CHAPTER 2

Background and Literature Review

Typhoid fever had been known long time ago, but only until XVIII century name, clinical feature, pathogens were described clearly.

1793 Huxhmann recognized that some patients with diarrhea have prolonged fever and symptoms of nervous system, he isolated this group for research.

1804 Prost found out an ulcer in small intestine of the patients that had clinical symptoms like above.

1829. Louis (France) described clinical feature, pathology and named the disease typhoid fever.

1880 Graffky (Germany) isolated Salmonella typhi and found out biological characteristics of organism.

1886 Wright's initial study of vaccine began on intensive investigation lasting for nearly 60 years.

1890 Schattmuler (Germany) isolated organism from blood. It helped to diagnose early stage and also distinguish Salmonella typhi and para typhi A, B, C.

1896 Widal found out the coagulated serum from some patients that had recovered and he used this method for diagnosis and named it Widal.

1948 Chloramphenicol appeared and became the drug of choice in treating typhoid fever and helping to reduce mortality to ten times.

2.1 Organism.

2.1.1 Bio-chemical characteristics of Salmonella typhi.

Salmonella typhi is negative gram bacillus-bacteria of salmonella group, movement and belong to Enterobacteriacae family.

They ferment an aerogenic glucose, manitol, sorbitol, and non-lactose, saccarose, adonitol, salixin- fermenting. They do not produce indol and do not have urease for creating H_2S. They have lysin decarboxylase enzym, do not create axeton and can not grow in environment with KCN (cyanua kali).

2.1.2. Construction of Salmonella typhi.

1974 Costeron and Colleagues [6] determined the construction of outer membrane of Salmonella typhi. It had two layers.

- The interlayer is cytoplasma layer. It is constructed by protein and phospholipid. The function of this layer is the translation of needed substance for vitality and exchanging some factors that constructed outer membrane with outside.
- The outerlayer is constructed by phospholipid, special proteins and lipopolysaccharide.
- There is another layer in the middle. It contains peptidoglycan that makes the membrane rigid, and stick with outerlayer by lipoprotein.

Through the components of outer membrane people has determined antigen of Salmonella.

2.1.3 *Construction of antigen of Salmonella typhi*

- Antigen O (somatic antigen) is constructed by glucido-lipido-polipeptide and presented at all species of Salmonella. It relates to endotoxin. It is stable with t^0 (100^0C) and alcohol, but easy to be ruined by formalin 0.5%.

- Antigen Vi is only present in Salmonella typhi and para typhi C. It is capsular polysaccharide antigen and covers antigen O.

- Antigen H (flagellar antigen) is one protein that is sensitive with t^0 and easily ruined by t^0 and alcohol. But it is stable with formalin 5%.

Nowadays with the improvement of high technology some proteins located on outer membrane have found to be important antigens that used in serodiagnosis of typhoid fever, such as: 50 kDa OMP , Baber protein....

2.2 Diagnosis of typhoid fever.

2.2.1 Isolation:

Right now the gold standard of diagnosis of typhoid fever is still the isolation of bacteria from blood, stool, urine, bone marrow, other body fluid. But the isolation can only be done in big hospitals with good laboratories and the results are still low: the rate of positive blood culture in epidemic of Kien Giang province in 1993 was 50.7% and stool culture was 32,4%. In Long Phu the blood culture rate was only 15%, stool culture was 0% [(4)]. According to study of Gilman,R.H: the rate of isolation was 40% [(7)]. The reason of low results of isolation is that it is influenced by so many factors, such as:

- Isolated point time.
- Stage of disease.
- The use of antibiotics before entering hospitals.
- Quality of technicians.
- Quality of equipment.
- Quality of isolated environment and chemical agents.

Besides the diagnosis by isolating organism, people had found out many other methods to help in diagnosing typhoid fever.

