ORIGINAL RESEARCH:

Antibiotic sensitivity on pathogenic bacteria causing bacterial vaginosis

Shiwi Linggarjati[1*], Dita Diana Parti[2], Elly Nurus Sakinah[3*]
[1]Medical Program, University of Jember, Jember, Indonesia, [2]Laboratory of Obstetrics Gynecology, Faculty of Medicine, University of Jember, Jember, Indonesia, [3]Laboratory of Pharmacology, Faculty of Medicine, University of Jember, Jember, Indonesia

ABSTRACT

Objectives: To identify the sensitivity of antibiotics to pathogenic bacteria that cause Bacterial Vaginosis (BV).
Materials and Methods: This type of research was an observational study with a sample of six specimens. The data were taken using primary data from patients who were swabbed in the vagina and then diagnosed BV with amsel criteria on vaginal secretion specimens carried out at Tanggul health center on January 23-February 23, 2020. The specimens were sent to Parahita Clinical Laboratory for bacterial identification and adjusted for sensitivity with CLSI using vitek 2 compact tool.
Results: The results of this study identified the bacteria that caused bacterial vaginosis, the *E. coli* and *K. pneumoniae* with one sample of suspected ESBL. ESBL is a beta lactamase enzyme produced by bacteria and can induce bacterial resistance to penicillin, cephalosporin generation 1, 2, and 3. The types of bacteria found were *E. coli* and *K. pneumoniae* with high sensitivity antibiotics tested including piperacillin/tazobactam, ceftazidime, cefepime, ertapenem, meropenem, amikacin, gentamicin, tigecycline, and nitrofurantoin. Antibiotics with high levels of resistance tested against these bacteria included: ampicillin, amoxicillin, and ampicillin/sulbactam due to the mechanism of beta-lactam antibiotic resistance in the production of beta lactamase from bacteria.
Conclusion: The type of bacteria found was *E. coli* and *K. pneumoniae* with high resistance levels in beta lactam antibiotics.

Keywords: Bacterial vaginosis; sensitivity; antibiotics; amsel criteria

ABSTRAK

Tujuan: Mengetahui sensitivitas antibiotik terhadap bakteri patogen penyebab Vaginosis Bakterialis khususnya Jember.
Bahan dan Metode: Jenis penelitian yang digunakan merupakan penelitian observasional dengan jumlah sampel 6 spesimen. Data diambil menggunakan data primer dari pasien yang di swab vaginanya lalu didiagnosis BV dengan kriteria amsel pada spesimen sekret vagina yang dilakukan di Puskesmas Tanggul pada tanggal 23 Januari-23 Februari 2020. Spesimen kemudian dikirim ke Laboratorium Klinik Parahita untuk identifikasi bakteri serta melihat sensitivitasnya yang disesuaikan dengan CLSI menggunakan alat vitek 2 compact
Hasil: Hasil penelitian ini teridentifikasi bakteri penyebab vaginosis bakterialis yaitu *E. coli* dan *K. pneumoniae* dengan terdapat satu sampel suspek ESBL dimana ESBL merupakan enzim beta laktamase yang diproduksi oleh bakteri dan dapat menginduksi resistensi bakteri terhadap penisilin, sefalosporin generasi 1, 2, dan 3. Jenis bakteri yang ditemukan adalah *E. coli* dan *K. pneumoniae* dengan antibiotik dengan sensitivitas tinggi yang diujikan antara lain: piperacilin/tazobactam, ceftazidime, cefepime, ertapenem, meropenem, amikacin, gentamicin, tigecycline, dan nitrofurantoin. Antibiotik dengan tingkat resistensi tinggi yang diujikan terhadap bakteri tersebut antara lain: ampicillin, amoxicillin, dan ampicillin/sulbactam akibat mekanisme resistensi antibiotik beta-laktam pada produksi beta lactamase dari bakteri.
Simpulan: Jenis bakteri yang ditemukan adalah *E. coli* dan *K. pneumoniae* dengan tingkat resistensi tinggi pada antibiotik golongan beta lactam.

