных условиях в региональной больнице Dr. Soebandi, Jember, Индонезия. В качестве образцов использовались пациенты с диагнозом диабетическая стопа с 1 января 2014 года по 31 декабря 2018 года. Данные в этом исследовании были подвергнуты унивариантному анализу. Сорок три патогена были выделены от 40 пациентов с 12 изолятами бета-лактамаз расширенного спектра. Наиболее часто встречались грамотрицательные бактерии (90,7%), включая Escherichia coli (33,33%), Klebsiella pneumoniae (12,82%) Pseudomonas aeruginosa (12,82%), Enterobacter cloacae (7,69%) и Proteus mirabilis (7,69%). Кроме того, среди всех изолятов было 9,3% грамположительных бактерий, причем Staphylococcus aureus был наиболее доминирующим видом (50%). Анализ на чувствительность к антибиотикам также показал, что наиболее чувствительными антибиотиками были имипенем, амикацин, фосфомицин, цефокситин и нетилмицин. Наиболее распространенным типом обнаруженных бактерий была кишечная палочка, а антибиотиком, сохранившим чувствительность у большинства бактерий, был имипенем.

Ключевые слова: Диабетическая стопа; антибиограмма; резистентность антибиотиков

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INTRODUCTION

Diabetic foot are diabetic complications in the form of sores on the feet.[1] The data from the WHO showed that there were 422 million adults who lived with diabetes in 2014 worldwide. Diabetes increases the risk of lower limb amputations due to the foot and gangrene that are difficult to cure. The amputation rates in the populations diagnosed with diabetes are usually 10 to 20 times higher than non-diabetic populations, and over the past decade have ranged from 1.5 to 3.5 events per 1000 people per year.[2]

The main therapy for diabetic foot is the infection control using antibiotics, but nowadays many bacteria have antibiotic resistance.[3] In the western countries, bacterial resistance was in the form of methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant Enterococci (VRE), extended spectrum β-lactamase (ESBL) in *E. coli* and *K. pneumoniae*, and carbapenem-resistant Enterobacteriaceae (CRE).[4] Meanwhile, in Indonesia, the gene for New Delhi metallo-β-lactamase was found in *K. Pneumoniae* samples in 2009.[5]

The spread of antibiotic resistance can actually be prevented by using several ways, one of which is by using rational therapy based on the results of the bacterial culture and antibiotic sensitivity testing or antibiogram. The previous studies, conducted in Arifin Achmad Hospital of Pekanbaru in 2012 showed that the most bacteria found was *Acinetobacter baumanii* (34.8%). The ampicillin was resistant (0%), while the highest antibiotic sensitivity was meropenem (100%).[3] Another research conducted in dr. Soetomo General Hospital of Surabaya in 2013 showed that *Pseudomonas sp*. (20.3%) the most was founded. Imipenem antibiotics showed the highest sensitivity of 99.2%,while the most resistant one was ciprofloxacin 33.5%.[6] In addition, the selection of the rational therapy could also save costs used by patients for the antibiotic use.[7,8]

At the present time, many empirical therapies performed by medical staff on patients with diabetic foot do not use an antibiogram. This study was designed to determine the antibiogram of patients with diabetic foot in the period of 2014 - 2018 in dr. Soebandi Regional Hospital of Jember.

MATERIALS AND METHODS

This research used descriptive research design by collecting secondary data, which were the unlink medical records data from the wound swab culture and sensitivity test on the patients with diabetic foot with pus from January 1st, 2014, to December 31st, 2018. This study was approved by the Ethics Commission of the Faculty of Medicine, University of Jember. The research was carried out in the Medical Records section of dr. Soebandi Regional Hospital of Jember from February to March 2019. The data obtained was processed by using the univariate descriptive statistical analysis.

