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SHORT COMMUNICATION

Role of Chloramphenicol Acetyltransferase (CAT) Enzyme for Early Detection of Chloramphenicol Resistant *Salmonella typhi*

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Microbial resistance to antibiotic is a major problem in the treatment of infectious diseases. The purpose of this study was to identification the role of CAT enzyme for early detection of chloramphenicol resistant *Salmonella typhi*. Observation was performed on *Salmonella typhi* isolated from Dr. Sutomo Hospital, Surabaya Health Laboratory, and Microbiology Laboratory-Airlangga University, Surabaya in 2009 and had been sub-cultured. The sub-culture was then tested for its susceptibility to chloramphenicol by using anti-chloramphenicol acetyl transferase (CAT) antibody from Sigma (catalog number C.9336). Susceptibility test using dilution and diffusion methods proved that resistant *Salmonella typhi* could change to sensitive and vice versa. It seems that the CAT enzyme can bind anti-CAT so reversing the resistant *Salmonella typhi* into sensitive and conversely, sensitive *Salmonella typhi* into resistant one.

Key words: CAT enzyme, chloramphenicol, resistant *Salmonella typhi*

Resistensi mikroorganisme terhadap antibiotika merupakan masalah besar dalam penanganan penyakit infeksi. Tujuan penelitian ini adalah untuk mengidentifikasi peran enzim CAT untuk deteksi dini *Salmonella typhi* yang resisten terhadap kloramfenikol. Observasi terhadap kuman *Salmonella typhi* yang diisolasi dari RSUD Dr. Sutomo Surabaya, Laboratorium Kesehatan daerah Surabaya dan Laboratorium Mikrobiologi Unair Surabaya pada tahun 2009 dan telah di sub kultur lagi . Hasil sub kultur kemudian dilakukan uji kepekaan dengan anti-chloramphenicol acetyl transferase (CAT) *antibody.* Hasil uji kepekaan secara dilusi dan difusi membuktikan *Salmonella typhi* yang resisten dapat berubah kepekaannya menjadi sensitif dan sensitif dapat berubah kepekaannya menjadi resisten. Kesimpulan dalam penelitian ini anti CAT berperan mengikat aktivitas enzim CAT sehingga *Salmonella typhi* yang resisten menjadi sensitif dan sebaliknya *Salmonella typhi* sensitif yang diberi kloramfenikol dapat berubah menjadi resisten.

Kata kunci : enzim CAT, kloramfenikol, *Salmonella typhi* resisten

Microbial resistance to antibiotic is a major problem in the treatment of infectious diseases. Since 1972, resistance of *Salmonella typhi* to chloramphenicol was widely reported around the world. Resistance mechanisms of microorganisms to antimicrobial drugs might occur through changing of drug receptor, decreasing the drug amount that reaches the receptor, damaging or deactivating the drug, and developing resistance metabolic pathways. Bacteria may have one or more abilities to establish the resistance mechanism. One of the *Salmonella typhi* abilities is to produce CAT enzyme that can deactivate chloramphenicol (Baron S 2005).The purpose of this study was to identify the role of CAT enzyme for early detection of *Salmonella typhi* resistant to chloramphenicol.

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Observation was performed on *Salmonella typhi* isolated from Dr. Sutomo Hospital, Surabaya Health Laboratory, and Microbiology Laboratory-Airlangga University, Surabaya in 2009.The sub-cultured bacteria were then tested for their susceptibility to chloramphenicol by using anti-chloramphenicol acetyl transferase (CAT) antibody from Sigma (catalog number C.9336). The study was conducted in the biology laboratory, Unirow Tuban and the Institute of Tropical Disease, Airlangga University (ITD-Airlangga University), Surabaya. Fifteen isolates taken from blood specimens were firstly tested using chloramphenicol. *Salmonella typhi* strains that consistently showed chloramphenicol sensitivity and resistance were used for susceptibility test using dilution and diffusion methods.