Kata kunci: Vaginosis bakterialis; sensitivitas; antibiotik; kriteria amsel

***Correspondence:** Elly Nurus Sakinah, ellyns.fk@unej.ac.id ; Shiwi Linggarjati, Cempaka Street No. 17A, E-mail: shiwilinggarjati@gmail.com

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INTRODUCTION

Bacterial vaginosis (BV) is a disease in vaginal infections caused by infection with a group of pathogenic bacteria due to an imbalance of the normal flora in the vaginal mucosa in the form of a shift in the number of *Lactobacillus bacteria* colonies that are replaced with various pathogenic bacteria such as *E. coli, Gardenella vaginalis, Mycoplasma hominis,* and *Mycoplasma curtisii*.[1] The first-line therapy in BV management is metronidazole and can also use clindamycin as a second-line therapy.[2] The prevalence and distribution of bacteria that cause BV varies in populations throughout the world. 20-30% of women with vaginal discharge experience BV even though the prevalence can increase to 50-60% in populations of high-risk sexual behavior.[3] Success rates for therapy are often low due to antibiotic resistance or the use of antibiotics that are not in accordance with the etiology so an antibiotic sensitivity test is needed.[4] This study aims to determine the sensitivity of antibiotics to pathogenic bacteria that cause Bacterial Vaginosis, especially in Jember so that it can be a guideline for clinicians in using the medical choice as a choice of empirical BV therapy and can reduce antibiotic resistance caused besides to determine the characteristics of age, occupation, use of tools contraception, and personal hygiene of BV patients.

MATERIALS AND METHODS

This type of research was an observational study with a sample of 6 specimens that had inclusion criteria as follows: married, did not take antibiotics for fourteen days, and signed an informed consent. The exclusion criteria were damaged and unreadable sample.[5] Data were taken using primary data from patients who were swabbed in the vagina and then diagnosed with BV with amsel criteria, namely by looking at the color of abnormal vaginal secretions, pH, whiff tests, and clue cells in vaginal secretion specimens on vaginal secretion specimens conducted at Tanggul Public Health Center on January 23 - February 23, 2020.[6] The specimens are then sent to the Parahita Clinical Laboratory for bacterial identification and see which sensitivity is said to be sensitive if the bacteria are able to be inhibited by antibiotics with minimal concentrations (MIC) adjusted to CLSI using vitek-2 compact tool.[7]

RESULTS AND DISCUSSION

This research was conducted on January 23 to February 23, 2020 at Tanggul public health center and Parahita Jember Clinical Laboratory. The characteristics of the sample were obtained from six patients who met the criteria of the study sample with the largest age range of four people (66.6%) at the age of 46-55 years. The most occupational distribution of BV patients was found four people (66.7%) had jobs as housewives. Diagnosis with amsel criteria is positive if 3 out of 4 positive criteria are obtained. Table 3 shows six colors of 100% homogeneous white vaginal discharge, pH above 4.5 (100%), positive whiff test (16.7%), negative whiff test (83.3%), and positive clue cell (100%).

Table 1. Distribution of characteristics of the study sample

Characteristics	Total (n)	Percentage (%)
Ages		
26-35	1	16.7
36-45	1	16.7
46-55	4	66.6
Occupation		
Housewife	4	66.7
Farmer	2	33.3
Amsel criteria		
Vaginal discharge color		
Clear	0	0
Yellowish white and homogeneous	6	100%
pH		
<4.5	0	0
>4.5	6	100%
Whiff test		
Positive	1	16.7%
Negative	5	83.3%
Clue Cell		
Positive	6	100%
Negative	0	0
Type of contraception		
IUD	0	-
Hormonal	4	66.7%
No contraception	2	33.3%
Personal Hyegine		
Smoke		
Yes	0	0%
No	6	100%
Douching		
Yes	3	50%
No	3	50%
Change panties		
≥3x a day	0	0%
<3x a day	6	100%
Use of antibiotic		
Metronidazole	3	50%
Amoxicillin	1	16.6%
Not given antibiotic	2	33.3%

The results of the most use contraceptives in BV patients found four people using hormonal contraception by 66.7. The distribution of personal hygiene risk factors for BV patients found as many as six patients did not smoke (100%), as many as 50% of patients did douching, as well as the frequency of changing panties every day <3x a day by 100%. There are 2 types of antibiotics given to patients, namely metronidazole

(50%) and amoxicillin (16.6%) and not given antibiotics as many as 2 people (33.3%) which can be seen in Table 1.

In this study obtained two bacterial species from culture results with positive growth which can be seen in Table 2. The culture results from vaginal secretion specimens found five species of *E. coli* bacteria (83.3%) and one species of bacteria *K. pneumoniae* (16.6%). Based on gram staining obtained overall six gram negative bacteria.

