1.0 INTRODUCTION

*Salmonella enterica* subspecies *enterica* serotype *Typhi* (*S. Typhi*) is the causative agent of typhoid fever that is still a major challenge for public health in developing countries. The bacterium is transmitted by faeco-oral route, through contaminated water or food. *Salmonella Typhi* is highly adapted to human host; the only reservoir is man. The diagnosis of typhoid becomes difficult as discriminating typhoid from other febrile disease is still unclear.

1.1 Epidemiology of Typhoid Fever

Typhoid fever is a major cause of morbidity and mortality among humans with an estimated global incidence of 21.6 million cases and 216510 deaths per year. In developing countries, its annual incidence ranges from 12 to 622/100 000 persons (Gaind *et al.*, 2006).

Typhoid fever is a major health problem in Southeast Asian countries including Malaysia, Thailand, and Indonesia (Thong, 1995). In 2007, the Communicable Disease Centre (CDC) of Indonesia reported a prevalence of 358-810 per 100,000 populations for typhoid fever with 64% occurring in 3 to 19 year old (Moehario, 2009). In South Sulawesi, the case detection rate increased from 257 per 100,000 population in 1991 to 386 per 100,000 population in 2007 (Hatta, 2008).

In Jakarta, typhoid fever was the second leading infectious disease after gastroenteritis and caused the highest mortality. The mortality rate
varied from 3.1-10.4% among hospitalized patients with cases occurring throughout the year, peaking during the dry season (Moehario, 2009).

1.2 Typhoid in India

Enteric fever, although not common in industrialized countries, remains an important and persistent health problem in developing nations. Hospital based studies and outbreak reports from India indicate that enteric fever is a major public health problem, with *Salmonella enterica* serovar *Typhi* (*S. Typhi*) as the most common aetiological agent (Kanungo et al., 2008). India, South and Central America and Africa are the regions where the disease is endemic, due to the rapid population increase, increasing urbanization, restricted water resources and insufficient infrastructure and health services (Kuvandik et al., 2009).

Incidence of typhoid fever varies substantially within Asia. Very high typhoid fever incidence has been found in India and Pakistan (Kothari, 2008). Risk factors such as poor sanitation, lack of safe drinking water supply and low socio economic conditions in resource poor countries are amplified by the evolution of multidrug resistant *Salmonellae* with reduced susceptibility to fluoroquinolones and treatment failure leading to increased mortality and morbidity (Kanungo et al., 2008). Antimicrobial resistance in enteric pathogens is of great importance in the developing nations, where the rate of diarrhoeal diseases is highest and treating these organisms is tough. Enteric fever due to infection with *Salmonella enterica* serovar *Typhi* is a major health hazard, even with the introduction of newer antimicrobial drugs. In the last decade *S. enterica* serovar *Typhi* has rapidly developed
resistance to antibiotics such as ampicillin, chloramphenicol, and cotrimoxazole, and also to ciprofloxacin (Mandal et al., 2009).

Hence constant monitoring of the susceptibility pattern is important, which would provide suitable guidelines for treatment. Study of the phenotype and genotype of these strains would result in deeper insight of the epidemiology of them. Due to growing awareness of different factors that influence the spread of human and animal pathogens in various environments, typing methods for microorganisms were developed. Among the conventional typing methods, the major methods based on phenotype are biotyping, serotyping, bacteriocin typing and typing through antibiotic susceptibility pattern. These methods have been used for typing of a variety of microorganisms (Tsen, 2002). Other than this, Salmonella Typhi can be differentiated based on fermentation of xylose and arabinose. According to the classification proposed by Kristensen and Henrikson S. Typhi can be classified as biotypes I (arabinose -, xylose +), II (arabinose -, xylose -), III (arabinose +, xylose +) and IV (arabinose +, xylose -) (Quintaes, 2002).

Genotypic study can also be done for the rapid and accurate typing of the strains based on molecular methods like RAPD, PFGE, Ribotyping, etc. A PCR based typing method, RAPD-PCR (Random Amplified Polymorphic DNA), has been described as a simple and rapid method for detailed fingerprinting of the genomic composition of the organism. The success of this method is due to the fact that no prior sequence information is needed and a short 10-mer oligonucleotide primer can be employed. The amplification happens at low stringency, allowing the primers to anneal to several locations on the two strands of the DNA (Quintaes, 2002).

Early detection and identification of the etiological agent, Salmonella Typhi, is essential in diagnosis of enteric fever and to reduce morbidity and
mortality rates. The definitive diagnosis of the disease requires the isolation of *Salmonella Typhi* from the blood, faeces, urine, or other body fluids (Rego, 1994). Since typhoid is a severe, systemic infection, there is a clear need for a sensitive and specific test that will permit its rapid laboratory diagnosis. *Salmonellae* can be characterized by their somatic (O) and flagellar (H) antigens and an envelope antigen called Vi (virulence). These antigens were used in various diagnostic kits (including widal) for the diagnosis of typhoid.

Various methods like blood culture, urine culture and immunological methods including Widal were employed in diagnosis of typhoid fever. WIDAL is a serological test, to detect antibodies from patient, still the conventional standard diagnostic tool for enteric fever but, serological diagnosis of typhoid fever (Widal test) has been found by some investigators to be unreliable (Levine, 1978). In areas with endemic typhoid; serodiagnosis requires both acute and convalescent sera, since the prevalence of O and H antibodies in the adult and adolescent populations is high. In this case, diagnosis is made purely retrospective. Clearly, there is a need for a sensitive and specific test that will permit rapid laboratory diagnosis of typhoid fever (Levine, 1978).

