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Detection of Gen Chloramphenicol Acetyl Transferase (CAT) *Salmonella typhi* Resistant Chloramphenicol by Polymerase Chain Reaction (PCR) Technique

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ABSTRACT

Objective: *Salmonella typhi* as a causative agent of typhoid abdominalis many militias, which was resistant to the drug, one of which is chloramphenicol. Detection of *Salmonella typhi* serologically generally done so if there is a problem in the diagnosis difficult to overcome so it needs a faster diagnosis techniques with accurate results. PCR can be used as an alternative solution to these problems. The purpose of this study was to detect the DNA of *Salmonella typhi* resistant to chloramphenicol by PCR.
Material and methods: The method in this population experimental laboratory conducted in the laboratory biology and laboratory Unirow Tuban ITD Airlangga University Surabaya. Experiment done using PCR from blood samples. Using primer from CAT gene to detect this gene.
Result: The results of this study with tank PCR can detect DNA of chloramphenicol resistant *Salmonella typhi* with a length of 293 bp.
Conclusion: The conclusion PCR technique can detect DNA of *Salmonella typhi* resistant to chloramphenicol.

INTRODUCTION

Salmonella typhi bacteria as the agent of typhoid fever resistant to many antibiotics, one of the antibiotic chloramphenicol. This is also consistent with the results of the study Sambrook (2001) and Wain et al (2003) that this resistance is encoded by a gene located on Tn 9 with 1102 bp long and encodes an enzyme that asetyltransferase Chloramphenicol (CAT) length of 293 bp. So that should be done detection the CAT genes generally detection *Salmonella typhi* serologic only be done, for example The Widal test has limitations determine the positive or negative typhoid necessitating techniques that more valid and accurate detection of *Salmonella typhi* resistant to chloramphenicol particular thing. Polymerase Chain Reaction (PCR) can be used as an alternative to the solution of this problem because it has excess can directly detect the DNA of *Salmonella typhi* as a causative agent of disease with results

more quickly and accurately. The purpose of this study to detect DNA encoding the CAT gene in *Salmonella typhi* resistant to chloramphenicol.

MATERIAL AND METHODS

This result was a cross sectional study. *Salmonella typhi* DNA obtained from blood samples of abdominal typhus patients treatment at a hospital in the district of Tuban. Diagnosis is done by a specialist in internal medicine who served in the hospital. The samples in this study were 30 samples. The method used in this research was a laboratory experiment. Research carried out in the laboratory at the University Ronggolawe biology (Unirow) Tuban and Institute of Tropical Disease, Airlangga University (ITD Airlangga University), Surabaya from July to October 2012.

Examination Widal

Widal examination of the serum. Serum total 20µl was mixed with 150µl reagent Remel Widal brands of each type is O, H, A, and B. Each one drop of each reagent was mixed with 20µl serum is then shaken and see aglunation based instructions adauntuk determine the result is negative, 1 / 80 , 1/100 , 1/160 , 1/320 and 1/400.

PCR *Salmonella typhi*

PCR *Salmonella typhi* with primmer *S.typhi*

The study was conducted on blood samples of patients with abdominal typhus, for *Salmonella typhi* given blood coagulant, then do take centrifugation for blood cells. Blood cells were transferred into a sterile tube ependrof 1.5 ml size sterile and stored in -80 °C similarly to date examination of *Salmonella typhi* DNA by PCR. The stages of *Salmonella typhi* DNA PCR are as follows:a. Ekstraksi DNA *Salmonella typhi*

Extraction of DNA of blod cell with DNA Kit (*Roche*) solution for kit procedure.

b. Amplification Reaction by PCR

Amplification reaction used for *Salmonella typhi* DNA PCR: buffer solution 10x Tth, enzyme Tth DNA polymerase, dNTPs solution. Enzim *Tth DNA polymerase,* larutan *dNTPs*. Primer set 1 and set 2 correspond to genomic area *Salmonella typhi* . Sequence nucleotida primer 1(*sense*): 5'-GCTGCGCGCGAACGGCGAAG - 3' and primer 2 (*antisense*): 5'-TCCCGGCAGAGTTCCCATT-3' (Ferretti, *et al*., 2001). Result PCR positive with nucleotide 389 *base pairs* (bp). PCR temperatures used for denaturation was 94°C for 1 min, annealing 50°C for 1 min, extension 72°C for 2 min, PCR cycles was 35.

c. **Electrophoresis**

DNA *Salmonella typhi* amplification result then performed electrophoresis using 2% agarose in 0.5 X TBE buffer solution containing ethidium bromide . DNA *Salmonella typhi* from the samples, negative control (used distilled water), positive control and markers (Ø x 174/Hae III digest) that already separated can be viewed under ultraviolet light.

d. **Photo of Electrophoresis Result**

The result was documented using digital camera.