Table 2. Types of bacteria that cause vaginosis bacterialis

Bacterial species	Total	Percentage
E. coli	5	83.3%
K. pneumoniaee	1	16.6%

The results of the sensitivity test of bacteria that grew came from the results of bacterial culture using vitek 2 compact. The antibiotics tested were different for each bacterial species as shown in Table 3. Of the five species of *E. coli* identified, two *E. coli* bacteria are sensitive to Amoxicillin, Ampicillin, Ampicillin/ Sulbactam, and Trimethoprim/Sulfamethoxazole. Three out of five bacteria are sensitive to Ciprofloxacin. Four of the five bacteria are sensitive to Cefazolin Urine, Cefotaxime, Ceftriaxone, and Azretonam. Five *E. coli* bacteria are sensitive to Piperacillin/Tacobactam, Ceftazidine, Cefepime, Ertapenem, Meropenem, Amikacin, Gentamicin, Tigecyline, and Nitrofurantoin. Four of the five *E. coli* bacteria found to have intermediate sensitivity levels to other cefazolin antibiotics. While one in five bacteria have resistance to urine Cefazolin, Cefazolin other, cefotaxime, ceftriaxone, and azretonam. Two out of five bacteria have resistance to ciprofloxacin, and three out of five bacteria have resistance to amoxicillin, ampicillin, ampicillin/ sulbactam, and trimethoprim/sulfamethoxazole. One in five *E. coli* bacteria suspected ESBL.

The results of the bacterial sensitivity test for *K. pneumoniae* to bacteria are shown in Table 3. In this bacterium found resistance to Amoxicillin and Amicillin. The sensitivity results showed intermediates on other cefazolin antibiotics as well as being sensitive to antibiotics Ampicillin/Sulbactam, Piperacillin/ Tazobactam, cefazolin urine, cefotaxime, ceftazidime, ceftriaxone, cefepime, aztreonam, ertapenilin, meropenem, cefazolin tincture, cefotaxime, ceftazidime, ceftriaxone, cefepime, aztreonam, ertapenem, meropenem, cefazolin tint, cefotaxime, ceftazidime, ceftriaxone, cefepime, aztreonam, ertapenem, meropenem, amylacinline, cefotazidime, amikacin line, cycloxin, cycloxin/sulfamethoxazole. The bacterium *K. Pneumoniae* is also negative for ESBL suspects.

Overall the sensitivity level of *E. coli* bacteria to antibiotics is 75.79%, the intermediate level is 4.21%, and the resistance level is 20% with a positive suspicion of ESBL of 20% and negative ESBL of 80% while the sensitivity level of bacteria *K. pneumoniae* for antibiotics at 84.21%, intermediate levels at 5.26%, and resistance levels at 20% with positive ESBL suspects of 0% and negative ESBL at 100% as shown in Tabel 4.

Table 3. Results for bacterial sensitivity to antibiotics

Antibiotic		*E. coli*					*K. pneumoniae*
ESBL		NEG	NEG	POS	NEG	NEG	NEG
AMC	Amoxicillin	R	S	R	S	R	R
AMP	Ampicillin	R	S	R	S	R	R
AMS	Ampicillin/Sulbactam	R	S	R	S	R	S
PTZ	Piperacilin/Tazobactam	S	S	S	S	S	S
CFZ URINE	Cefazolin Urine	S	S	R	S	S	S
CFZ OTHER	Cefazolin Other	I	I	R	I	I	I
CTZ	Cefotaxime	S	S	R	S	S	S
CAZ	Ceftazidime	S	S	S	S	S	S
CTR	Ceftriaxone	S	S	R	S	S	S
CPM	Cefepime	S	S	S	S	S	S
AZT	Aztreonam	S	S	R	S	S	S
ETP	Ertapenem	S	S	S	S	S	S
MEM	Meropenem	S	S	S	S	S	S
AMK	Amikacin	S	S	S	S	S	S
GM	Gentamicin	S	S	S	S	S	S
CIP	Ciprofloxacin	S	S	R	S	R	S
TGC	Tigecycline	S	S	S	S	S	S
FD	Nitrofurantoin	S	S	S	S	S	S
SXT	Trimethoprim/Sulfamethoxazole	S	R	R	S	R	S

Table 4. Antibiotic sensitivity level

Bacterial species	S (%)	I (%)	R (%)	ESBL + (%)	ESBL - (%)
E. coli	75.79	4.21	20	20	80
K. pneumoniaee	84.21	5.26	20	0	100

