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Animal Modeling Try Strain Balb/c Mice with Gardnerella Vaginalis

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Abstract

Gardnerella vaginalis is the causative agent of sexually transmitted infections (STIs) of the bacterial class and is the main species of bacterial vaginosis (BV), classified as gram-negative bacteria, and is an anaerobic facultative. The incidence of bacterial vaginosis increased rapidly in Indonesia with a prevalence of approximately 20-30% of women of childbearing age suffering from sexually transmitted infections diagnosed BV and an increase of 50-60%. BV is the leading cause of vaginal infection in women of childbearing age and is one of the most common infectious diseases in the gynecological obstetrics. Balb-C is a trying animal with a human-like cervical vagina. The Objective of this study is to identify the dose of *Gardnerella vaginalis* bacteria that has the potential to live on the vagina of experimental animals. Balb/c female strain, adult age 8-12 weeks, weight 20-25 g. Using the patient's urine specimen suspect Bacterial vaginosis was inoculated on intravaginal Balb-c each of 10 µl doses of 3×10^6 , 3×10^5 , and 3×10^4 . Prior to inoculation (H0), a vaginal swab was taken and continued at H1 ± 18-24 h post-infection. After inoculation, 3x checks per day to control the health status include movement, respiration, food, fur state (neat or messy), for a period of 5 days and weight measured 1 day before and 5 days after infection. Vaginal ecosystem changes 18-24 hours post inoculation of intravaginal *Gardnerella vaginalis* indicated by growth of cultured culture colony on Plat count agar (PCA) ie 21 cfu / ml for dose 3×10^4 ; 106 cfu / ml for a dose of 3×10^5 ; And too much to count (± 10000) for a dose of 3×10^6 . In conclusions; the dose 'Concentration 3×10^4 can be recommended as a concentration of treatment of infection of experimental animals using *Gardnerella vaginalis* in subsequent studies.

Keywords: *Gardnerella Vaginalis*; Bacterial Vaginosis; Balb-c; Intravaginal Inoculation; Plant Count Agar (PCA); Colony count.

1. Introduction

Gardnerella vaginalis (*G.vaginalis*) discovered by Hermann L. Gardner and Dukes in 1955 was one of the *Haemophilus* species, growing and small in diameter 1-1.5 mm, circular, gray colony, with clue cells, ie Epithelial cells that blanket the bacteria, do not form spores, non-motile bacteria, and have no capsules. *G.vaginalis* has a scientific classification such as: Kingdom Bacteria; Phylum Actinobacteria; Class Acuty'tinobacteria; Order Bifidobakteriales; Family Bifidobacteriaceae; Genus *Gardnerella*; Species *G. vaginalis*; Binomial name *Gardnerella vaginalis*. *G. vaginalis* has Gram-positive cell walls, but because cell walls are very thin, may appear either Gram-positive or Gram-negative under a microscope. It is associated with microscopic epithelial cells that are covered in bacteria [1, 2]. This organism was first known as *Haemophilus vaginalis* then converted into the genus *Gardnerella* on the basis of an investigation of the phenotype and deoxy-ribonucleic acid. *Gardnerella* can be isolated from the genital tract, urine, blood, and pharynx; Is a bacterium closely related to Bacterial vaginosis; As well as a major cause of Bacterial vaginosis [3,16], however *G. vaginalis* may also be isolated from women without Bacterial vaginosis. Bacterial vaginosis (BV) incidence is rapidly increasing and is the leading cause of vaginal infection in women of childbearing age and is one of the most common infectious diseases in the gynecological obstetrics. Worldwide, including Indonesia, it is estimated that 20-30% of women of reproductive age suffer from sexually transmitted infections diagnosed with BV and it is found that a prevalence of 50-60% occurs in high-risk populations including commercial sex