The chloramphenicol susceptibility was performed by administering an anti- CAT antibody (10 μg mL^{-1})

and 3.125 µg of chloramphenicol to the resistant strains. Sensitive *Salmonella typhi* strains treated without anti CAT was used as control. The treatment was conducted on *Salmonella typhi* taken from 15 stock tubes. Three isolates from tubes 3, 9, 12, which consistently showed resistance, were then coded as R3, R9, and R12. Three isolates from tubes number 13, 14, 15, which consistently showed sensitivity, were then coded as S13, S14, and S15. Whereas, from tubes 1,2,4,5,6,7,8,10,11 no results found.

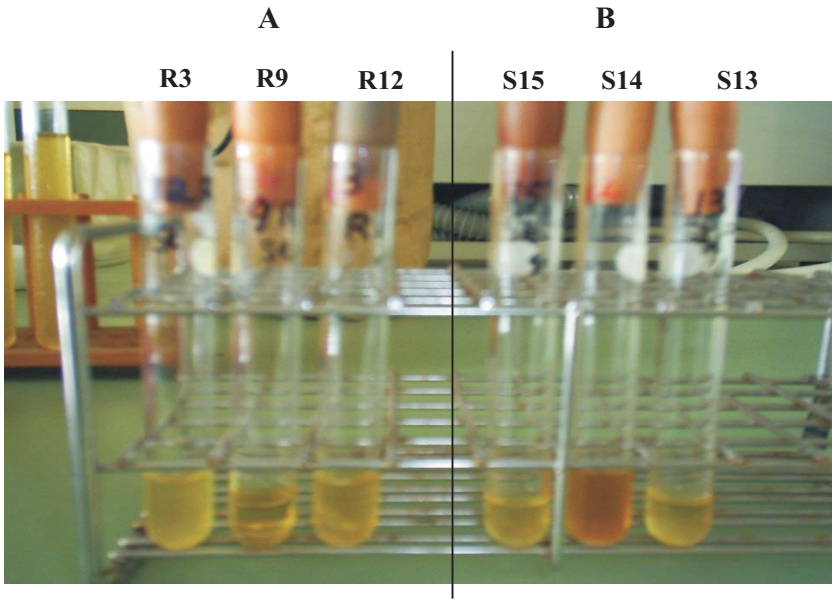

Fig 1 Dilution susceptibility test to determine the sensitivity and resistance of the *Salmonella typhi* strains after overnight incubation at 37 °C.

Clear culture of *Salmonella typhi* means no bacteria growth, whereas turbid cultures means there was bacteria growth. *Salmonella typhi* resistant strains (R3, R9, and R12) were treated with anti - CAT 10 µg and 3.125 µg chloramphenicol (Fig 1A). Cultures of *Salmonella typhi* resistant strains R3 and R12 turned turbid, while R9 culture remained clear. Sensitive *Salmonella typhi* (strains S13, S14, S15) were treated only with 3.125 µg of chloramphenicol, without anti CAT (Figure 1B). Cultures of *Salmonella typhi* sensitive strains S15 and S13 turned turbid; whereas S14 culture remained clear. Clear culture of *Salmonella typhi* meaning means there was no bacteria growth in negative control medium (R9) and turbid cultures showed growth (R3 and R12) (Fig 2).

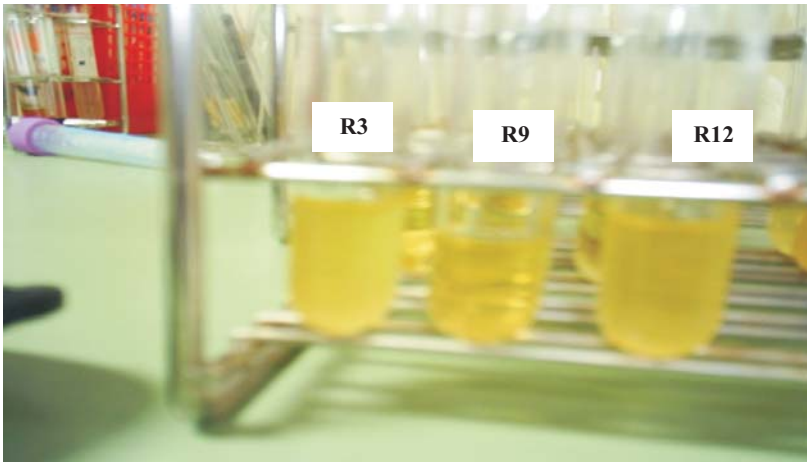

Fig 2 Cultures of chloramphenicol resistant *Salmonella typhi* strains treated with anti-CAT after overnight incubation at 37 °C.

