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## **Z66Ind gene detection and its relationship with the titer of antibodies to *Salmonella typhi* flagella antibody in patients with typhoid fever in the city of Jayapura**

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### **ABSTRACT**

*Typhoid fever is an acute infectious disease caused by *Salmonella typhi*, is characterized by fever, dirty tongue, abdominal pain, diarrhea, vomiting, headaches, seizures and impaired consciousness. The purpose of this study was to determine the relationship between antibody titer results with the presence of *Salmonella typhi* z66Ind gene in patients with typhoid fever in the city of Jayapura, Papua province of Indonesia. This study uses laboratory exploration studies with a total sample of 92. The test is used to detect the presence of *Salmonella typhi* z66Ind genes that widal test, lateral flow test, culture test and analysis of PCR amplification. The results showed that discovered the gene z66Ind *Salmonella typhi* in the Widal test titre Ag O 1/320 and Ag H 1/320, lateral flow test (2+), a culture test (+) *Salmonella typhi* and positive PCR results (+) on electrophoresis will formed 0.6 kb fragment of DNA bands in patients with typhoid fever in the city of Jayapura.*

**Keywords:** Z66Ind gene, titers of antibodies, *S. typhi*, typhoid fever, city of Jayapura

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### **INTRODUCTION**

Typhoid fever is an acute infectious disease caused by *S. typhi*, is characterized by fever, dirty tongue, abdominal pain, diarrhea, vomiting, headaches, seizures and impaired consciousness. This disease is also an important public health problem because of its spread is closely related to urbanization, population density, environmental health, water sources and poor sanitation and hygiene standards of food processing industry is still low [1].

*Salmonella* is a group of organisms belonging to the family *Enterobacteriaceae* are rod-shaped, gram-negative, moving with flagella Peritrics, easy to grow in the medium hatchery bile [2]. The amount of the exact numbers of cases of typhoid fever in the world is very difficult to determine because the disease is known to have clinical symptoms with a very broad spectrum. Data from the World Health Organization (WHO) in 2003 estimated that there are approximately 17 million cases of typhoid fever worldwide with an incidence of 600,000 deaths annually [3].

In developing countries, cases of typhoid fever reported as endemic disease, of which 95% are outpatient cases so that the actual incidence is 15-25 times greater than reported in the hospital inpatient. In Indonesia, the disease is an endemic disease with high incidence rates including that between 358-810/100,000 population per year. The prevalence of typhoid fever in the province of Papua is one of the twelve provinces that have a prevalence above the national average of around 1.6% (from 0.3 to 3.0%). [4-5]

One of the factors that cause typhoid fever is acute even cause death is a virulence trait flagella. Flagella is a tool movement *Salmonella typhi* bacteria are composed of a protein called flagellin. Flagella improve the motility and invasiveness of *Salmonella typhi*, which can lead to perforation of the bowel (lymphoid tissue). In the process of bacterial invasion requires direct contact with the surface of host cells, attachment to the cell surface receptor and then enter the cell by endocytosis [6]. Flagella function thought to be associated with the first stage of invasion and chemotaxis power causing bacteria make direct contact with host cells [7].

Serotype motility functions *HI-j* is reduced due to changes in the function of flagella, so invasiveness and clinical levels of disease can also be reduced. Reduced motility serotype *HI-j* be an important contributor to the decline in invasive [8]. As reported by other studies, which compared with *HI-j* serotype and *z66*, serotype *HI-d* cause more severe clinical disease, has a stronger and more invasive motile in vitro assays in semisolid medium [9-10].

Flagella antigen variation caused by the accumulation of genetic processes in the flagella genes, such as point mutations, deletions, and insertions, as a phenomenon that can describe the lateral transfer of genetic material that produces interspecific recombination between flagellin genes [11-12]. Gen *Hd*, *Hj* and *z66* is a variation of the gene found in *Salmonella typhi* flagella is one of the factors that cause acute the disease and even cause death [13-14].

At the bacteria *Salmonella typhi* flagella are *fliC* genes that encode or phase 1 antigen *Hi-d*. Flagelin *Hi-d* gene is a gene potentially invasive power of the host and is owned by *Salmonella typhi* in typhoid fever patients worldwide [11]. Other studies report that *Salmonella typhi* flagella find different serotypes of the *HI-j* and about 10-50% are only found in isolates of *Salmonella typhi* from Indonesia and there are no data that indicate its existence in other countries [15].

Apparently, in addition to antigen *Hi-d* and *Hi-j* in Indonesia also found a different antigen that is *z66*. Further research results indicate that *z66* antigen in the code on the linear plasmid called pBBBS1 while *fliC* genes that encode antigen *Hi-d* and *Hi-j* located on chromosome. Other studies, further found *Salmonella typhi* antigen *z66Ind* and allegedly associated with higher risk of severe clinical typhoid fever [16]. Then to detect the presence of *Salmonella typhi z66Ind* gene using serological tests, test culture and PCR test.