This study was conducted in patients with a diagnosis of bacterial vaginosis at the Tanggul Health Center in Jember. The number of research samples with female sex 46 years and over (66.6%) more than the age of 46 years and under. This is supported by a research by Bitew et al. (2017) which states that the proportion of bacterial vaginosis is highest in women aged over 46 years.[5]

In the distribution of amsel criteria, the color of yellowish white to gray secretions is found as well as homogeneous white color, this is supported by the theoretical basis stated.[8] In the pH criteria it is found that the pH is more than 4.5, the positive result is supported by the research of Mohammadzadeh et al. (2015) which states that the measurement of pH ≥4.5 is the second highest sensitivity after the presence of a clue cell with a sensitivity value of 97% this is due to a decrease in the number of normal flora of Lactobacillus. The clue cell criteria were obtained 100% in 6 samples where the clue cell was a buildup of bacteria in the vaginal epithelium so that the vaginal epithelium appeared granulated under a microscope. In the research of Mohammadzadeh et al. (2015) stated that clue cells in vaginal secretions have the highest sensitivity for diagnosis of BV, which is 97.6%.[6,8]

In the results obtained the use of vaginal douching by (50%). This is consistent with research by Ranjit et al., 2018 which states that douching was found in 55.8% of cases of BV with a significant result of 0.015%. The use of vaginal douching on a regular basis can cause disruption of the normal vaginal flora ecosystem, Lactobacillus so that it can increase vaginal pH and be a risk factor for bacterial vaginosis.[8] The results obtained as many as 100% of samples to change the underwear ≤3x a day. This is supported by research by Ernawati et al. (2013) about the risk of changing underpants to the incidence of bacterial vaginosis which shows a significant relationship with the specific frequency of changing underpants at least 3 times a day with the incidence of BV.[9,10]

The use of antibiotics in the samples examined is in accordance with the theory of using metronidazole (75%). Research conducted by Ara et al. (2017) states that metronidazole resistance in *Gardnerella vaginalis* is 52.63% where this resistance is quite high and always increases every day, this can be caused by the use of antibiotics that are not rational or due to the use of antibiotics that are not in accordance with the etiology due to the presence of pathogenic bacteria other. In this study the pathogenic bacteria found were *E. coli* and *K. pneumoniae*. Research on the sensitivity of metronidazole to *E. coli* has been carried out and the results obtained from 31 samples examined, only 5 samples that are sensitive to metronidazole, while 26 others are resistant so that in the treatment of metronidazole against *E. coli* can often recur.[11,12]

The highest levels of *E. coli* resistance found in this study were broad spectrum antibiotics that were often used including amoxicillin (60%), ampicillin (60%), Ampicillin/sulbactam (60%), and trimethoprim sulfamethoxazole (60%). High levels of resistance to amoxicillin, ampicillin, or ampicillin/sulbactam are supported by Anago et al. (2015) in which the study mentioned resistance to amoxicillin (95.2%) and ampicillin (97.6%) due to the mechanism of beta-lactam antibiotic resistance in the production of beta lactamase from *E. coli*. In addition to the beta lactam group, it was also mentioned in the results that the resistance of *E. coli* to ciprofloxacin was (40%). Ciprofloxacin resistance to Gram-negative bacteria has been reported to increase in recent years due to excessive and inappropriate use of drugs, especially at low doses and single therapy.[13,14]

K. pneumoniae is one of the bacteria that currently has a high level of antibiotic resistance due to changes in the genome of the organism's core and can produce beta-lactamase which can cause hydrolysis of beta-lactam ring on antibiotics, this is found in the results that isolate *K. pneumoniae* is resistant against amoxicillin and ampicillin (20%). In addition, ESBL in *K. pneumoniae* has also been reported in Europe in 1983 and the United States in 1989 where this ESBL can hydrolyze oxyimino cephalosporin which causes third generation cephalosporins are no longer effective against ESBL treatment.[15]

In the identification results found a suspicion of ESBL (Extended-spectrum -lactamase) positive where ESBL is a beta lactamase enzyme produced by bacteria and can induce bacterial resistance to penicillin, generation 1, 2, and 3 cephalosporins, and aztreonam (except cephwhiff and carbapenem) by hydrolyzing antibiotics where the antibiotic can inhibit β-lactamase with β-lactamase inhibitors for example clavulanic acid. ESBL ESBL is produced by gram-negative bacteria, for

example the family Enterobacteriaceae which is a normal intestinal flora where this bacterium has been resistant to beta lactam antibiotics and is one of the main causes of bacterial infections in hospitals or in the community.[16]