The diffusion susceptibility test results using chloramphenicol-disk concentration 30 µg (C30) method for resistant *Salmonella typhi* (R3, R9, R12) indicated the presence of inhibition zone, meaning the resistant bacteria change to be sensitive to chloramphenicol (Fig 3).

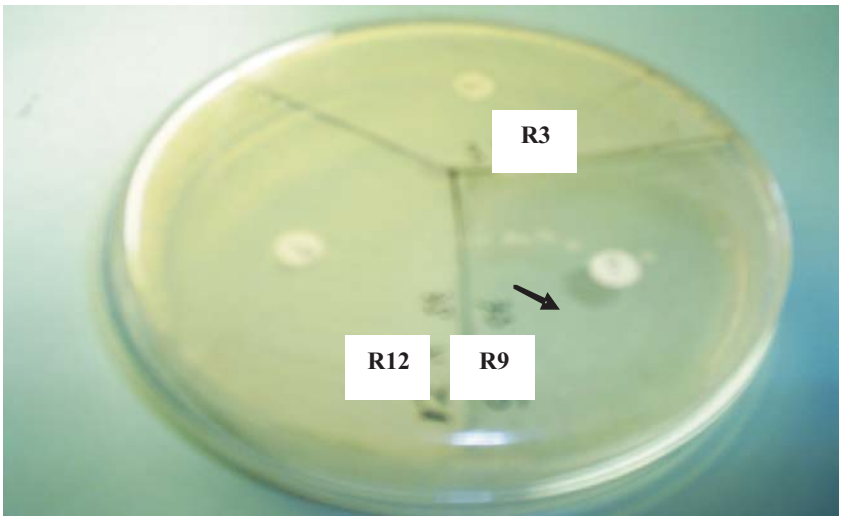

Fig 3 Diffusion susceptibility test results *Salmonella typhi* resistant the same strain using chloramphenicol-disk method (C30) after overnight incubation at 37 ºC inside petridish.

The diffusion susceptibility test results using chloramphenicol-disk method for sensitive *Salmonella typhi* (S13, S14, S15), arrow indicated the presence of inhibition zone (Fig 4). The inhibition zone showed that the bacteria still sensitive to chloramphenicol. *Salmonella typhi*; no inhibition zone means the bacteria is resistant to chloramphenicol. It showed that sensitive *Salmonella typhi* changed to be resistant to chloramphenicol.