Based on the background of the problems described above, it is for the detection of gene *z66Ind* and relationships antibody titers against *Salmonella typhi* flagella antigen in patients with typhoid fever in the city of Jayapura it is necessary to test serology, culture and amplification of DNA.

## MATERIALS AND METHODS

This study was conducted in January-March 2015 in the Hospitals in the city of Jayapura, namely: Naval Hospital, Regional General Hospital of Dok II, Police Hospitals of Bhayangkara and Laboratory of Molecular Biology and Immunology, Division of Microbiology, Faculty of Medicine, University of Hasanuddin, Makassar-South Sulawesi.

### Population and sample

Population is suspected typhoid fever patients who visit the emergency department or outpatient clinic at the hospital in the city of Jayapura ie Dok II Hospital, the Naval Hospital and Police Hospital of Bhayangkara. With referral the doctor to request laboratory tests. This type of research is the exploration of the laboratory is to evaluate the results of tests used to detect IgM antibodies and using lateral flow and widal while to detect the presence of *Salmonella typhi Hz66Ind* gene PCR method. This study uses 4 testing is Widal, Lateral flow, culture and PCR [17]. Meanwhile, samples such as serum and red blood cells are taken in the event of fever in patients with suspected typhoid fever from the sample population. The patient has not received antibiotic therapy. Accidental sampling technique using sampling that samples taken in cases of typhoid fever patients in accordance with the criteria of the sample [17].

## RESULTS AND DISCUSSION

Widal test of 92 samples of the study showed O antigen negative (22.8%), O antigen positive with titer 1/160 (16.3%), titer 1/320 (53.3%), and titer 1/640 (7.6%). While the results of H antigen negative (56.5%), H antigen with positive titer 1/160 (17.4%), titer 1/320 (19.6%) and the titer 1/640 (6.5%). Sample of 92 suspected typhoid fever lateral flow test showed negative (51.1%), positive result (+) (40.2%) and positive result (++) (8.7%). Positive samples from the lateral flow test as many as 45 test cultures obtained negative results (82.2%) and a positive result (17.8%). Further samples test positive cultures as many 8 *Salmonella typhi* PCR test to detect the presence of *Salmonella typhi z66Ind* gene.

From blood culture results in suspected typhoid fever is obtained from the media *Salmonella Shigella* Agar: a small colony, black, Media Mac Conkey form colonies smooth, clear, fermentation of lactose (-). At the gram stain obtained shaped bacillus bacteria, red, and are gram-negative.

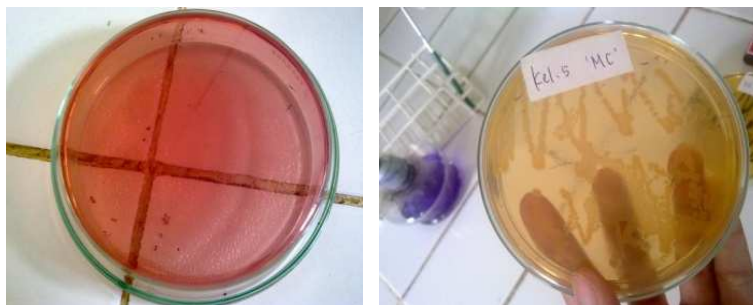


Fig 1. Media Mac conkey

Samples that test positive on culture as many 8 *S. typhi* PCR test to detect the presence of *Salmonella typhi* *z66Ind* gene. In the PCR test showed negative (87.5%) and positive (12.5%) found the gene *z66Ind Salmonella typhi*. This proves that the highly sensitive PCR test to detect the presence of *Salmonella typhi* *z66Ind* gene. The results of electrophoresis analysis of PCR products, as shown in the figure below (Figure 2).