CONCLUSION

The types of bacteria that cause bacterial vaginosis found were *E. coli* (83.3%) and *Klebsiella pneumoniae* (16, 6%). High sensitivity antibiotics tested against *E. coli* and *Klebsiella pneumoniae* bacteria include piperacillin/tazobactam, ceftazidime, cefepime, ertapenem, meropenem, amikacin, gentamicin, tigecycline, and nitrofurantoin. Antibiotics with a high level of resistance tested against *E. coli* and *Klebsiella pneumoniae* bacteria include ampicillin, amoxicillin, trimethoprim/sulfamethoxazole, and ampicillin/sulbactam.

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REFERENCES

1. Kumar K, Behera B, Sagiri SS, et al. Bacterial vaginosis: Etiology and modalities of treatment-abrief note. Journal of Pharmacy and Broallied Science. 2011;3(4):496-503.
2. Machado D, Castro J, Palmeira-de-Olieveira A, et al. Bacterial vaginosis biofilms: Challenges to current therapies and emerging solutions. Frontiers in Microbiology. 2016;6(1528):1-13.
3. Bautista CT, Wurapa E, Sateren WB, et al. Bacterial vaginosis: a synthesis of the literature on etiology, prevalence, risk factors, and relationship with chlamydia and gonorrhea infections. Military Medical Research. 2016;3(4):1-10.
4. Permanasari DA, Sakinah EN, Santosa A. Aktivitas ekstrak etanol daun cincau hijau (*Cyclea barbata* Miers) sebagai penghambat pembentukan biofilm bakteri *Salmonella typhi*. Journal of Agromedicine and Medical Science. 2016;2(2): 24-27.
5. Bitew A, Abebaw Y, Bekele D, et al. Prevalence of bacterial vaginosis and associated risk factors among women complaining of genital tract infection. International Journal of Microbiology. 2017:1-8.
6. Mohammadzadeh F, Dolatian M, Jorjani M, et al. Diagnostic value of amsel's clinical criteria for diagnosis of bacterial vaginosis. Global Journal of Health Science. 2015;7(3):7-14.
7. Prihatini, Aryanti, Hetty. Identifikasi cepat mikroorganisme menggunakan alat Vitek-2. Majalah Patologi Klinik dan Laboratorium Medik. 2007;13(3):129-32.
8. Pujiastuti AT, Murtiastutik D. Studi retrospektif: Vaginosis bakterial. Berkala Ilmu Kesehatan Kulit dan Kelamin. 2014;26(2):127-33.
9. Ranjit E, Raghubanshi BR, Maskey S, Parajuli P. Prevalence of bacterial vaginosis and its association with risk factors among nonpregnant women: A hospital based study. Int J Microbiol. 2018;2018:8349601
10. Ernawati. Faktor determinan terjadinya vaginosis bakterial pada wanita usia subur di kota Makassar. STIKES Nani Hasanuddin Makassar. 2013;3(5):70-7.
11. Ara NNR, Husain MA, Akter N, et al. Detection and antibiotic sensitivity pattern of *Gardnerella vaginalis* isolated from bacterial vaginosis patients attending Chittagong Medical College Hospital. Chattagram Maa-O-Shishu Hospital College Journal. 2017;16(1):48-53.
12. Iswara, A. Pola sensitivitas *Eschericia coli* terhadap antibiotik metronidazole. The 2nd University Research Coloquium 2015. 273-377.
13. Anago E, Ayi-Fanou L, Akpovi CD, et al. Antibiotik resistance and genotype of beta-lactamase producing *Escherichia coli* in nosocomial infections in Cotonou, Benin. Annals of Clinical Microbiology and Antimicrobials. 2015;14(5):1-6.
14. Drago L, Nicola L, Mattina R, et al. In vitro selection of resistance in *Escherichia coli* and *Klebsiella spp*. at in vivo fluoroquinolone concentrations. BMC Microbiology. 2010;10:119.
15. StatPearls [Internet]. *Klebsiella pneumoniae* [Updated 2020 March 25; cited 2020 February 24]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK519004/
16. Naelasari DN, Koendhori EB, Dewanti L, et al. The prevalence of Extended Spectrum Beta-Lactamase (ESBL) producing gut bacterial flora among patients in Dr. Soetomo Hospital and Primary Health Center in Surabaya. Fol Med Indones. 2018;54(4):256-262.