2.2.2 Other methods.

Widal is the oldest test in serodiagnosis of typhoid fever . The performance of Widal test is based on the reaction between antibody with antigen H, O. The results are positive when the result of the second time increases four times compared to the first or single time but can meet the standard titer. Standard titer depends on each country or each area. In Vietnam Widal test is considered to be positive when titer of O, H is 1:100 [4]. In Bangladesh the Widal is considered positive when O and H agglutinin titers > 1:80 and > 1:160.[8]. Right now Widal test is still the most commonly used in many countries, especially in developing countries where there are lack of high technology and knowledge. Many studies were done to re-evaluate the value of Widal test in diagnosis of typhoid fever.

Chen SK, Crur Ms assessed the diagnostic value of Widal test in patients with typhoid fever. The study showed that: a single Widal test is useful in the diagnosis of typhoid fever in Singapore.[9]

- A Quino KL and Lansang MA. evaluated the usefulness of single Widal test in an endemic area and the results of sensitivity was 61% and specificity was 88%.[10]
- Rai GP and Zachariah.K. compared the diagnostic value for typhoid fever between Widal test and indirect fluorescent antibody test. The study showed that the indirect fluorescent was better than Widal test.[11]
- Saha SK and Ruhulamin M assessed the Widal test in diagnosing typhoid fever of children age between 1-10 years in Bangladesh. The result showed that Salmonella typhi O and H agglutinin titers > 1 : 80 and > 1:160 were considered to be significant with 88% sensitivity and 98% specificity. This study suggested that for children in an endemic areas, a positive Widal test is considerable important in diagnosing typhoid fever. Furthermore, negative results should be interpreted with caution and both the agglutinins must be considered equally important.[8]
- The study of re-evaluation of the Widal agglutination test in response to the changing pattern of typhoid fever in highlands of Papua New Guinea by Clegg A, Passey M.in 1998 showed that: the O cut-off titre greater than or equal 40 was appropriated for this population. But in 1992 new O antibody titer of > or = 160 was recommended as a diagnostic titer for typhoid fever in Papua New Guinea.[12]

Koeleman JG conducted a study to determine the diagnostic value of Widal test in non-endemic country. The results showed that: routine use of the Widal test is of limited value and should only be used for patients in whom repeated culture remain negative

However people try to find other test to replace Widal because of it's many limitations.

After the Widal test plenty following tests studies were:

- ELISA
- RIA
- Hemagglutination assay
- Coagglutination
- DNA probes for detection of salmonella in blood.
- Latex agglutination
- Indirect hemagglutination with lyophilized cells.

Although these tests have high sensitivity and can process many samples at the same time, their uses have been restricted to relatively large hospitals and laboratories because of equipment requirement.

The dot enzyme immunoassay (typhi dot) is a qualitative antibody detection test for diagnosis of typhoid fever and is a product of 8-year research in Malaysia. It detected serum antibody to specific antigen (50KD) of Salmonella typhi dotted on nitrocellulose membrane.

The 50 kDa outer membrane protein (OMP) of Salmonella typhi was isolated via preparative sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and electroelution. SDS-PAGE was performed under reducing condition using the discontinuous buffer systems with a vertical slab electrophoresis unit. The stacking and separating gels contained 4.5% and 9% acrylamide respectively. Each preparative

gel was loaded with 500 µg of OMP and was run at a constant current setting of 25 mA per plate at 4^0 C for 4 hours. The separated OMPs were stained with Coomassie blue, and the molecular weights were established with molecular weight markers of 14.4-94 kDa. The location of the 50 kDa OMP was determined and the band of interest was excised and minced to small pieces. The 50 kDa OMP was then eluted from the gel pieces via electroelution with a mini electroeluter unit consisting of glass tubes fitted with membrane caps with a molecular weight cut-off 15 kDa..

Electroelution was performed at 10 mA per tube for 6 hours in an elution buffer containing Tris (0.3%), glycine (1.44%), and SDS (0.1%). The eluted protein was collected from the membrane caps and further concentrated by precipitation with two volumes of iced-cold ethanol, overnight, at 4^0C. The precipitated proteins were pooled, resuspended in 0.03 M tris and stored at – 20^0C until required.

