

Effect of Antibiotic Applications on Salivary Amylase and Catalase Kinetic Parameters on Neonatal at Risk of Sepsis *In Vitro*

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ABSTRACT

In this present study, we try to demonstrate the effect of antibiotic applications to salivary amylase and catalase kinetic parameters on neonatal at risk of sepsis. This present study was performed at February-June 2015. Saliva samples were taken from 20 newborns (5 from normal newborn and 15 from infants were a risk of sepsis) treated in Ulin General Hospital, Banjarmasin, South Kalimantan, Indonesia. Saliva samples then divided into two groups, one group for salivary amylase and another group for salivary catalase kinetic parameter analysis, respectively. Each group will be divided into 4 subgroups with; T0 served as control which contains saliva+starch or H₂O₂, T1 which contains saliva+meropenem+starch or H₂O₂; T2 which contains saliva+amikacin+starch or H₂O₂; and T3 which contains saliva+diazole+starch or H₂O₂. Solutions then incubated at 37°C for 1 hours and then was prepared for kinetic parameter analysis. The kinetics parameters (Km and Vmax) were determined using Lineweaver-Burk plot. The results showed that antibiotic treatments could decrease the affinity of both amylum-amylase and H₂O₂-catalase complex which expressed by the higher Km and Vmax values. From this results, it can be concluded that antibiotic works not just by the common mechanisms, but work with another mechanism, ie. by decreased glucose and increased H₂O₂ concentrations that will have a negatif effect for bacterial living in neonatal at risk of sepsis.

Key Words: Antibiotic, Amylase, Catalase, Kinetic Parameters, Neonatal Sepsis, Saliva

INTRODUCTION

Neonatal sepsis (NS) remains a leading cause of neonatal morbidity and mortality despite extraordinary progress in the field of neonatology in recent years¹. World Health Organization (WHO) estimates that there are about 5 million neonatal deaths a year especially in developing countries². Another previous report also showed that in the developing countries neonatal mortality resulting from NS is about 34 per 1000 live births, occurring mainly in the first week of life³. In Indonesia, infant mortality rate is 19/1000 live births or around 236/days and 10 people per hour. NS becomes the biggest cause with the presentation of 20.6% from age 0-28 days and around 12% of age 0-6 days⁴.

NS is a treatable condition and there are specific guidelines for the treatment of the condition⁵. Standard treatments for NS is antibiotic therapy⁶. WHO currently recommended ampicillin and gentamicin as first-line antimicrobials for NS. However, the initial choice of antibiotic therapy will depend on the clinical context and local bacterial epidemiology⁷. Alternative therapeutic such as meropenem, amikacin and metronidazole can be used to treat NS⁸. Still, there is a few data about the interaction

between these antibiotic and neonatal host defence mechanism.

Saliva is a body fluid that is of high importance for determining physiological and pathological situations of the human body. Saliva is primarily composed of water, proteins, electrolytes, mucins, nitrogenous products, and enzyme including amylase and catalase⁹. Several studies have demonstrated that salivary amylase is one of the antimicrobial proteins¹⁰. Amylase is important to host defence in oral-respiratory mucosal immunity by inhibiting the adherence and growth of certain bacteria¹¹. Beside amylase, saliva also contained catalase. The presence of catalase in saliva could maintain the oxidative status by decomposing hydrogen peroxide to water and oxygen¹².

It is well documented that saliva can be used as a marker for several disease and to monitor an individual's health¹³⁻¹⁴. Our previous study demonstrated the use of saliva to detect an early onset of NS by measured the neutrophil levels and TLR2 expression in saliva¹³. Also in our previous study saliva had used to investigated the expression of TLR-2 and TLR-4, the level of neutrophil and Reactive Oxygen Species (ROS), antioxidant enzyme