RESULT

In this study, it was found that there were more female patients than male. The female patients were 23 people or 57.5%, while male patients were 17 people or 42.5%, out of 40 samples of patients with pus. In terms of age, it was found that 10 people (25%) were 56-60 years old, while 3 people each (2.5%) were 16-20 years old, 71-75 years old, and 76-80 years old.

Furthermore, from 40 patient samples, there were 43 bacterial isolates (12 ESBL isolates), which consisted of 37 patients mono-infection while 3 other patients with poly-infection All of the 43 isolates were known to have 16 different species of bacteria, including 4 (9.3%) Gram-positive and 12 (39.7%) Gram-negative (Table.1)

Most species found were *E. coli*, 12 isolates (27.91%), 3 of which were ESBL. Other ESBL species include *P. aeruginosa, E. cloacae, P. mirabilis, S. liquefaciens, Salmonella sp., S. marcescens, K. ornithinolytica, A. baumannii,* and *S. hominis* (Table.1) and furthermore, 12 bacteria are known to produce Extended spectrum beta-lactamases (ESBLs) (Table.1).

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Data on antibiotic sensitivity test results showed five antibiotics that have high sensitivity, namely imipenem, amikacin, fosfomycin, cefoxitin and netilmicin (Figure.1). while tetracyclin, ampicillin-sulbactam, levofloxacin, cotrimoxazole and gentamycin are resistant antibiotics (Figure.2).

Table 1 The Distribution of Types of Bacteria that Caused the Diabetic Foot

Type of bacteria	Number of Isolates	%	Number of ESBL Isolates	% of ESBL Isolates out of the Total Isolates
Gram-Positive				
S. aureus	2	4,65	-	-
S. xylosus	1	2,33	-	-
S. hominis	1	2,33	1	2,33
Gram-Negative				
E. coli	12	27,91	3	6,97
K. pneumoniae	5	11,63	-	
P. aeruginosa	5	11,63	1	2,33
E. cloacae	3	6,97	1	2,33
P. mirabilis	3	6,97	1	2,33
S. liquefaciens	2	4,65	1	2,33
Salmonella sp.	2	4,65	1	2,33
S.marcescens	2	4,65	1	2,33
E. aerogenes	1	2,33	-	-
K.ornithinolytica	1	2,33	1	2,33
A. hydrophila	1	2,33	-	-
A. baumannii	1	2,33	1	2,33
S. odorifera	1	2,33	-	-
Total	43	100	12	27,9

