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

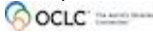

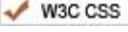

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C-1562T polymorphism of matrix metalloproteinase-9 (MMP-9) gene associated with elevated level of plasma MMP-9 concentration in patient with acute myocardial infarction (AMI) in Denpasar-Bali

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Increase in bacteroides/firmicutes ratio after early-life repeated administration of Cefixime in Rat



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Komang Januartha Putra Pinatih,^{1*} Ketut Suastika,² I Dewa Made Sukrama,¹
I Nengah Sujaya³

ABSTRACT

The antibiotic administration could potentially to affects metabolism and physiology by altering the gut microbiota composition and diversity. As early life is a critical period for metabolic development, microbiota disruption during this window period could have a significant implication to the health status. In humans, early-life microbiota disruption has been proven to be associated with increased risk of overweight later in childhood. As the use of cefixime is rising in pediatric clinical infections, it is important to determine the changes in gut microbiota composition after early-life exposure of this antibiotic. In this study, six-week old male Wistar rats were used as an animal model, divided equally into a control group and cefixime

group. Both groups were transplanted orally with feces from 7-months old infant boy prior to antibiotic administration. Cefixime group was given cefixime orally twice a day for five days in 3 consecutive periods with one-week interval. Microbiome analysis from caecal content was conducted to determine the changes in gut microbiota composition. This study found an increase in relative abundance of Bacteroides and decrease in relative abundance of Firmicutes. An increase in Bacteroidetes/Firmicutes ratio by 32% was also observed. It was concluded that early-life-repeated-administration of cefixime in rat has a significant increase in Bacteroidetes/Firmicutes ratio.

Keywords: Cefixime, Dysbiosis, Bacteroidetes/Firmicutes Ratio

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INTRODUCTION

Antibiotics are widely used in various clinical infections and saved the millions life of people worldwide every year. Despite their beneficial properties to combat infections, the use of antibiotic is one of the common and significant causes of major alterations in normal gut microbiota.¹ This event could potentially result in dysbiosis between host and microbes which could be described as a state in which the microbial community in the gut produces harm, through qualitative and quantitative changes in the intestinal microbiota, bacterial metabolic activity and the local distribution of bacteria.² Broad-spectrum antibiotics can significantly causing rapid drops in bacterial diversity. Short-term disturbances in the human gut microbiota have been observed after antibiotics treatment, and incomplete recovery of the microbiota to its initial composition has been found.³⁻⁶

Evidence in the last decade has shown that gut microbiota had a significant impact on host physiology.^{7,8} It significantly associated with some of the most notorious chronic diseases such as obesity, type II diabetes mellitus, inflammatory bowel disease, and colorectal cancer.^{7,9} It even implicated in the effectiveness of cancer chemotherapy as it modulates host immune system.¹⁰ Because of its wide clinical implications, gut microbiota quickly

becomes the frontier in the various field of research. However, study about gut microbiota in Asia or developing countries is still scarce while populations in these regions have increased the risk of chronic diseases related to changes in gut microbiota composition due to change in diet or antibiotics overused.

Early life is a critical period for metabolic development. Thus, microbiota disruption during this window period could lead to changes in body composition.¹¹ In humans, early-life microbiota disruption is associated with increased risk of overweight status later in childhood.¹²⁻¹⁵ Since antibiotic is widely used in childhood, especially in Indonesia, it can be postulated that antibiotics could potentially affect host metabolism and physiology by altering the composition of gut microbiota.

Recently, there is an increasing trend of the usage of cefixime in a clinical setting, especially in children. Cefixime is a beta-lactam antibiotic with broad-spectrum activity and it usually used to treat respiratory tract and gastrointestinal infection, two of the most common diseases in children. Unfortunately, there is no report regarding the effect of this antibiotic to gut microbial diversity. Therefore, this study was aimed to reveal the

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change in the gut microbial diversity after repeated exposure of cefixime in early-life.

RESEARCH DESIGN AND METHODS

Animal model

Six 6-weeks old male Wistar rats were used as an animal model. The rats randomly divided into two groups (3 rats per group), i.e., control group (receiving no antibiotic treatment) and cefixime group. All rats were singly housed under controlled conditions (12:12 light-dark cycle, 22-24°C and 50% relative humidity) with free access to water and standard rodent chow. Prior to antibiotic exposure, all rats were transplanted orally with feces from 7-months old infant boy once a day for five days.

Antibiotic Administration

One week after last fecal transplant, antibiotic treatment was started. Cefixime 8 mg/100 grams of body weight were given orally by gastric gavage, twice a day for five days. Antibiotics was given in 3 consecutive periods with a one-week interval. Two weeks after last antibiotic administration, rats were sacrificed and caecal content was collected for microbiome analysis.

Microbiome Analysis

Microbiome analysis was conducted by using Next Generation Sequencing on Illumina Nextseq platform. The 16S rRNA genes were amplified at V3-V4 hypervariable regions and the resulted amplicon library was sequenced in paired-end (PE) mode with length 150 bases. The surviving paired-end

reads were clustered into Operational Taxonomic Units (OTUs) based on 97% of sequence identity using USEARCH v.5.2.236 algorithm within QIIME v.1.8.0 software. After OTU picking, representative sequence set was extracted from the clustered OTUs. Both extracted reads 1 and reads 2 were separately aligned onto the Greengenes v.13.8 database to obtain the taxonomy annotation. The abundance of each microbial taxon is estimated from the absolute abundance values of the OTUs which further normalized by the expected number of copies of 16S rRNA genes present in the genome of each microbial species.

