INTRODUCTION

Typhoid fever Is an acute systemic infection caused by the bacterium Salmonella enterica serovars typhi. Salmonella enterica serovars paratyphi A, B and C cause the clinically similar condition, paratyphoid fever. Typhoid and paratyphoid fevers are collectively referred to as enteric fevers. In most endemic areas, approximately 90% of enteric fever is typhoid ***(Parry, 2006),***According to the most recent nomenclature adopted by the Center for Disease Control and prevention (CDC), the genus Salmonella contains only two species, Salmonella enterica and Salmonella bongori ***(CDC: Salmonella surveillance, the annual surveillance, 2006).*** The incidence of typhoid fever, however, varies substantially between countries. High incidence estimates (more than 100 cases per 100.000 inhabitants per year) were calculated in south central Asia and south-east Asia while low incidence (less than 10 cases per 100.000 Inhabitants per year) was reported in Europe, Australia and New Zealand and North America ***(Nadi et al., 2009).*** In Africans countries, estimates have been difficult to calculate, in part due to the use of antibody testing as a measure of infection detection that may result in false positive episodes ***(Mweu and English, 2008).***In Egypt, the reported cases are far below the real one because many cases are treated in private practice without notification. It's estimated that one third of a million

cases occur annually ***(Abdel-Wahab et al .,2004).*** Current estimates of typhoid fever incidence are derived from case reports received from passive hospital-based surveillance, without laboratory confirmation of the disease. In the year 2000, the estimated Incidence of typhoid In Egypt was 15 cases per 100.000 persons per year ***(Padmini et al., 2006).*** However, this estimate may not reflect the true incidence of disease, as less than 1% of these cases were much lower than initially anticipated animal and human infections in developing as well as industrialized countries ***(Crump et al., 2003).***

Typhoid is usually contracted by ingestion of food or water contaminated by fecal or urinary carriers excreting S. enterica serovar typhi. In addition, these bacteria can survive for prolonged periods in water, ice, dust and dried sewage and these may become sources of infection. Transmission of Typhoid has also been attributed to flies, laboratory mishaps, unsterile instruments and anal intercourse. ***(Parry, 2006)***

Osier, 1892 wrote a compelling description of typhoid fever, He described the rose-colored spots and splenomegaly[5] and emphasized the temprature-pulse relationship (relative bradycardia) and apathetic face as key features of the disease. Symptoms may include headache, diarrhea or constipation and abdominal pain. Other non-specific complaint includes chills, loss of appetite, cough, or mayalgia ***(Patel et al, 2010).***On

examination, patient may have fever, coated tongue, bradycardia (less than 50% of cases), Rose spots (in 30% of cases). Other findings include splenomegaly, hepatomegaly, and mental status changes ***(Kuvandik et ai, 2009).***

Blood test findings may be nonspecific with relative neutropenia, lymphopenia, thrombocytopenia, and increased C-reactive protein, and increased erythrocyle sedimentation rate, Abnormal liver function tests could be observed ***(Kuvandik et al., 2009).***The lack of specific clinical signs complicates the diagnosis of typhoid, which must be distinguished from other endemic acute and subacute febrile illnesses as malaria., deep absceses, tuberculosis, amoebic liver abscesses, encephalitis,, influenza, dengue fever, infectious mononucleosis, Infectious hepatitis, toxoplasmosis, and brucellosis ***(Wain et al, 2005).***

Salmonella spp. may be isolated from blood, bone marrow aspirates, urine, stool and other sterile sites. Blood or stool cultures followed by conventional microbiological identification and serology are the mainstay salmonella infection testing***(World Health Organization, 2003).*** Blood cultures have low sensitivity as only 40-60% are positive in enteric fever cases, it: contrast, the sensitivity of bone marrow aspirates cultures is more than 80%, making this type of culture the gold standard for diagnosis of enteric fever ***(Baker et al., 2010 and***

Parry et al., 2011).Stool cultures are positive in only 30-35% of cases. The urine culture sensitivity is also low (7-10%). Blood culture is the corner stone of definitive diagnosis of acute typhoid but serological tests still widely adopted in all fever hospitals in Egypt ***(Abdelwahab et al, 1999).***

