ASSESSMENT OF LABORATORY DIAGNOSTIC TESTS AND ANTIMICROBIAL TREATMENT OF TYPHOID AT THE UNIVERSITY OF NAIROBI HEALTH SERVICES

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DECLARATION

I certify that this dissertation is my original work and has not been presented for a degree in any other University.

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ABBREVIATIONS

DNA	Deoxy ribonucleic acid
KNH	Kenyatta National Hospital
MDR	Multi drug resistant
PCR	Polymerase Chain Reaction
QRDR	Quinolone Resistant Determining Region
S. typhi	Salmonella enterica serovar Typhi
SPSS	Statistical package for social sciences
UHS	University Health Services
UON	University of Nairobi

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ABSTRACT

Typhoid is a systemic disease caused by *Salmonella enterica* serovar typhi. It causes significant morbidity and mortality especially in developing countries with estimated 12 to 33 million cases and 200,000 to 600,000 deaths annually. Proper diagnosis of the disease will enable clinicians to initiate the right treatment thereby contributing to the well being of the patient and minimizing the development of antimicrobial resistance. It will also give epidemiologists accurate data on the local prevalence of typhoid.

Main Objective: Assessment of laboratory diagnostic tests and antimicrobial treatment of Typhoid at the University of Nairobi Health services.

Study Design: Retrospective study

Study Site: University of Nairobi health services, off State House road, Nairobi.

Study Population: All patients sent to the University of Nairobi health services laboratory for typhoid diagnostic tests from January 2010 to Dec 2010.

Methodology: The laboratory and medical records of 384 randomly selected patients were analyzed. Information of laboratory tests done and antimicrobial treatment given was abstracted from laboratory records and patients files. This information was documented on serialized data collection forms.

Results: The Widal test was the most commonly used in the diagnosis of typhoid at 95.55%. Stool culture was 3.40%, blood culture 0.5% and Salmonella stool antigen 0.1%. Antimicrobial treatment was given at all levels of Widal titres from O negative H negative to O1:160 H 1:160 (78.87%) and at O 1:320 H 1:320 (21.12%). Quinolones (especially ciprofloxacin) were the mostly used antibiotics (52.11%) then cephalosporins (28.16%) and penicillins (19.71%)

Conclusion: The high utilization of widal test coupled with the poor interpretation of the results and the low use of cultures can lead to inappropriate antimicrobial treatment and worsening of multidrug resistant *Salmonella enterica* serovar Typhi.

Recommendations: Urgent designing and implementation of a policy targeting health care providers within the University of Nairobi Health Services on rational utilization of laboratory diagnostic tests of typhoid and antimicrobial treatment.

CHAPTER 1: INTRODUCTION

Typhoid is a systemic illness that is caused by a bacterium, *Salmonella enterica* serovar Typhi and is spread through ingestion of contaminated food or drinks[1]. Typhoid has a wide range of symptoms and is usually confused with other infections[2]. These symptoms include fever, abdominal pains, constipation, generalized body aches and diarrhea. Infections that can be confused with typhoid are many and include acute bacterial infections, viral infections and parasitic infections like malaria[2].When patients in developing countries including Kenya visit healthcare facilities, laboratory tests for typhoid are often requested before initiation of antimicrobial treatment.

Culture of different specimens like blood, bone marrow, stool and urine is considered the gold standard of diagnosis. However, the widal test is most commonly done due to lack of resources[3,4] to do the cultures.

Proper interpretation of widal test before initiation of antibiotic treatment is very important because patients may be repeatedly sent for the test and subsequently receive several courses of antibiotics due to persistent or high widal titres[4].

Some infections may be associated with false positive widal test for example beta hemolytic *Streptococci*, *Treponema pallidum*, other intestinal bacterial infections (enterobacteriaceae) and brucellosis[5]. Non infective conditions like rheumatoid arthritis, ulcerative colitis and nephrotic syndrome can also produce false positive widal tests[2].These emphasize the need for proper widal interpretation as every inappropriate antimicrobial treatment worsens the antimicrobial resistance which was detected from the early 1980s[6,7,8].

The widespread availability of unprescribed antibiotics in the developing countries together with inappropriate typhoid antimicrobial treatment[9] both need to be reduced to stem the potential worsening of antimicrobial resistance. This is important so as to prevent more expenses in use of fewer and less available antibiotics[10].

This study was done in a setting where both widal and culture facilities are available. There was an assessment of the tests done and the drugs given. The outcome of the study

will help to influence policy to target healthcare providers on appropriate diagnostic and treatment strategies for typhoid.

CHAPTER 2: LITERATURE REVIEW

Typhoid is caused by the gram negative bacillus, *Salmonellae enterica* serovar Typhi. Salmonella is one of the genera under the family Enterobacteriaceae.