Detection of antigen from the body fluids like blood, CSF aspirates will help in early diagnosis. Antigens can also be detected from urine and other samples. Simple, sensitive and cost effective method should be adopted for the diagnosis of typhoid organisms. Rockhill *et al.* (1980) have reported a test that detected soluble *Salmonella Typhi* antigens Vi and D in the urine of Indonesian patients with typhoid fever. Antibodies to these antigens were attached to protein A-rich *Staphylococci*, and antigen was detected by mixing urine with the antibody-coated cells on a slide and
observing for co-agglutination. The test showed high sensitivity (59 of 61 urine samples from culture-proved typhoid patients were positive) but low specificity (8 of 46 control urines were positive).

1.3 Immunoglobulin Y Antibody (IgY)

‘IgY’ antibody represents the antibodies from the egg yolk, hence the term immunoglobulin of egg yolk (IgY). Various advantages of IgY are as follows:

(i) In conventional method, the animals which were immunized with antigens have to be sacrificed to purify the antibody. But in avian system, collection of eggs is sufficient to purify the antibodies and there is no need for sacrificing the animal.

(ii) The birds being closely related to mammals exhibit enhanced immunogenicity of conserved mammalian proteins than other animal models (Gassmann et al., 1990).

(iii) In addition, IgY antibodies tend to recognize the same protein in a number of mammalian species, making them more widely applicable.

(iv) The yield of IgY antibodies can be compared to that of IgG antibodies obtained by conventional immunization methods; 200 mg of IgG can be obtained monthly, with approximately 5% constituting the specific antibody. Whereas in the case of chicken, the IgY production is 18 times more efficient in laying hens (Schade et al., 1996).

Taken together, chicken antibody collection and isolation can be described as noninvasive, rapid and economical. Compared to antibody production in rabbits, the IgY technology offers other advantages like easy isolation, very low antigen requirement and long lasting titres (Gassmann et
al., 1990). Despite the similarities between IgY and IgG antibodies, there are also some profound differences in their structure. The IgY heavy chain is 67-70 kDa, whereas the molecular mass of mammalian IgG heavy chain is approximately 50 kDa (Warr et al., 1995). The greater molecular mass of IgY is due to an increased number of heavy-chain constant domains and carbohydrate chains. In addition, the hinge region of IgY is hydrophobic than IgG (Davalos–Pantoja et al., 2000).

With regard to function, four important differences between IgY and IgG need to be considered. Firstly, IgY does not bind to protein A or G, an important feature of IgG that allows simple IgG isolation. However, there are several procedures equally simple for IgY isolation (Schwarzkopf, 1996). Secondly, IgY does not bind the rheumatoid factor (RF). IgG molecules often result in false positives due to their interaction with RF in immunoassays (Davalos-Pantoja et al., 2000). Thirdly, chicken egg-yolk immunoglobulin does not interfere with mammalian IgG; and finally, they do not activate mammalian complement (Carlander et al., 2000). These differences bring great advantages to the applications of IgY technology in many medical areas, such as xenotransplantation (Fryer et al., 1999). Chicken antibodies have been used in many recent diagnostic applications, including detection of gastric cancer (Noack et al., 1999), determination of hepatocyte growth factor (HGF) in serum and urine (Ohinishi et al., 2000) and for the detection of human serum antigens using surface plasma resonance (SPR) (Vikinge et al., 1998). The dot blot test to detect IgG and/or IgM against flagellar antigen of serovar Typhi can be used in laboratories in rural areas in developing countries where typhoid fever is endemic.
1.4 Fourier Transform-Infra Red (FTIR)

FTIR is one of the preferred methods of infrared spectroscopy. In infrared spectroscopy, IR radiation is passed through a sample; some infrared radiation is absorbed by the sample and some of it is transmitted. The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample. Like any fingerprint, no two unique molecular structures produce the same infrared spectrum. This makes infrared spectroscopy useful for several types of analysis.

FTIR spectroscopy is one of the several whole-organism fingerprinting techniques whose potential for simple, rapid and accurate characterization and identification of microorganisms has recently been investigated. FTIR spectroscopy measures the vibrations of chemical bonds within all the biochemical constituents of cells (i.e.) proteins, lipids, polysaccharides and nucleic acids; thus providing quantitative information about the total biochemical composition of the intact whole microbial cell (Helm, 1991). FTIR spectroscopy has been demonstrated to be a highly sensitive and reproducible method for microbial analysis and process control (Amiali, 2007).

Modern techniques like FTIR constitute radically different approaches for the identification of microorganisms. The IR spectra of intact bacteria provide highly specific patterns that allow microbial cells to be distinguished at different taxonomic levels and have frequently been employed for the rapid and accurate identification of microorganisms even at the strain level. FTIR is easy to implement; allows the analysis of small quantities of biomass; and requires no consumables or reagents (Bosch, 2008).
1.5 Plant extract against *Salmonella Typhi*

From the Stone Age, plant source has contributed a lot to human population in various aspects like food and shelter. The herbs have been used in medicine for a long time to control many diseases and pathogens. World Health Organization (WHO) too has not systematically evaluated traditional medicines despite the fact that they have been used for primary health care by about 80% of the world population (Kamboj, 2000).

Neem is a very well known tree which stands as an ideal example for its applications in medicine. Neem (*Azadirachta indica*) belongs to the family of *Melilaceae*, so also *Swietenia mahagoni*. Both these plants can be used to study the antibacterial potency particularly against *Salmonella Typhi*. The *S. mahagoni* has been used as traditional medicine in some countries for malaria treatment and other diseases. Few active compounds have also been isolated whose antimicrobial activity has been studied though only very few reports were available for the antimicrobial activity of *S. mahagoni* against *S. Typhi* (Ekimoto et al., 1991).