PCR *Salmonella typhi* with *primer* gen CAT

Salmonella typhi PCR with primers CAT gene performed on blood samples of patients with abdominal typhus that has given positive results with the Widal test and PCR with primers common *Salmonella typhi*. *Salmonella typhi* plasmid DNA is used for PCR in an attempt *Salmonella typhi* sensitive and resistant to antibiotics (chloramphenicol). The primary use for the region that codes for the enzyme chloramphenicol acetyltransferase (CAT). Stages same PCR performed with *Salmonella typhi* PCR with primers common but used *primer* set1 and set 2 corespond area gen CAT *Salmonella typhi*. Sequence nucleotida primer CAT-F (*sense*) : 5' TCCCAATGGCATCGTAAAGAAC - 3' and primer CAT-R (*antisense*): 5'-TCGTGGTATTCACTCCAGAGCG -3'. PCR temperatures used for denaturation 94^{o}C for 1 min, *annealing* **55**^{o}C for 1 min, extension 72 ^{o}C for 1,5 min, and siklus PCR 35.

RESULT

Research data on the detection of CAT gene DNA in *Salmonella typi* can be seen in table 1, in this table are presented the data produced by PCR with primers CAT gene.

Table 1. Result of PCR DNA *Salmonella typhi* resistant chloramphenicol of Tifus Abdominalis Patient in Tuban Distric

No.	Sex	Age X ±SD	Sample	Result PCR Positive
1.	Male	31± 14,51	13	1/13
2.	Female	33± 18,30	17	0/17
	Total		30	1/30 (3,3%)

Table 1 shows from 30 sample only one sample of amplicon has positive resistant chloramphenicol. In percentage it is 3,3%. Figure result PCR gen CAT for *Salomella typhi* showed figure 1.

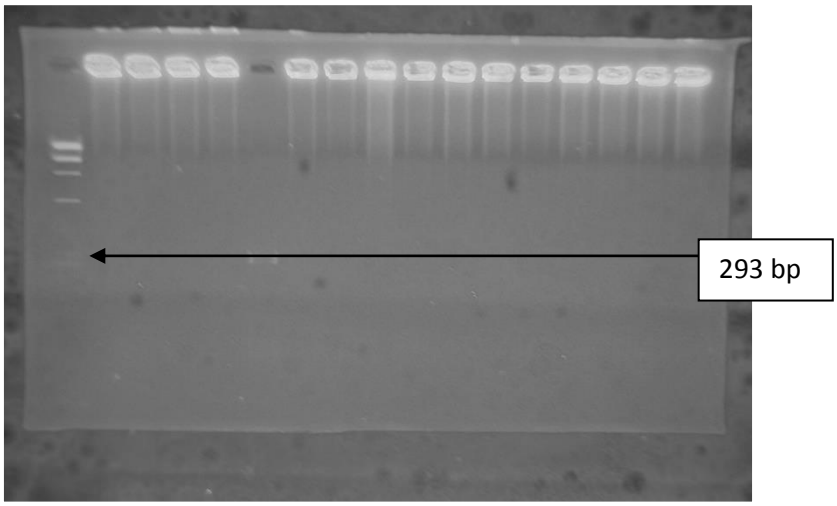

Figure1. Result PCR DNA *Salmonella typhi* resistant chloramphenicol 293 bp

Figure 1 PCR DNA *Salmonella typhi* resistant chloramphenicol lenght 293 bp. Markers (Ø x 174/Hae III digest. These markers are used to define a standard for determining bp long samples in the search

DISCUSSION

From the research, the next stage (second year) from 30 samples of patients with abdominal typhus in Tuban after PCR only 1 (3,33%) DNA samples of the *Salmonella typhi* resistant to chloramphenicol. This happens because of the sensitivity of chloramphenicol still have thingy so that only one sample was found resistant to chloramphenicol, although at the time of sampling was conducted after getting treatment for all hospitalized patients. (Puhr, Tauxe, & Mintz, 2000) and Ivannof (2002) suggests that currently has a lot of *Salmonella typhi* resistant to multidrug one of them resistant to chloramphenicol. Sambrook (2001) and Wain et al (2003) also suggests that the factors that play a role in resistance to chloramphenicol is encoded by a gene located on Tn 9 with long 1102 bp and coding enzyme *Chloramphenicol Asetil Transferase (CAT)*. Fragmen DNA coding enzyme CAT in research by PCR examination with primer for method resulting nukleotida 293 bp.

In this study, although only one sample detected *Salmonella typhi* resistant to chloramphenicol but PCR techniques can help early diagnosis when the patient is suspected to be resistant to chloramphenicol because PCR can detect quickly with more accurate results than the sociological.

CONCLUSION

PCR technique can detect DNA CAT gene in *Salmonella typhi* resistant to chloramphenicol

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