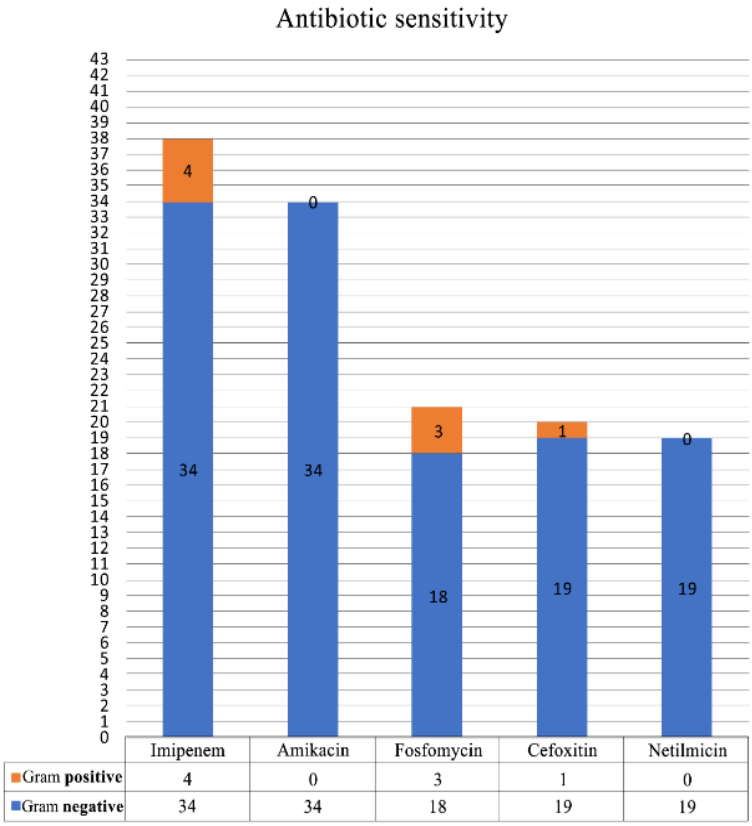

Figure 1 The Data of Antibiotic which had the Highest Sensitivity

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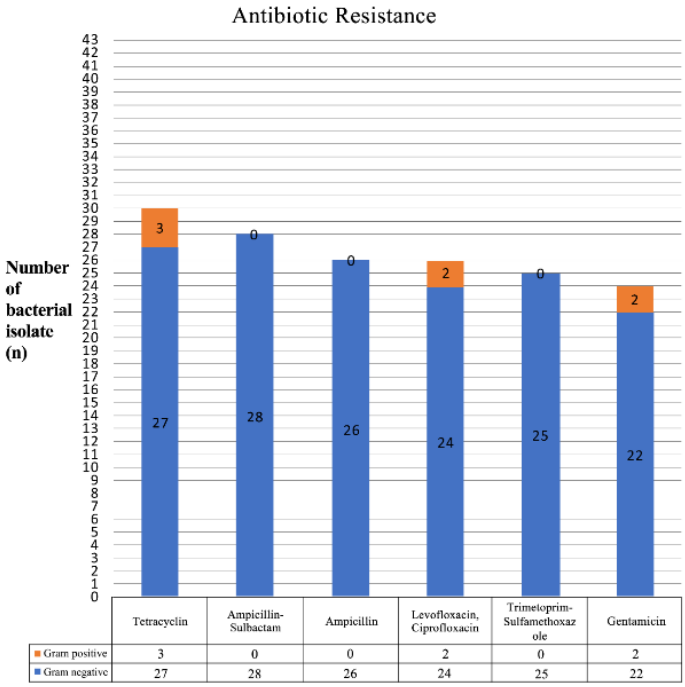

Figure 2: The Data of Antibiotic which had the highest number of resistance

The preparation of the antibiogram is based on current guidelines, data obtained from the clinical pathology laboratory of dr. Soebandi Jember (see Table 3a and 3b in supplementary materials).

DISCUSSION

In this study, 12 bacteria were found to produce Extended spectrum beta-lactamases (ESBLs) (Figure.2). ESBL is a mutated β-lactamase enzyme that causes an increase in the enzymatic activity of β-lactamase. The ESBL coding gene is in a non-chromosomal genetic material called a plasmid and can move from one bacterium to another. The transfer of genetic material or ESBL encoding genes will cause the spread of resistance.[9] ESBL produced by bacteria is able to hydrolyze broad-spectrum cephalosporin, so the treatment choice is antibiotics, which, in addition to these groups, the examples that are still sensitive are carbapenem and aminoglicoside groups.[10]

The bacterial data distribution in this study showed that the bacterial results were mostly from Gram negative that was 39 out of 43 isolates or 90.7%, while Gram-positive bacteria were fewer in number, only 4 out of 43 isolates or 9.3%. The mostly found bacteria were *P. mirabilis* and *E. coli* (12/43 or 27.91%). The result was almost the same as the research conducted by Nurwahidiah et al.,[11] in Makassar City Hospital, which found that the number of Gram-negative bacteria dominated the study, 30 of 34 isolates or 88.3%. Similar research was also carried out by Syahputra et al.,[12] which obtained 16 types of gram-negative bacteria from 18 types of bacteria obtained.