workers (CSWs) and / or engaging with CSWs Number of sexual partners more than one, douching action and women involved in sexual intercourse at a younger age [4]. BVs include public health concerns as they relate to other sexually transmitted infections (IMS) such as human immunodeficiency virus (HIV), herpes simplex virus type 2 (HSV-2), Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (NG). Women with BV had 1.8 and 1.9-fold infections with NG and CT. A man who has sex with a woman is three times more likely to get HIV if her partner has a BV [5,15]. The delay in treating BV is due to dependent on complaints of patients who complain of after vaginal irritation with dysuria / dyspareunia and even abdominal pain that is often associated with pelvic inflammatory disease (PID) [6]. *G. vaginalis* is a facultative anaerobic bacteria, meaning bacterial progression occurs by using oxygen if available and / or in areas with little or no oxygen. Anaerobic bacteria in the female genital tract cause infertility, pelvic abscess, pelvic inflammatory disease (PID), uterine lining inflammation (endometritis) and pelvic infection followed by chorioamnionitis, amnionitis, miscarriage, preterm delivery, neonatal infections and infant, to stillbirth in infants. These conditions also influence the high maternal and infant mortality rates [7, 8]. *G. vaginalis* produces pores that form toxins (vaginolysin) affect only humans and live in vaginal epithelial cells [9]. Balb-C is an experimental species of mice (*Mus musculus*) that has a similar sensitivity to humans [10]. Mice have better disease resistance than other test animals. Mice are often used in research because mice represent animals from the mammalian class, the reproductive system, respiration, blood circulation, excretion and other organs resembling humans. Male and female mice reach sexual maturity (ready to be mated) at 8 weeks old and are said to be adult mice at 8-12 weeks with a weighted average of 20-25 grams. Adult mouse vaginas are coated with epithelial cells and have secreted mucus [11, 12, 13].

2. Materials and Methods

Experimental procedures carried out in Molecular Microbiology and Immunology Laboratory, Medical Faculty, Hasanuddin University, Makassar.

2.1. Animal care and use

For in vivo experiments, female BALB-C mice (8-12 weeks old, weighing 20-25 g) were obtained from laboratory biofarma Surabaya. Food and water were given ad libitum. The Animal Care Committee of Hasanuddin University approved the experimental protocol used in this study. Adaptation of experimental animals for 7 days in veterinary laboratory of Hasanuddin University Medical School, then the animals were weighed to get the initial average weight. The samples for the trials consisted of 3 tails and the marking on the experimental animals to distinguish each dose using a 10% picric acid solution in alcohol on head, back and tail [10].

2.2. *Gardnerella vaginalis* and infection

First stage: taking urine specimen suspect Bacterial vaginosis and taken to microbiology laboratory Faculty of Medicine Hasanuddin University. The second stage: the specimens were centrifuged to produce sediment and microscopically analyzed, found gram negative bacteria in the form of cocobacil and the presence of clue cells so that positive *Gardnerella vaginalis* and specimen were stored at -800. Third stage: division of three doses of

concentration ie 3×10^6 , 3×10^5 , and 3×10^4 [12]. For infection of 3 mice the Gardnerella vaginalis inoculation intravaginal Balb-c each 10 μ l concentration 3×10^6 , dose 3×10^5 , and dose 3×10^4 . Prior to inoculation (H0) the vaginal swab was taken and continued at H1 \pm 18- 24 hours post infection. After inoculation, 3x checks per day to control the health status include movement, respiration, food, fur state (neat or messy), for a period of 5 days and weight measured 1 day before and 5 days after infection. The procedure for handling rats, according to the guidelines of Animal Care and Usage Committee from Hasanuddin University Makassar.

3. Results



Figure 1: PCA appearance of vaginal discharge of mice before inoculation of intravaginal Gardnerella vaginalis (H0)

Figure 1 above shows that prior to inoculation of intravaginal Gardnerella vaginalis (H0) from vaginal secretions of Balb-C after culturing on PCA (Plat Count Agar) there was no colony growth. This means that the vaginal ecosystem of mice there is no growth of pathogenic bacteria.