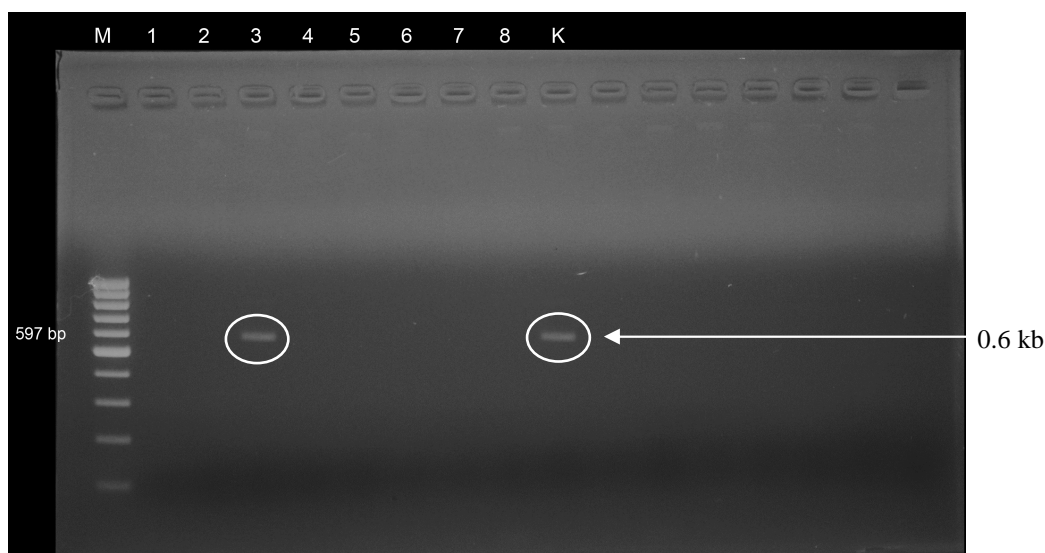


Fig 2. PCR amplification results are very sensitive to detect the presence of *Salmonella typhi* *z66Ind* genes along the 0.6 kb

PCR test research results on 8 samples showed positive culture 1 PCR positive samples found any *z66Ind* gene *Salmonella typhi*. In the above image reading of the results of electrophoresis of *Salmonella typhi* is characterized by the existence of the band of 0.6 kb, Marker (M). Gene *z66Ind* found any *Salmonella typhi* in the sample S03. While (K) shows the positive control and the band (N) shows the negative control band. Results of research on the sample S03 with positive test widal Ag O titers of 1/320 and Ag H 1/320 titer positive, lateral flow test positive one (1+), test positive culture (+), and found a positive PCR test genes of *z66Ind Salmonella typhi*.

According to previous studies, basically antigen *Hi-j* highly homologous with *Hi-d*, except for deletion of 261 bp in the central parts of the determinant antigen gene *fliC* from strain *Hi-j* is responsible for flagella antigen variation. This deletion occurs as a result of homologous recombination intragenic involving the repetition of 11 bp. Intragenic recombination in the central sections of *Hi-d* gene, damaging specificity d and forming new specificity in flagella fragments. However, further research is done in some isolates from Indonesia find any special or additional antigen which was then known as the *z66* antigen [18]. The analysis showed differences in expression *z66* compared *fliC* under osmotic pressure, pressure bile acids and oxidative stress. *Salmonella typhi* antigen expression *z66* 10 times higher than *fliC* in the osmotic conditions of low and medium.

Food and drink contaminated is the source of transmission of *Salmonella typhi*. Career in humans is the source of the infection. *Salmonella typhi* usually in water, ice, dust, and when dry waste into the vesicles are suitable for example meat, shells and so on. *Salmonella typhi* will proliferate reach infective dose. Transmission of typhoid fever by bacillus *Salmonella typhi* to humans through food and drink that has been contaminated by feces or urine. There are two sources of the transmission of typhoid fever patients were either on sick or recovering.

This is in line with that proposed by previous researchers that the increasing cases of typhoid fever in urban areas affected by several factors, such as increasing urbanization, population density and rapid spread of populations, poor sanitation and asymptomatic cases were not detected [19-20].

Flagellin antigen biodiversity caused by the accumulation of genetic processes between flagellin genes for example point mutation, deletion and insertion as a phenomenon that can describe the lateral transfer of genetic material that produces interspecific recombination between flagellin genes [21]. In addition to these processes, the interaction between genes *fliC* and *fliB* also considered one of the causes of biodiversity antigen *Salmonella* serovar.

Flagellin gene variations found in this study indicate that there is a process of adaptation that is carried by the bacteria *Salmonella typhi* either DNA or plasmids. Changes in the DNA of *Salmonella typhi* as a form of adaptation in bacteria population in defending themselves gradually and continuously. This is explained by other studies, that the adaptation of living organisms including bacteria to environmental changes are a response to natural selection to survive [22]. This adaptation may include behavioral and physical. This phenomenon is called microevolution and the accumulation of these changes in the long term lead to the macroevolution and ultimately produce new species.

## CONCLUSION

Based on the results and discussion can be concluded that *Salmonella typhi* *z66Ind* gene found in patients with typhoid fever in the city of Jayapura and found to have the gene of *Salmonella typhi* *z66Ind* on the titer Widal test with Ag O 1/320 and Ag H 1/320, Lateral Flow test (2+), culture test (+) found the bacteria *Salmonella typhi* and positive PCR results (+) on 0.6 kb band reads electrophoresis in patients with typhoid fever in the city of Jayapura, Papua province of Indonesia.

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