Serological tests have been used for the diagnosis. The Widal test, utilizing a suspension of killed S.typhi as antigen to detect serum antibodies against the flagellar and somatic antigens, has a controversial role in diagnosis as it has a low sensitivity and specificity and requires two samples taken approximately 10 days apart. The diagnosis based only on Widal test is frequently inaccurate as false positive and false negative results are common ***(Olopoenia et al., 2000 and Wain et al., 2008).*** In contrast, rapid diagnostic testing using PCR stool and blood specimens were reported to have high specificity and sensitivity ***(Hatta et al., 2007;Hatta et al., 2008 and Gomez-Duarte et al., 2009),*** but they are too expensive to be used for the diagnosis. Recently, it was reported that specific antibodies have been demonstrated in saliva from patients with viral, bacterial and parasitic infections. Sampling for salivary antibodies can be done conveniently with modest training and involves simple and cheap equipments. Salivary immunoglobulin A (IgA) against the lipopolysaccharide antigen (LPS) of S.typhi has been measured in some studies ***(Herath et***

al., 2003).From these preliminary data on adults this technique appeared to hold considerable promise for diagnosis of typhoid. A recent study suggests that this method has the potential to replace the more invasive traditional serological techniques for the diagnosis of typhoid, particulary in developing countries where bacterial culture facilities are limited ***(Zaka et al., 2012***).

AIM OF THE WORK

Compare the sensitivity and specificity of salivary and serum anti-salmonella typhi lipopolysaccharide IgA with the widely used Widal test for serodiagnosis of typhoid fever.

AETIOLOGY

Salmonellais a genus of rod-shaped, gram- negative, non-spore forming, predominantly motile enterobacteria with diameters around 0.8 to 1.5um, lengths from 2 to 5 um, and peritrichous flagella, (flagella that are all around the cell body). They are chemoorganotrophs, obtaining their energy from oxidation and reduction reactions using organic sources, and are facultative anearobes***(Clarkand Barret, 1987).***

The bacterial genus Salmonella is divided into two species, Salmonella bongori and S.enterica. S. enterica itself is comprised of six subspecies: they are S.entrica subsp. enterica, S.enterica subsp.salamae, S. enterica subsp.arizonae, S.enterica subsp.diarizonae, S.entrica subsp.indica, and S.entericasubsp houtenae, or I, II, IIIa, IIIb, IV, and VI, respectively. ***(Popoff and Minor, 1997).***

Both Salmonella species and subspecies are serotyped for further identification***(CDC, Salmonella surveillance, 2006).***The only types of strains of Salmonella spp that cause clinical syndromes in human subjects, typhoid Salmonella and non-typhoid Salmonella(NTS). The former species are the causative agents of enteric fever and they include S.enterica subsp.enterica, serotypes Typhi and Paratyphi (Salmonella Typhi and Salmonella Paratyphi). The latter group includes the remaining strains(Table1).

Table 1: Comparison between typhoid Salmonella and non-typhoid Salmonella infections.

Features	Typhoid Salmonella	NTS*	References
Serotypes	S.Typhi, S.Paratyphi	Remaining strains	***(Crump, and Mintz.2010)***
Reservoir	Humans	Animals	***Dion, Ikumapayi., et.al.2011)***
Transmission	predominantly Water	Predominantly food	***(Linam, and Gerber.2007)***
Location	Developing countries	World wide	***(Hardy.2004)***
Disease	Systemic	Local or systemic	***(Khan, Ochiai., et al.2010) (Huang, and DuPont.2005)***
HIV Infection risk	No higher risk	Increased risk	***(Kariuki.2008) (Gordon, Banda, et al.2002)***
Carrier rate	1-4%	<1%	***(Gonzalez-Escobedo, Marshal., et al.2011)***

*NTS : Non- typhoid salmonella.

Salmonella is found worldwide in both cold-blooded and warm-blooded animals, and in the environment ***(Ryanand Ray, 2004).***

Salmonella infection are zoonotic and can be transferred between humans and animals ***(Rothschild, 2011).***

History

The genus Salmonella was named after Daniel Elmer Salmon, an American veterinary pathologist. While Theobald Smith was the actual discoverer of the type bacterium (Salmonella enterica var.Choleraesuis) in 1885, Dr.Salmon was the adminstrator of the USDA research programm, and thus the organism was named after him by Smith. ***(FDA-Center for food safety and applied nutrition.2008***).

