

**PENGARUH AEROBIOSIS DAN ANTIBIOTIK TERHADAP
KARAKTERISTIK; DAYA TAHAN, VIRULENSI DAN
ULTRASTRUKTUR BENTUK KOKOID *HELICBACTER PYLORI***

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RINGKASAN

Kuman *H. pylori* bersifat mikroaerofilik, berbentuk pleomorfik yaitu spiral, batang bengkok dan bila mendapat stres akan berubah bentuk menjadi kokoid. Kuman ini penghasil urease yang kuat, karenanya sanggup hidup pada lambung yang mempunyai pH rendah. Ada beberapa sarjana yang menganggap bahwa bentuk kokoid adalah bentuk kuman mati, sedangkan pendapat lain mengatakan bahwa bentuk kokoid adalah bentuk pertahanan menghadapi lingkungan yang buruk. Kokoid pertahanan dapat terdiri dari kokoid hidup dan dapat dikultur (*Viable Culturable*) serta kokoid yang masih hidup tetapi tidak dapat dikultur kembali (*Viable Non Culturable*) serta kokoid mati (*Non Viable*).

Penelitian epidemiologik menunjukkan bahwa infeksi *H. pylori* dapat ditularkan secara fekal-oral. Cara penularan ini melibatkan kokoid *H. pylori*, sebab kuman *H. pylori* di lingkungan secara teoritik berbentuk kokoid. Demikian pula terdapatnya bukti bahwa kokoid ternyata lebih kebal pada antibiotik dibandingkan dengan bentuk spiral. Serta banyak pendapat bahwa kokoid bertanggung jawab terhadap kekambuhan infeksi *H. pylori* pada kasus yang telah mendapat terapi eradikasi. Sehingga harus diupayakan agar kuman tidak terbentuk kokoid sebelum pengobatan antibiotik diberikan.

Tujuan dari penelitian ini adalah 1). Membuktikan bahwa stresor antibiotik berpengaruh lebih besar terhadap karakteristik bentuk kokoid kuman *H. pylori* dari pada stresor aerobiosis 2). Membuktikan bahwa stresor antibiotik berpengaruh lebih besar dalam menurunkan daya tahan (survival) bentuk kokoid kuman *H. pylori* dari pada stresor aerobiosis 3). menganalisis stresor aerobiosis yang berpengaruh lebih besar dalam menurunkan daya virulensi kokoid kuman *H. pylori* dari pada stresor antibiotik 4). membandingkan pengaruh stresor aerobiosis dan antibiotik terhadap variasi ultrastruktur bentuk kokoid kuman *H. pylori*. Penelitian eksperimental laboratoris dengan rancangan randomized post test only ini dengan variabel suasana mikroaerofilik, aerobiosis, dan pemberian amoksisilin pada suasana mikroaerofilik

Sampel didapatkan dari lambung penderita gastritis kronis. Jumlah sampel adalah 81 tabung berisi kuman *H. pylori* dengan 10^9 CFU/ml. Pemeriksaan dilakukan terhadap jenis protein, kadar DNA, integritas DNA, integritas RNA, mRNA urease, gen *ureA* dan gen *cagA*. Demikian pula dilakukan pemeriksaan dengan *transmission electron microscope* untuk melihat perbedaan ultrastruktur pada kokoid yang terbentuk. Dilakukan pula pemeriksaan spiral yang diperlukan sebagai kontrol. Analisis statistik dilakukan dengan manova dan analisis faktor.