A nitrocellulose membrane, 0.5 cm by 1 cm, was used. 1 µl containing 0.03 µg of the extracted 50 kDa OMP was dotted on to the nitrocellulose using a microsyringe and allowed to dry. The strips were dipped in to blocking buffer (3% skimmed milk, 0.9% NaCl, 10 mM Tris-HCl, pH 7.4), and placed on a rocker platform for 30 minutes, at room temperature. The blocked strips were rinsed 3 times for 15 minutes with 0.15 MNETG buffer (150 mM NaCl, 50 mM Tris-HCl, 5 mM EDTA and 0.25% gelatin), allowed to dry and keep at 4^0C until use.

The typhi dot test was evaluated in Pakistan, Malaysia, Philippines, India…

- Asma Ismail, Ong kok Hai and Zainoondin conducted a study to determine the presence of a specific antigenic protein on the outer membrane of Salmonella typhi. The results indicated the usefulness of specific antigen in the development of serodiagnostic

test for typhoid fever since antibodies of both IgM and IgG class responses were obtained (Proceeding of the South East Asia Seminar on Immunology and Workshop on Biotechnology, USM, Feb 2 – 10, 1992).

- Javaid Usman, KA karamat, Tuhizaziz Ahmed evaluated the diagnostic value of typhi dot test. The results showed that: the sensitivity and specificity was above 90% (Second International Biennial Conference of Pakistan Society for Microbiology Burban, Pakistan,1997).
- Essa M.Abdullah, Sharia tahassum, Farhan Essa conducted study with consideration of practical value to compare the efficacy of the typhi dot with the old Widal test. Results indicated that the typhi dot had predictive value of over 95% when both IgM and IgG were seen, gave results often as early as 1-3 days of fever, especially with IgM antibodies, while the Widal was positive in 40.3% of these patients with titers especially of " H ''seen not earlier then 5 th day of fever.(Second International Biennial Conference of Pakistan Society for Microbiology Burban, Pakistan,1997).
- In Philippines M.Lu-Fong, A.C Ludan.... They evaluated the diagnostic value of typhi dot test. The results indicated that Dot EIA has a sensitivity of 100% and specificity of 87,7%, positive predictive value of 62,3%, negative predictive value of 100%.[13]
- Other study was conducted by K.E.Choo, T.M.E.Davis, A. Ismail and K.H.Ong to see the longevity of antibody responses to a Salmonella typhi- specific outer membrane protein. The results showed that mean persistence of IgM dot EIA positive was 2.6 months (95%CI=2.0-3.1months) and that of IgG was 5.4 months (4.5-6.3 months)[14]

With the usefulness of typhi dot test, it is more implemented in many countries, especially developing countries where typhoid fever is still common. It is expected that can show the diagnostic value of typhi dot test for typhoid fever in Vietnamese patients. In Vietnam because of the limitation of technique, equipment and knowledge, the physicians in many areas are still making decision for treating typhoid fever based on clinical symptoms.

2.3. Clinical manifestation:

Le Dang Ha and college studied clinical manifestation of 203 typhoid fever cases with age from 5 to 64 years old. The results showed that:

- Fever: 100%.
- Abdominal ileus 78.3%
- Diarrhea: 82.3%
- Hepatomegaly: 71.4%
- Splenomegaly: 57.6%
- Rose spots: 44,8%
- Headache: 47.3%
- Complication: 22%
  + Heart complication: 12.3% in children, 2.1% in adult.
  + Intestinal bleeding: 6.8% in adult, 3.5 in children
  + Acute renal failure: 1.8% in children, 2.1% in adult
  + Intestinal perforation: 0.7%

2.4 Treatment of typhoid fever

In 1948 with the implementation of Chloramphenicol in treating typhoid fever, the mortality, complication and duration of fever reduced a lot. It was the first drug of choice for treatment of typhoid fever for long period. After Chloramphenicol, Cotrimoxazol and Ampicillin were implemented. Step by step Salmonella typhi resistant to Chloramphenicol, Cotrimoxazol and Ampicillin have occurred.