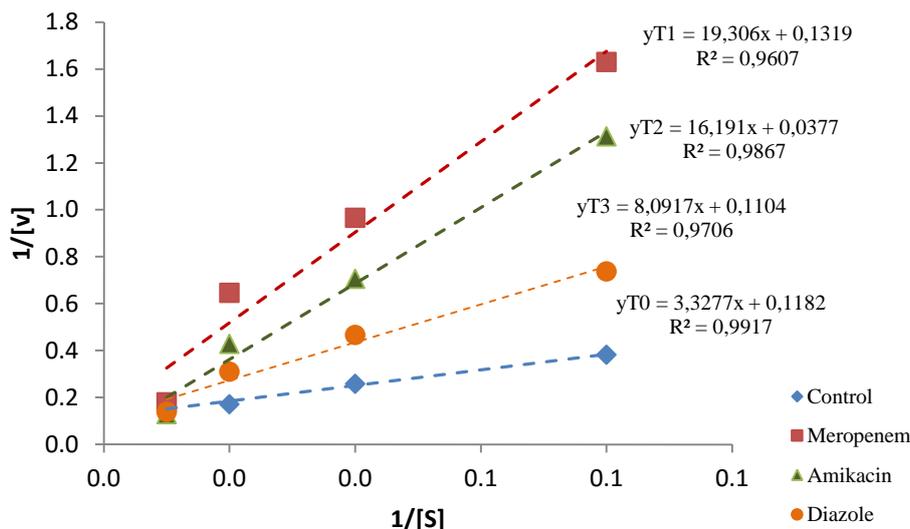


Figure 1: The Lineweaver-Burke plot for salivary amylase in different groups of treatment.

Table 1: Kinetic parameters and coefficient correlation for salivary amylase in different group of treatments

Group	Parameters		
	Vmax	Km	r
T0	8.458	28.145	0.996
T1	7.584	146.421	0.980
T2	26.518	429.345	0.993
T3	9.056	73.275	0.999

T0: Control; T1: Meropenem; T2: Amikacin; T3: Diazole

activity, and antioxidative index in early onset NS and newborn with risk of sepsis¹⁵⁻¹⁷. However, there is no study in the literature examining the effect of antibiotic applications on the salivary enzyme in newborns at risk of sepsis. Therefore, the present experimental study aimed to determine the effects of the antibiotic applications on salivary amylase and catalase kinetic parameters in newborns at risk of sepsis.

MATERIAL AND METHODS

This study is a prospective cross-sectional study. Saliva samples were collected from 20 newborns, of which 15 newborns were at risk of sepsis and 5 from healthy newborns. The samples were collected in the Neonatal Intensive Care Unit (NICU), Ulin General Hospital, Banjarmasin, South Kalimantan, Indonesia from February to June, 2015. The data were primarily collected to determine the effect of antibiotics application on salivary amylase and catalase in newborn at risk of sepsis. For the present study, the data from the original study were used. This study was approved by the ethics committee of University of Lambung Mangkurat, Banjarmasin, South Kalimantan, Indonesia.

Samples collection: Saliva samples were taken from newborn at risk of sepsis and normal newborn. Subjects in the sepsis risk group must have at least 1 major criteria or 2 minor criteria for sepsis as per ACOG guidelines. Major

risk criteria were premature ruptured of membranes (PROM) for > 24 hours, maternal fever with intrapartum temperature > 38°C, chorioamnionitis, fetal heart rate persisting at > 160 times/min or bad smelling of amniotic fluid. Minor risk criteria were PROM for > 12 hours, maternal fever with intrapartum temperature > 37.5°C, low Apgar score (<5 at the 1st min, <7 at the 5th min), very low birth weight baby (VLBW) of <1500 gr, gestational age < 37 weeks, multiple pregnancy, bad smelling of vaginal discharge, maternal urinary tract infection (UTI) or suspected untreated maternal UTI. Saliva specimens (3 ml each) were taken via suction from the oropharynx according to standard procedures for neonatal resuscitation.

Experimental models : Samples were divided into 2 groups. Group 1 for amylase and group 2 for catalase kinetic parameter analysis. Each of groups were divided again into 4 subgroups (1 control (T0) and 3 treatments group (T1, T2, and T3). Group 1: T0: Saliva + starch; T1: Saliva + starch + Meropenem; T2: Saliva + starch + Amikacin; and T3: Saliva + starch + Diazole. Group 2: T0: Saliva + H₂O₂, T1: Saliva + H₂O₂ + Meropenem; T2: Saliva + H₂O₂ + Amikacin; and T3: Saliva + H₂O₂ + Diazole. Then, each of solutions was incubated at 37°C for 1 hour and undergo to amylase and catalase kinetic parameter analysis