Hasil penelitian menunjukkan bahwa lama pembentukan kokoid paling cepat terjadi pada stresor antibiotik, kemudian stresor aerobiosis dan terakhir stresor mikroaerofilik. Penurunan kadar DNA kokoid VC dan kokoid VNC paling cepat terjadi pada stresor antibiotik, kemudian stresor aerobiosis dan terakhir pada stresor mikroaerofilik. Penurunan kadar DNA kokoid NV paling cepat terjadi pada stresor mikroaerofilik, kemudian stresor antibiotik dan terakhir stresor aerobiosis. Aktivitas enzim urease pada semua bentuk kokoid negatif, tetapi protein ureaseA, protein ureaseB

THE EFFECTS OF AEROBIOSIS AND ANTIBIOTIC ON THE CHARACTERISTICS; SURVIVAL, VIRULENCE AND THE ULTRASTRUKTUR OF *HELICOBACTER PYLORI* COCCOID FORM

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SUMMARY

H. pylori is a microaerophilic bacteria, and has pleomorphic appearances. It can be in spiral or curved form, and in unfavourable condition it changes into coccoid form. This bacteria produces strong urease that enable it to survive in the stomach with very low pH. In the past years some scientist thought that coccoid form is dead bacteria, while many others believe that coccoid form is a defensive form to survive the unfavourable environment. Defensive coccoid can be present as living and culturable bacteria (Viable Culturable) or living but no longer culturable (Viable Non Culturable) or dead bacteria (Non Viable)

Epidemiological studies showed that *H.pylori* infection can be transmitted through fecal-oral route. This fecal oral transmission mode involves *H. pylori* coccoid form, as theoretically most of *H.pylori* in the environment is in the coccoid form. Coccoid form is more resistant to antibiotic treatment compared to spiral form. Many scientist believe that coccoid form is responsible for the recurrence of the infection after eradication. So the formation of coccoid form should be avoided before the administration of antibiotic in eradication treatment.

The objectives of the study were 1) to prove that antibiotic stressor has stronger effect on the characteristics of coccoid form of *H. pylori* than aerobiosis stressor 2) to prove that antibiotic stressor has stronger effect on decrease of survival of the coccoid form of *H. pylori* than aerobiosis stressor 3) to analyse aerobiosis as a stressor with stronger effect on decrease of virulence coccoid form of *H. pylori* than antibiotic stressor and 4) to compare the effects of aerobiosis and antibiotic stressors on the ultrastructure variation of the coccoid form of *H. pylori*. This study is an experimental laboratory study using randomized post test only design with three variables: microaerophilic condition, aerobiosis and the addition of amoxycillin in microaerophilic condition.

The material used for this study is *H. pylori* cultured from endoscopic biopsy sample taken from a patient suffering from chronic gastritis. Sample of this study consisted of 81 tubes each containing 10^9 CFU/ml of bacteria. The assays done on the coccoid form sample and spiral were protein analysis, DNA content, DNA integrity, RNA integrity, mRNA urease, *ureA* gene and *cagA* gene. Serial ultramicroscopic examination was also done to observe ultrastructural differences of formed coccoid. Statistical analysis used in this study is manova and factor analysis.

Result of this study showed that the formation of coccoid form was the fastest in antibiotic condition followed by aerobiosis and slowest in the microaerophilic condition. Rate of the decrease of DNA content of VC coccoid and VNC coccoid was the fastest in antibiotic condition, followed by aerobiosis and microaerophilic condition. The urease enzymatic activity was absent in all coccoid form, despite the positive urease A protein, urease B protein and *ureA* gene. mRNA urease in VC coccoid and VNC coccoid in microaerophilic and antibiotic condition was positive but it was negative in all coccoid formed in aerobiosis.

The longer the time needed for the formation of coccoid form, the decrease of DNA and RNA integrity is more clearly observed. The most rapid decrease of DNA integrity in VC coccoid form occurred in aerobiosis, followed by microaerophilic condition and then antibiotic condition. The decrease of the DNA integrity in VNC and NV coccoid occurred most rapidly in antibiotic condition followed by aerobiosis and then microaerophilic. The decrease of RNA integrity in coccoid form occurred in aerobiosis, followed by microaerophilic and antibiotic condition. CagA protein was negative in all coccoid form although *cagA* gene was positive in all coccoid form, except in NV coccoid of microaerophilic condition.

Antibiotic condition has the strongest effects on the characteristics of coccoid compared with aerobiosis. Aerobiosis has more effects on the decrease of the survival of VC coccoid, the difference was not seen in VNC coccoid. In NV coccoid antibiotic condition showed stronger effects on the decrease of survival compared with aerobiosis. Antibiotic condition showed the strongest effects on the virulence.