Table 2: Status of resistance of Salmonella typhi in Clinical and Research Tropical Diseases Institute from 1988 to 1994

Antibiotics	1988 n=78	1990 n=102	1993 n=139	1994 n=524
Chloramphenicol	45%	49%	75%	84%
Ampicillin	0%	3%	68%	79%
Sulfamethoxazole		46%	79%	86%
Trimethoprim		2%	71%	82%
Ceftriaxone	0%	0%	0%	0%
Quinolone		0%	0%	0%

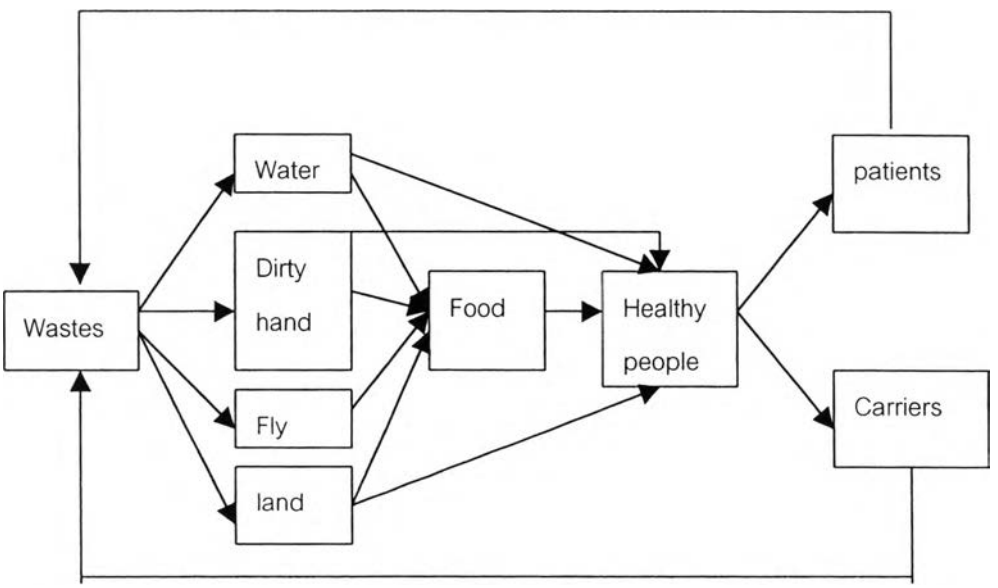
EI Sherbini A 1991 [15]; P.S. Rao [16] 1993 met 80% Salmonella resistant to Chloramphenicol and Cotrimoxazol.

Right now the drug of choice in treating typhoid fever is Ciprofloxacin (30-50mg/kg/day x 5-7 days or 14 days) and Cephalosporine with third generation (100mg/kg/day x 7 day).

- Gu XJ, Zhang MF, Wang F. conducted a study of efficacy of Ofloxacin in treating typhoid fever and the results showed that: 100% patients were cured.[17]
- Hajjim, Elmdaghrin… evaluated the efficacy of Ciprofloxacin with dose 400mg x2/day x 14 days. The conclusion was: 100% cure without experiencing of relapse or becoming a Salmonella carrier.[18]
- Ti Ty, Moterio EH, Lam S. had used Ceftriaxone for treatment of typhoid fever with dose was 50-60mg/kg/day. The results concluded that mean period of defervescence was 4 days, the advantages were rapid clinical response, short course of treatment and lack of serious adverse drug reaction.[19]

2.5 Prevention:

Figure 1 : ***The ways of infection of Salmonella typhi***



To prevent typhoid fever we need to do:

- Improvement of hygiene, sanitation.
- Health education.
- Changing the behaviors.
- Vaccination for population in endemic areas.