Catalase and amylase kinetic parameter measurements: kinetic parameters were determined by using 4 different concentrations of the substrate, starch for amylase and H₂O₂ for catalase. The starch concentrations were 100, 50, 25, and 12.5 mmol and the H₂O₂ concentrations were 50, 25, 12.5, and 6.25 mmol. The kinetic parameters Vmax and Km were determined using the Lineweaver-Burk version of the Michaelis-Menten equation¹⁸, as follows:

$$\frac{1}{V} = \frac{km}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}}$$

where V is the reaction velocity, Vmax is the maximum reaction velocity, Km is the Michaelis constant (the

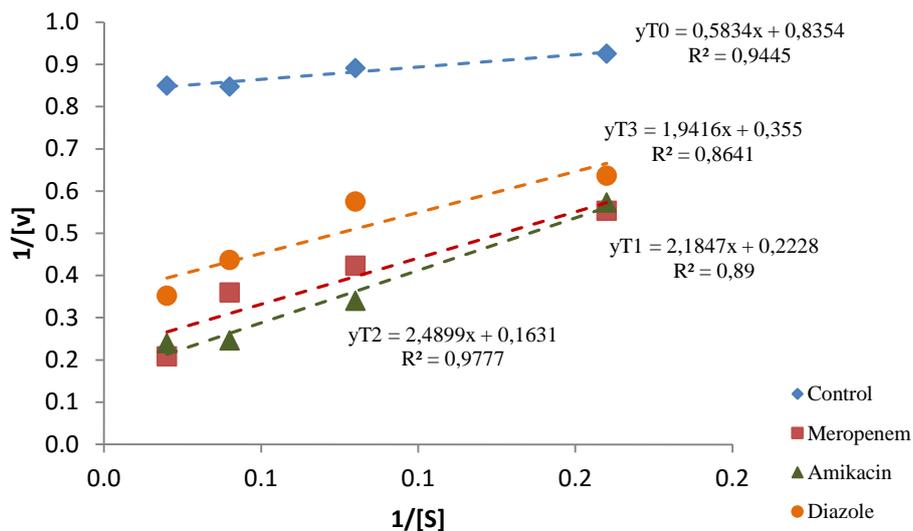


Figure 2: The Lineweaver-Burke plot for salivary catalase in different groups of treatment

Table 2. Kinetic parameters and coefficient correlation for salivary catalase in different group of treatments

Group	Parameters		
	Vmax	Km	r
T0	1.197	0.698	0.972
T1	4.624	10.041	0.950
T2	6.130	15.263	0.989
T3	2.815	5.463	0.929

T0: Control; T1: Meropenem; T2: Amikacin; T3: Diazole

substrate concentration at half-maximal reaction velocity) and [S] is the substrate concentration.

RESULTS AND DISCUSSION

This present study which was undertaken to assess the effects of meropenem, amikacin, and diazole on the kinetics parameters of salivary amylase on newborn with risk of sepsis. Kinetic parameters of salivary amylase were determined from Lineweaver-Burke plot drawn between the inverse of the concentrations of the substrate (12.5-100 mg/ml) against the mean rising rate for reaction. This plot, along with the equation of the linear regression line and the correlation index of r² from each group of treatment can be seen in figure 1. From this equation, the Km and Vmax values for each group of treatment can be calculated. As calculated from the Lineweaver-Burke plot, the Vmax of the salivary amylase in different group of treatments ranges from 7.854-26.518 sec⁻¹ amylum, while the Km values are within the range of 28.145-429.345 μmol/sec (table 1). The highest Vmax dan Km was found in the T2 group, while the lowest was the T1 group for Vmax and T0 for Km. Table 1 also showed that the Km and Vmax values are higher in all treatments groups (T1, T2, and T3) compared to control, except for Vmax value in T1 group. Results from the figure and table 1 suggest that antibiotic applications increase the values of Km and Vmax, except for meropenem application. Meropenem application can

increase the value of Km but not the Vmax. Results also suggest that antibiotic applications increase the Km at greater proportion than Vmax. This suggests that antibiotic works by decreased amylase-starch affinity instead of damage the catalytic ability of amylase.