Statistical Analysis

The data were analyzed using a statistical computer program. Shapiro-Wilk test was used to determine normality of data. Homogeneity of data was analyzed using Levene's test. Normal data presented as mean \pm SD, and median (minimum-maximum) was used for data that were not distributed normally. Statistical significance between the two groups was determined using Independent t-test, with p value of < 0.05 was considered significant.

RESULTS

At the end of the study, the rats were sacrificed and the gut microbiome data were analyzed using the caecal content. Before proceeding to bivariate analysis, all data obtained were first tested for normality and homogeneity. Shapiro-Wilk's test showed that all data were normally distributed. Meanwhile, Levene's test showed homogeneity in all data except for Firmicutes relative proportion (Table 1).

Microbiome analysis revealed that community of gut microbiota in control group was dominated by phyla Firmicutes (66,22% \pm 3,52) and Bacteroides (14,78% \pm 0,78), with a Bacteroides/Firmicutes ratio 0,22 \pm 0,02. Cefixime exposure significantly increase the relative proportion of Bacteroides to 17,40% \pm 0,40 ($p=0,011$) and Bacteroides/Firmicutes ratio to 0,29 \pm 0,01 ($p=0,005$). A decrease in the relative proportion of Firmicutes (58,69% \pm 0,60) was also observed but not statistically significant ($p = 0,062$). All results were presented in Table 2.

DISCUSSION

Besides genetics, age and dietary habits, antibiotic treatment is a major factor contributing to alteration in gut microbiota composition. Because of non-specific nature of most antibiotics, their administration is not only affecting the targeted pathogen but also the normal microbe of the gut³. The potency of an antimicrobial agent to influence the gut microbiota is related to its spectrum of

Table 1 Normality and variance analysis of the research data

	Normality test/ Kolmogorov Smirnov		Levene Test	
	Statistic	p-value	F	P- value
Firmicutes	0,264	0.200	9,234	0,038
Bacteroidetes	0,232	0.200	2,483	0,190
Bacteroidetes/Firmicutes Ratio	0,252	0.200	0,617	0,476

Table 2 Relative Abundance of Firmicutes, Bacteroidetes, and Bacteroidetes/Firmicutes Ratio

	Group	
	Control (n=3)	Cefixime (n=3)
Firmicutes	66,22% \pm 3,52	58,69% \pm 0,60
Bacteroidetes	14,78% \pm 0,78	17,40% \pm 0,40 *
Bacteroidetes/Firmicutes Ratio	0,22 \pm 0,02	0,29 \pm 0,01 *

Data is presented in Mean and Standard Deviation

* $p < 0,05$

activity, pharmacokinetics, and dosage, modes of action, and length of administration.¹⁶⁻¹⁹

Cefixime, a beta-lactam antibiotic with broad-spectrum activity, is widely used in various clinical infections. This study found that repeated cefixime administration increased relative abundance of Bacteroidetes and decreased relative abundance of Firmicutes. An increase in Bacteroidetes/Firmicutes ratio by 32% was also observed. Findings in this study are consistent with result obtain from several studies using beta-lactam antibiotics as the exposure agent. Panda et al. (2014) conducted a study to determine the short effect of several beta-lactam antibiotics (amoxicillin/clavulanic acid, ceftriaxone and piperacillin/tazobactam) against gut microbial community⁶. They found a 20% decrease in microbial diversity and increase in Bacteroidetes/Firmicutes ratio in one week after antibiotics exposure. Perez Cobas et al. (2013) found a shift toward Bacteroidetes after administration of antibiotic combination containing ampicillin/sulbactam and cefazolin in a patient with cardiac pacemaker infection.²⁰ Another study conducted by Vrieze et al. (2013) found alteration in gut microbiota composition, with a decrease in Firmicutes and increased the proportion of Proteobacteria after one-week administration of vancomycin.²¹

Because of its essential role, gut microbial disturbance during the early-life period (under 3 years of life) has potential health implications later in life. Some observational studies found that antibiotic-induced dysbiosis in early-life increase the risk of overweight during adolescent.¹²⁻¹⁵ Infants with broad-spectrum antibiotic exposure, on average, have a higher body mass compared to unexposed infants.²² The frequency of the exposure also found to be associated with increased in the body mass index. Infants who received 4 or more antibiotic exposures will have a higher increase in body mass index compared to 1-3 exposure. It was concluded that early-life or repeated antibiotic exposure would increase body mass and height later in life which started from 24 months. Another cohort study conducted by Bailey et al. (2014) also found a close relationship between cumulative antibiotic exposure and incidence of obesity later in life (RR: 1.11; 95% CI, 1.03-1.19), with stronger effect found when exposed to broad-spectrum antibiotics (RR: 1.16; 95% CI, 1.06-1.29).²³ Time of exposure also correlates with the risk to become obese. The study showed that the exposure at 0-5 months of age had a significantly higher risk than at 6-11 month of age.

All of the evidence clearly demonstrated that antibiotic administration should carefully regulated especially considering the increasing trend of

obesity and type II DM in younger age. However, the exact mechanism between gut microbiota diversity with obesity is still poorly understood. Thus, further research is needed to evaluate the metabolic change in association with gut microbiota alteration.

CONCLUSION

Early-life repeated administration of cefixime in rat causes a significant increase in Bacteroidetes/Firmicutes ratio. However, we did not evaluate the physiological change associated with the ratio. Further research in this area is needed in order to increase the understanding of the physiological mechanism that would lead to proper intervention to prevent metabolic disorder in children receiving antibiotic treatment.

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