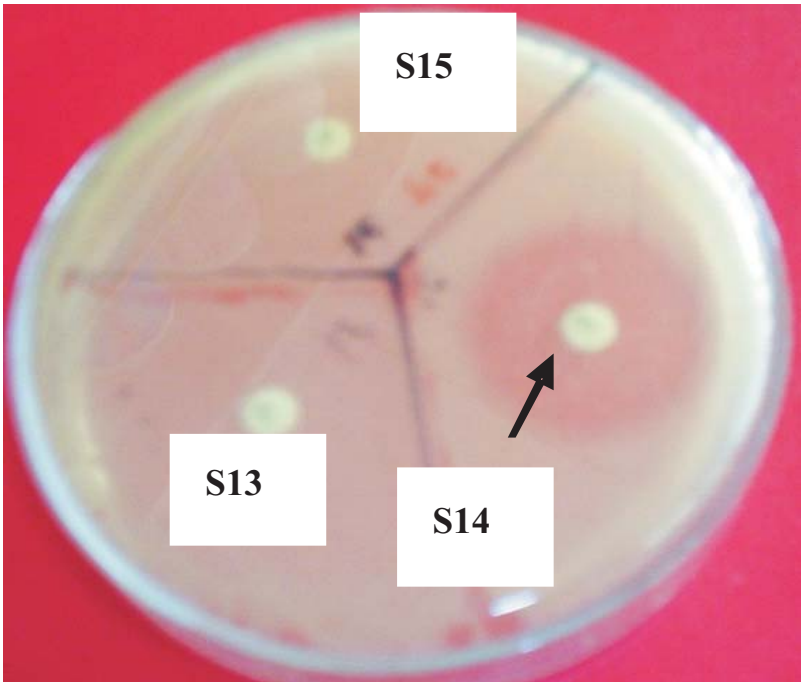

Fig 4 Difusion susceptibility test results *Salmonella typhi* sensitive the same strain using chloramphenicol-disk method (C30) after overnight incubation at 37 ^{0}C inside petridish.

The results indicate that change of the sensitivity of *Salmonella typhi*. Resistant *Salmonella typhi* is assumed to produce CAT enzyme that after given the anti-CAT, could change susceptibility become sensitive to chloramphenicol, and vice-versa. Sensitive *Salmonella typhi* that was given chloramphenicol could change susceptibility to become resistant. It demonstrates *Salmonella typhi* produce CAT enzymes that can inhibit chloramphenicol so that bacteria become resistant to chloramphenicol.

Similar results research by Juwita*. et al.* 2013. Out of 37 blood samples of typhoid fever patients in Paediatric Department of RSUD Ulin Banjarmasin, 20 samples were positive of *Salmonella typhi* isolate and the samples had undergone sensitivity test to antibiotic chloramphenicol, amoxicillin, and cotrimoxazole. The similar results were obtained on studies strain *Salmonella typhi* resistance to chloramphenicol (Mandal 2004).This is also shown by Mirza (2000) in Pakistan that at the same time genetically multidrug resistance has been reported to occure endemic, such as in Bangladesh. In India it was reported that 10% were found to be multidrug resistant (MDR) (defined as resistance to ampicillin, chloramphenicol and cotrimoxazole). There was a decrease in the susceptibility to ciprofloxacin of *S. Typhi* with MIC showing an upward trend (0.125-4 $\mu g\, mL^{-1}$) (Nagshetty *et al.* 2002).

Plasmids as intermediary for resistance genes to chloramphenicol, trimethoprim and ampicillin are type I CAT, type VII dihydrofolate reductase and TEM-1 ß-lactamase. Treatment with anti-CAT is based on the assumption that resistant bacteria capable of producing CAT enzyme and this enzyme may be expressed to the culture media so that when anti-CAT added to the media can bind the expressed CAT enzyme, this is mean CAT cannot function as a contributing factor in the resistance, so when exposed to chloramphenicol the bacteria will be sensitive. One out of three resistant isolates was turned into sensitive, this is supported by the statement of Wain *et al.* (2003) and Asma *et al.* (2005) that the genes responsible for resistance to chloramphenicol were brought by the plasmid and also performed by chromosomes, the catP gene.

Dumo and Adrian (1992) studied about in vitro resistance by Kirby-Bauer method found *Salmonella typhi* resistant to chloramphenicol (3.8%), ampicillin (6.7%), ceftriaxone (5.1%) and the highest resistance against cotrimoxazol (11.4%). Chloramphenicol resistance level, study is still low compared to other antibiotics so that when the CAT enzyme bind to anti-CAT as performed in this study, there is still a chance to turn out the bacteria to be sensitive to chloramphenicol, so the anti-CAT allegedly had a role in bacterial sensitivity, because it can change the resistant bacteria to be sensitive.

According to the Committee for Veterinary Medical Products, the term anti-microbial resistance refers to two terms, namely microbial resistance and clinical resistance. Microbial resistance is a biological process associated with various resistance mechanisms involving the role of resistance genes in bacteria. Microbial resistance is affected by an enzyme that makesanti-microbial agent is not active. Clinical resistance is bacterial resistance that dependent on the responds of bacterial infection to the treatment given (EMEA 1999; Fluit 2001).

We can conclude that the anti CAT enzyme can shutdown CAT activity, rendering the sensitivity of the resistance strains. So that CAT enzyme could be used as an early detection of *Salmonella typhi* resistant to chloramphenicol.

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