Salivary amylase is one of the most important enzymes in saliva. The enzyme was first described in saliva by Leuchs in 1831. It consists of two families of isoenzymes, of which one set is glycosylated and the other contains no carbohydrate¹⁹. The main function of this enzyme its hydrolytic activity that is responsible for the initial break down of starch to oligosaccharides²⁰. Another function of amylase is bound with several bacteria and this bacteria-bound amylase is capable of hydrolyzing starch to glucose, which can be used as a food source for bacteria²¹.

Results of this present study suggest that antibiotic applications could decrease the affinity between starch-amylase complex. It means the binding between starch and amylase might be weak by the presence of those antibiotics. This condition will increase the concentration starch as a substrate and decrease the concentration of oligosaccharides as a product. From this point of view, those antibiotics work not just as an antibacterial agent but also works to lower the production of oligosaccharides that can be used as a food source, and improved host immune system through its influence

on the amylase which is one component of immunity. It is indicated that the antibiotic applications can reduce the risk of sepsis in neonates through several mechanisms, as mentined above.

Besides salivary amylase, this present study also investigated the effects of meropenem, amikacin, and diazole on the kinetics parameters of salivary catalase on newborn with risk of sepsis. The kinetic parameters (Km and Vmax) gathered from the Michaelis-Menten and Lineweaver-Burke plots that are shown in figure and table 2. Results shows that the Vmax of the salivary catalase in different group of treatments ranges from 1.197-6.130 U/mg H₂O₂, while the Km values are within the range of

0.698-15.263 $\mu\text{mol sec}^{-1}$ (table 2). The highest V_{max} and K_m were found in the T2 group, while the lowest was in the T0 group. Table 1 also showed that the K_m and V_{max} values are higher in all treatments groups (T1, T2, and T3) compared to control.

Results from the figure and table 2 suggest that antibiotic applications increase the values of K_m and V_{max} of salivary catalase. Results also suggest that antibiotic applications increase the K_m at greater proportion than V_{max} . This suggests that antibiotic also works by decreased catalase- H_2O_2 affinity instead of damage the catalytic ability of catalase.

Human catalase belongs to the group of monofunctional haem-containing catalase; members of this large subgroup are found in almost all aerobically respiring organisms. Catalase is primarily an intracellular enzyme; its highest concentrations in mammals are found in erythrocytes, liver and occasionally in the kidney and saliva²²⁻²³.

Catalase is a tetrameric protein of 244 kDa with molecular 222 symmetry, comprising four identical subunits of 59.7 kDa. Each subunit contains 527 amino acid residues, one haem group, namely iron (III) protoporphyrin IX, and a tightly bound molecule of NADPH. The main function of catalase is the decomposition of hydrogen peroxide to water and oxygen-catalatic activity. During long-term exposure of catalase to H_2O_2 , the catalase-bound NADPH became oxidized to NADP^+ and the activity of catalase fell to about one-third of the initial activity²².

The results of this study suggest that antibiotic treatments, in this case meropenem, amikacin, and diazole could decrease the affinity between H_2O_2 -catalase complex. This condition indicated that H_2O_2 -catalase bound was weak and H_2O_2 reaction product, in this case H_2O and O_2 will decrease. On the other hand, the level of H_2O_2 in saliva increases. From this point of view, it seems those antibiotics not just attack the bacteria via common mechanism (disrupt protein synthesis or synthesis of cell wall), but working through other mechanisms, ie. by increasing the formation of reactive oxygen species including H_2O_2 . It seems the antibiotic applications could reduce the risk of sepsis in neonates via several mechanism, including oxidative pathway.

In conclusion, the present study demonstrated that antibiotic applications will disrupt the kinetic parameters (K_m and V_{max}) of salivary amylase and catalase. It seems those three antibiotics, ie. meropenem, amikacin, and diazole, increase the K_m and V_{max} value of salivary amylase and catalase. This indicated antibiotic applications decreased the affinity between starch-amylase and H_2O_2 -catalase complex. Together these observations lead us to conclude that the antibiotics works not just via common mechanisms, but working through another mechanism, such as decreased the concentration of oligosaccharides that will be used as a bacterial food source, increased immunity, and increased the concentration of H_2O_2 which is known as one of the ROS that will be used to attack the bacteria. This results indicated that antibiotic applications could reduce the risk of sepsis in neonates not just in common mechanism, but through several mechanisms.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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