Smith and Salmon had been searching for the cause of common hog cholera and proposed this organism as the causal agent. Later research, however, would show this organism(now known as Salmonella enterica) rarely cause enteric symptoms in pigs. (***http :// www. cgmh. org. tw/ chldhos/ intr/ c4a00/ academy/ bugs/ salchole. html),*** and was thus not the agent they were seeking(which was eventually shown to be a virus). However, related bacteria in the genus Salmonella were eventually shown to cause other important infectious diseases. The genus Salmonella was finaly formally adopted in 1900 by J.Lignie`res for the many species of Salmonella, after Smith`s first type-strain Salmonella cholera ***(Kauffmann, 1941).***

The disease has received various names, such as gastric fever, abdominal typhus, infantile remittant fever, slow fever, nervous fever or pythogenic fever. The name"typhoid" means

"resembling typhus" and comes from neuropsychiatric symptoms common to typhoid and typhus ***(Oxford English Dictionary, 2011***). Despite this similarity of their names, typhoid fever and typhus are distinct diseases and are caused by different species of bacteria ***(Cunha, 2004).***

Salmonella nomenclature

Initially, each Salmonella "species" was named according to clinical considerations, e.g., S.Typhimurium(mouse typhoid fever). After it was recognized that host specificity did not existfor many species, new strains(or serovars, short for serological varients) received species names according to the location at which the new strain was isolated.. Later, molecular findings led to hypothesis that Salmonella consisted of two species***, (Leand Popoff, 1987***).

S.enterica, and the serovars were classified into six groups ***(Reeveset al., 1989***), two of which are medically relevant. But as this now formalized nomenclature ***(Grimont et al., 2005***) is not in harmony with the traditional usage familial to specialists in microbiology and infectologists, the traditional nomenclature is common. Currently, there are two recognized species: S.enterica, and S.bongori. In 2005 a third species was thought to be added Salmonella subterranean, but this has since been ruled out and is seen as another serovar ***(Agbajeet al., 2011).***

The serovar (i.e. serotype) is a classification of Salmonella into subspecies based on antigens that the organism presents. It is based on the Kauffman-White classification scheme that differentiates serological varieties from each other. Serotypes are usually put into subspecies groups after the genus and species, with the serovar Typhimurium. Newer methods for Salmonella typing and subtyping include genome-based methods such as pulsed field gel electrophoresis (PFGE), Multiple loci VNTR Analysis(MLVA), Multilocus sequence typing (MLST) and (multiplex-) PCR- based methods ***(Porwollik, 2011&Achtmanet al., 2012).***

Genome structure:

The genome for Salmonella typhi has been completely sequenced. There are about 204 pseudogenes encoded in Salmonella typhi. A majority of these genes have been activated by a stop codon, which shows that the genes were recently modified due to evolutionary changes. Seventy five are involved in house keeping functions and 46 of gene mutations havetodo with host interaction.

There are two commonly used strains of Salmonella typhi, CT18 and Ty2. Salmonella typhi CT18 has a large circular chromosome consisting of 4.8 Mb and two plasmids, p HCM1, and p HCM2, one of which has multiple drug resistance (p

HCM1). Salmonella typhi Ty2 has one large chromosome that is 4.7 Mb and unlike CT18, it does not have plasmids and can be affected by antibiotics. In fact, the current vaccine was developed using S.typhi Ty2. Out of the 204 pseudogenes in Salmonella, 195 genes are the same in both strains CT18 and Ty2, making them 98% identical ***(Denet al., 2003&Parkhill, et al., 2001).***

Cell structure and metabolism

Salmonella typhi is a rod-shaped, gram negative bacteria that contains features that separates itself from other types of bacteria which include: having two membranes (an outer and an inner), periplasm, and a lipopolysaccharide chain that consists of &-d-galactosyl-(1-2)-&-d-mannosyl-(1-4)-1-rhamnosyl-(1-30- repeating units, and has short branches of single 3, 6-dideoxyhexose residues ***(Kitaet al., 1973).***