Escherichia coli is a Gram-negative, rod-shaped, non-spore-forming, no capsule, and 37°C optimum growth temperature. *E. coli* belongs to the Coliform group, that normally lives in the colon, human, and animals feces. *Escherichia coli* is an opportunistic bacteria, that occur more often or are more severe in people with weakened immune systems than in people with healthy immune systems. *E. coli* can survive for months in soil and water polluted by human faeces.[13] Moreover, *E. coli* easily infects the feet of patients with diabetic foot due to repeated trauma and open wounds that are in contact with human feces, soil, or

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polluted water. *E. coli* is thought to be related to the clinical features of patients with poor degrees of pedis ranging from local signs, such as foul-smelling wounds, necrosis, soft tissue infections, to systemic signs, such as leukocytosis.[14]

The data on the antibiotic resistance patterns showed that tetracycline was the most antibiotic resistant isolate with the total of 30 isolates out of 43 isolates or 68%. Tetracycline can inhibit bacterial protein synthesis by binding to the 30S ribosome subunit, so that it can inhibit the aminoacyl-tRNA bonding on the A ribosome side, which will distrub the peptide bonds. The gram-negative bacteria, such as *E. Coli*, use specific mechanisms, such as efflux pumps, ribosomal protection, and enzymatic inactivation to avoid tetracycline. Based on the latest surveillance, tetracycline resistance in several European countries was found to be 66.9% and 44.9% for extended-spectrum b-lactamase (ESBL) in *Klebsiella sp*. and *E. coli*. While the percentage of tetracycline resistance globally was 8.7% and 24.3% for methicillin-resistant *S. aureus* (MRSA) and *S. pneumoniae*.[15]

Antibiotics with the second highest number of resistant isolates were ampicillin-sulbactam (28/43) or 65.12%. Ampicillin is a penicillin class of antibiotics that has a beta lactam ring, whereas sulbactam is a strong inhibitor of the bacterial β-lactamase enzyme. This combined antibiotic resistance is caused by several mechanisms by the bacteria so that the enzyme β-lactamase remains effective, for example: hyperproduction of class A β-lactamases such as TEM-1 and SHV-1 (plasmid-mediated), AmpC-type β-lactamase (plasmid-chromosomal mediated), the production of resistance inhibitors in TEM-1 (IRT), other variants of β-lactamase OXA-1 (plasmid-mediated), and mutant complex enzymes in TEM that do combination / substitution with IRT or ESBL type (ESBL) extended-spectrum β-lactamase).[16] A study of antibiotic resistance reported that the level of the resistance of *E. coli* to ampicillin-sulbactam increased from 23% to 45%. The other Gram-negative rods, such as *Morganella sp*., *Enterobacter sp*. and *Serratia spp*, had higher levels of resistance to ampicillin-sulbactam. Ampicillin-sulbactam also did not have anti-bacterial activity against *P. aeruginosa* and ESBL-Enterobacter.[17]

The pattern of antibiotic sensitivity showed that the imipenem antibiotic had the greatest number of sensitive isolates with 38 sensitive isolates out of 43 isolates or 88.37%. The second highest number of the sensitive isolates was amikacin. Amikacin can inhibit the growth of bacteria for as much as 34 isolates out of 43 bacterial isolates or 79.07%. This is like research in Al-Azhar University Hospital in Egypt, which reported that imipenem had a sensitivity of 88.75% and amikacin at 87.83%.[18]

Imipenem is a potent antibiotic of the carbapenem group which is a family of β-lactam with a structure that resembles penicillin. Imipenem has a broad spectrum with fast bactericidal action, low levels of resistance, and high tolerability. Imipenem became the first carbapenem used by more than 26 million patients over the past two decades.[19] Imipenem is effective against the Gram-positive, Gram-negative, and anaerobic bacteria. The mechanism of action of imipenem begins with the infiltration of the drug into the bacterial cell wall, which then binds to an enzyme called penicillin-binding proteins (PBPs). The deadly effect results in inactivation of an autolytic enzyme inhibitor in cell walls that leads to bacterial lysis.[20]