The serial transmission electron microscopic examinations showed that the coccoid formation in aerobiosis and antibiotic condition occurred without typical U form and doughnut form seen in microaerophilic condition. VC coccoid form in microaerophilic condition showed various shapes: rounded, tubular and pear shaped. VC coccoid form in antibiotic condition showed bizarre form, with hook like structure. In the stage of VNC in antibiotic condition caused changes in cell wall with hook like structure not seen in the coccoid of microaerophilic condition. Periplasmic space in VNC coccoid of antibiotic condition was narrower compared with aerobiosis, while periplasmic space of NV coccoid of antibiotic condition is wider than aerobiosis condition.

This study confirmed a new concept that environmental stress is a very important factor in the induction of the coccoid. The morphology, characteristics, survival, virulence and the ultrastructure of the coccoid were variation with different stress condition. The results of this study also confirmed that coccoid form of *H. pylori* is not a live form without virulence.

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ABSTRACT

The objectives of the study were: 1). To prove that antibiotic stressor has stronger effect on the characteristics of the coccoid form of *H. pylori* than aerobiosis stressor 2). to prove that antibiotic stressor has stronger effect on decrease of survival coccoid form of *H. pylori* than aerobiosis stressor 3). to analyse aerobiosis as stressor with stronger effect on decrease of virulence of the coccoid form of *H. pylori* than antibiotic stressor 4). and to compare the effects of the aerobiosis and antibiotic stressor on the ultrastructure variation of the coccoid form of *H. pylori*.

Material of this study were 81 tubes containing samples of 10^9 CFU/ml spiral form of Cag A positive *H.pylori* grown from gastric biopsy of a patient with dyspepsia. The samples were divided into 3 groups. In the first group the coccoid form was induced by prolonged culture under microaerophilic stressor. In the second group the coccoid form was induced by aerobiosis, and the third group by the addition of 0.1 microgram amoxicillin /ml in the microaerophilic condition. Periodic sampling was done to calculate the percentage of coccoid form, to observe the possibility to regrow the spiral form, and to observe the presence of urease protein, urease activity, the presence of urease gene, the presence of mRNA urease, the presence of Cag A protein, presence of cag A gene, total DNA quantitation, DNA and RNA integration, and serial transmission electron microscopic observation.

The result of this study showed that different length of time was required for the transformation from spiral form to reach 100% of coccoid form. The viability of the coccoid form was the longest (49 days) in microaerophilic stressor, and in aerobiosis was 28 days while it was 13.5 days in antibiotic stressor. The urease protein and urease gene was positive in all viable coccoid, despite the negative urease enzymatic activity in all coccoid form. cag a gene was detected in all phases of viable coccoid, although the cag A protein was only positive in spiral form. The gradual decrease of total DNA content, DNA and RNA integration occurred and the lowest amount was reached in non viable coccoid form. The rate of the decrease was different among coccoid forms It was shown that antibiotic stressor exerted the strongest influence on the characteristic and virulence of the bacteria.

Serial ultramicroscopic observation in the microaerophilic stressor showed the intermediate U and doughnut form and the detachment of flagella. The rupture of cytoplasmic membrane was observed in unviable coccoid form. The similar ultrastructural sequence was not found in the aerobiosis and antibiotic stressor due to short time elapsed from spiral form to the death of coccoid form.

The conclusion of this study was the confirmation that environmental stress was an important factor in the induction of coccoid form of *Helicobacter pylori*. The morphology, characteristics, survival, virulence and the ultrastructure of the coccoid form were different with different stress condition. The possibility of regrowing into spiral

form and virulence is greater in aerobiosis compared with antibiotic environment. Microaerophilic stressor caused structural rupture and DNA damage, but this situation is caused by longer period needed for forming coccoid, it takes 80 days reach NV coccoid

Key words: *Helicobacter pylori*, coccoid form, characteristics, ultrastructure, stresses