Salmonella typhi has a complex regulatory system, which mediates its response to the changes in its external environment. Sigma factors, which are global regulators that alter the specificity of RNA polymerase, are examples of such regulation. Some sigma factors direct transcription to produce stress protiens, which increase the chances of the bacteria surviving environmental changes. RNA polymerase S is produced in response to starvation and changes in pH and temperature. It also

regulates the expression of up to50 other protiens and is also involved in the regulation of virulence plasmids. In order to survive in the intestinal organs of its hosts where there are low levels of oxygen, Salmonella typhi has to be able to learn to use other sources other than oxygen as an electron acceptor. Therefore, Salmonella has adapted to grow under both an aerobic and aneorobic conditions. Salmonella's most common source of electron acceptors is nitrogen. Examples of other electron acceptors are: nitrate, fumarate, and dimethyl sulphoxide. Global and specific regulatory systems of anaerobic gene expression, like the ones mentioned above, are implemented to make sure that the most energetically favorable metabolic process is used. Evidence showsthat the availability of oxygen is an environmental signal that controls Salmonella's virulence ***(Contreraset al., 1997).***

Enteritis Salmonella and Typhoid/ Paratyphoid Salmonella, because of a special virulence factor and a capsule protein (virulence antigen) can cause serious illnesses, such as Salmonella enterica subsp.enterica serovar typhi. Salmonella typhi is adapted to humans and doesn`t occur in other animals.***(Jantsch et al., 2011).***

Salmonella bacteria can survive for weeks outside a living body, they are not destroyed by freezing. ***(Sorrells, et al., 1970).*** Ultraviolet radiation and heat accelerate their demise; they perish

after being heated to 55°C(131 F) for 90 min, or to 60°C (140 F°) for 12 min ***(Goodfellow and Brown, 1978).***

Salmonella invasion:

A remarkable characteristic in salmonella pathogenesis is the invasion of non-phagocytic cells. Salmonella will penetrate into the intestinal epithelial cells by inducing their own uptake, in a complex and active process that morphologically resembles phagocytosis ***(Hansen-Westeret al., 2002&Takayaet al, 2003).***Virulence genes involved in invasion and required for intracellular survival are clustered in large chromosomal DNA regions designated Salmonella pathogenicity islands (SPIs). ***(Gerlachet al., 2007&GrassiandFinlay, 2008***).

SPI-1 and SPI-2 encode type III secretion systems, consisting of multiprotein complexes that build a contigous channel across both the bacterial and epithelial cell membranes, resulting in efficient traslocation of bacterial effectors directly into the epithelial cell cytoplasm. The secreted effectors interact with eukaryotic proteins to activate signal transduction pathways and rearrange the actin cytoskeleton and lead to membrane ruffling and bacterial engulfment. ***(Gala`n, and Wolf-Watz, 2006&Winnenet al., 2008&Dieyeet al., 2008)*** Once inside the host cell, these effectors are capable of alerting host cellular functions, such as cytoskeletal architecture, membrane

trafficking, signal transduction, and cytokine gene expression that result in bacterial intracellular survival and colonization.***(Kichi etal., 2004&Haraga et al., 2008).***

Intracellular survival

Salmonella spp. can infect both warm and cold- blooded hosts; this wide rang reflects the ability of this pathogen to sense and adapt to a range of different environments, including the interior of macrophages ***(Humphreyset al., 1999; Prostet al., 2007&Prostand Miller, 2008).***

Intracellular persistance in host cells is critical for pathgenesis, because strains defective in this property are avirulent ***(Raupachet al., 2003; Coburnet al., 2005&Coburnet al., 2007)***. Following invasion of host cells, Salmonella localize within a membrane compartment known as the Salmonella-containing vacuole ***(Bakowskiet al., 2008).***

The bacteria actively remodel this compartment and establish a niche where they are capable of survival and replication ***(Fieldset al., 1986&Ramsdenet al. 2007).*** The capacity of virulent S.Typhi to avoid fusion of Salmonella-containing vacuoles with dendritic cell lysosomes is the mechanism likely responsible for evasion of killing ***(Finket al., 2007).***