Amikacin is one of the semisynthetic aminoglycoside class of antibiotics synthesized from kanamycin A antibiotics. Amikacin works by inhibiting bacterial protein synthesis by binding to the A-site of the ribosomal 16S RNA from the small 30S ribosomal subunit of the bacterium. As a result of that bonding, antibiotics trigger the failure of the translation process by inducing errors in reading codons in the delivery of aminoacyl-RNAt. This protein synthesis error makes inapproMalete amino acids combine to become polypeptides and then is released. Eventually, there is damage in the cell

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membrane and also in other places.[21] Amikacin, alone or in combination with other antibiotics, is used to treat various serious infections caused by Gram-negative aerobic bacteria, Mycobacteria, and Nocardia. These antibiotics are also important in the treatment of life-threatening infections in neonates. Amikacin was reported to have succeeded in treating infections caused by strains that are resistant to various antibiotics.[22]

The preparation of the antibiogram in Table 3a and 3b was based on the current guidelines, but this could not be considered completely valid. Guidelines for Analysis and Presentation of Cumulative Antimicrobial Susceptibility Test Data from the CSLI (Clinical and Laboratory Standards Institute), only recommends entering the type of bacteria with test data ≥30 isolates. Meanwhile, the data obtained from the clinical pathology laboratory of dr. Soebandi Regional Hospital of Jember only had a maximum of 12 isolates, namely *Escherichia coli*. Responding to such a thing, CLSI provides a solution for the number of bacteria less than 30 isolates, so it can still be entered as long as it can still provide the correct data and must also be given the description of the sample limitations. The sensitivity data from a small sample size can cause antibiograms to be unreliable and unrepresentative. The small sample size allows the overall resistance pattern of a bacterium from other bacteria to be seen, which affects the results of the estimated sensitivity for the entire antibiogram. However, due to the large number of hospitals with patients who did not undergo a bacterial culture, there would still be a scarcity of isolates and the result was that many bacteria are not reported at all. Therefore, the bacteria with <30 isolates must be treated carefully, because such small amounts can cause a significant bias.[23]

Antibiogram is a type of germ map of culture results, while sensitivity test is used as a reference for empirical antibiotics in the hospital. The purpose of using an antibiogram in a hospital is to1) assist the selection of empirical antibiotics before obtaining culture results that will be the definitive therapeutic reference, 2) get a picture of the tendency of the antibiotic resistance, 3) show an extraordinary event due to the bacterial infection, 4) be a guide for the preparation of antibiotic formulary in the hospital, and as part of hospital regulation.[24]

CONCLUSIONS

The conclusion of the study at dr. Soebandi Regional Hospital of Jember in the 1st period (January 2014 - 31 December 2018) revealed that most bacteria found were *E. coli*. Antibiotics that were found to have the greatest number of resistant isolates against all types of bacteria were tetracycline, ampicillin-sulbactam, ampicillin, levofloxacin, ciprofloxacin, trimethoprim-sulfamethoxazole, and gentamicin. Antibiotics that were found to have the most sensitive towards all species of bacteriawere imipenem, amikacin, fosfomycin, cefoxitin, and netilmicin.

RECOMMENDATION

Based on the results of the study, further research should be carried out periodically every year for the purpose of preparing a hospital antibiogram. Bacteria are evolving very rapidly, and doctors need to use antibiograms to reduce the spread of bacterial resistance

DATA AVAILABILITY

The data used to support the findings of this study are included within the article.

CONFLICTS OF INTEREST

There is no conflict of interest regarding the publication of this paper.

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AUTHOR CONTRIBUTION

ES conceived and designed the study, conducted research, provided research materials. HM collected and organized data. BH analyzed and interpreted data. DA and ENS wrote initial and final draft of article and provided logistic support. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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Supplementary Materials

Antibiogram of isolates (table 3a and 3b)

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