Figure 2: PCA appearance of vaginal secretions of mice 18-24 hours post inoculation of intravaginal Gardnerella vaginalis (H1) at concentrations of 3×10^4

Figure 2 above shows that 18-24 hours post-inoculation of Gardnerella vaginal intravaginal (H1) concentration 3×10^4 from Balb-C vaginal secretion after culture done on PCA (Plat Count Agar) there is a growth of 21 cfu / ml colony, meaning that there is ecosystem change Vaginal mice and growth pathogenic bacteria.



Figure 3: PCA appearance of vaginal secretions of mice 18-24 hours post inoculation of intravaginal Gardnerella vaginalis (H1) at concentrations of 3×10^5

Figure 3 above shows that 18-24 hours post-inoculation of intravaginal *Gardnerella vaginalis* (H1) concentration of 3×10^5 Balb-C vaginal secretions after culturing on PCA (Plat Count Agar) has colonies of 106 cfu / ml growth. This means that there is a change in the vaginal ecosystem of mice and the growth of pathogenic bacteria that increased more than the concentration of 3×10^4 .



Figure 4: PCA appearance of vaginal secretions of mice 18-24 hours post inoculation of intravaginal *Gardnerella vaginalis* (H1) at concentration 3×10^6

Figure 4 above shows that 18-24 hours post-inoculation of intravaginal *Gardnerella vaginalis* (H1) concentration 3×10^6 on Balb-C vaginal secretion after culture on PCA (Plat Count Agar) has dense colony growth of ± 10000 cfu / ml. This means that there is a change in vaginal ecosystems of mice and the growth of pathogenic bacteria that increased too much from the concentrations of 3×10^4 and 3×10^5 . The results of observation during the experiments, that experimental animals inoculated *Gardnerella vaginalis* intravaginal concentration 3×10^5 weight loss of 2 grams, namely: 30 grams on the day I to 28 grams on the second day, experiencing abortion, and on day III To 26.3 grams; Inoculated animals *Gardnerella vaginalis* intravaginal concentration 3×10^6 had a fixed weight of 27.5 grams but showed vaginal secretions that many on the third day; Whereas experimental animals in *Gardnerella vaginalis* intravaginal 3×10^4 did not lose weight 30 g and no vaginal discharge occurred.

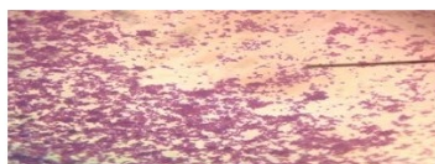


Figure 5: Microscopic view of gram negative coccobacil colonies

Figure 5 above shows that the appearance of coccobacil gram negative on the appearance of the colony gram painting preparation by microscopic examination means that *Gardnerella vaginalis* colonies grow on PCA at concentrations of 3×10^4 , 3×10^5 and 3×10^6

4. Discussion

The results show that *Gardnerella vaginalis* bacteria grow in Balb-C mice with various concentrations. This is evidenced in Plat Count Agar (PCA), ie: before *Gardnerella vaginalis* (H0) inoculation, Balb-c vaginal secretion showed no colony meaning no growth of pathogenic bacteria. In H1 (18-24 hours post inoculation of

intravaginal *Gardnerella vaginalis*), showed colony growth of 21 cfu / ml at concentrations of 3×10^4 , 106 cfu / ml at concentrations of 3×10^5 , and solid colony growth means too much to count (± 10000 cfu / ml) at a concentration of 3×10^6 . *Gardnerella vaginalis* is a gram-negative bacteria, anaerobic facultative meaning that bacteria survive despite oxygen. This supports that during the transport process, the inoculation process, as well as on the culture process in PCA, the colony continues to grow. The growth of colonies in PCA showed that the growth of pathogenic bacteria including *Gardnerella vaginalis* bacteria. This is evidenced by a microscopic examination that demonstrates the bacterial nature of *Gardnerella vaginalis*: coccobacil gram negative [14].

5. Conclusion

Mice (*Mus musculus*) Balb-C strains can be modeled for experimental animals for *Gardnerella vaginalis* anaerobic bacteria and concentrations of 3×10^4 can be recommended as treatment concentrations of experimental animals in subsequent studies.

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Conflict of Interest

The authors declare no conflict of interest

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