Salmonella enterica serovars are defined on the basis of the somatic (also called the cell wall or O antigen) (lipopolysaccharide) and flagellar or H antigens (Kaufmann White scheme)[1] The capsular antigen is called the Vi antigen[1].

Global impact and epidemiology

Typhoid fever continues to be a major public health problem in many developing countries. Globally it is an important cause of morbidity and mortality especially in endemic countries[1]. According to recent estimates, typhoid fever causes 216,000 deaths and 22 (range 16 to 33) million cases per year .This is predominantly in school age children and young adults[11].

Regions with high incidence of typhoid fever, >100/100,000 cases per year include South East and South Central Asia. Those with medium incidence, 10-100/100,000 cases per year include the rest of Asia, Africa, Latin America, Oceania except Australia and New Zealand. Low incidence typhoid fever (<10/100,000 cases per year) is found in Europe, North America and the rest of the developed world[11].

Africa has been shown to have lower rates of typhoid compared to South East Asia and South Central Asia.This is a paradox considering that socio-economic indices including known risk factors for typhoid like provision of safe drinking water and sanitation are much lower in most parts of Africa[12]. However population based surveillance showed incidence rates in Africa comparable to those reported in urban slums in Asia .The rate was highest in the youngest age groups 2-4 years of age(adjusted incidence >2000 per 100,000 per year)[13].

In developed countries, typhoid fever is a predominantly a travel related disease with greater risk following travel to the Indian subcontinent than to other areas[14,15].

Typhoid is an exclusively human disease and the bacteria are transmitted through contaminated urine or stool[1].Mary Mallon also known as Typhoid Mary, working as a

cook, was responsible for several outbreaks of typhoid fever in the United States in the early 20th Century while being asymptomatic[16].

Pathogenesis

Natural infection in enteric fever occurs by ingestion followed by penetration through the intestinal mucosa. Disease production is dependent on several factors including number of organisms swallowed, state of gastric acidity and possession of Vi capsular antigen by the organisms. The infecting dose that is large enough to produce disease is a dose of about 10,000 bacteria to 1,000 million bacteria. Possession of Vi capsular antigen is associated with increased infectivity. Gastric acidity is an important defense against enteric infections[1].

Once in the small intestinal lumen, the organisms multiply and penetrate the mucosa, then sub mucosa and are ingested by macrophages. They are then carried to the mesenteric lymph nodes where they multiply and enter circulation via the thoracic duct .This produces transient primary bacteremia and the organisms are carried to the liver and spleen where after more multiplication a heavier secondary bacteraemia is produced with onset of clinical symptoms. Infected bile renders stool cultures positive. Pre – existing gall bladder disease predisposes to chronic biliary infection, leading to chronic faecal carriage[1].

Invasion of the peyer's patches can occur during either the primary intestinal infection, or during the secondary bacteremia and further seeding occurs through infected bile. The peyer's patches become hyperplastic with infiltration of chronic inflammatory cells. Later, necrosis of the superficial layers leads to the formation of irregular ovoid ulcers along the long axis of the gut. When an ulcer erodes into a blood vessel, severe hemorrhage results and transmural perforation leads to peritonitis.[1]

Molecular basis of pathogenesis

To be effective pathogens, Salmonella must be able to invade epithelial cells and for organisms to cause enteric fever, they have to be adapted to survive in the cells of the reticulo-endothelial system. Genes necessary for invasion are encoded for in the Salmonella Pathogenicity Island 1 (SPI 1). Intracellular survival is controlled, by Salmonella genes, by a two component regulatory unit Pho Q / Pho R, which control the

activity of pag genes (genes A, B, C).The pag genes are expressed within the macrophage phagosome and are required for survival within it.[1]

Mechanism of immunity

Production of humoral immunity seems to play little role in recovery from acute infection as the patient often continues to deteriorate despite the appearance of O, H and Vi antibodies. Cell mediated immunity is probably the key factor in recovery. The ability of the Vi antibody to prevent infection is demonstrated by the efficiency of the Vi vaccine. However protection afforded by phenolized-killed vaccine, which does not contain the Vi antigen indicates the role of other antibodies. Local gut immunity may be important in preventing re-infection. Specific secretory IgG and IgA have been demonstrated in the gut. In the endemic countries, enteric fevers have the highest prevalence in the young, adults having acquired substantial immunity through previous exposure(s)[1].

Clinical manifestations

Diagnosis of typhoid fever on clinical grounds is difficult as many other febrile illnesses present similarly[2].Community based studies in areas of endemic disease show that many patients with typhoid especially children less than five years of age may have non specific illness that is not recognized as typhoid[17,18,19].

Patients present with varied manifestations depending on the stage of progression of the disease. The incubation period of Typhoid fever depends on the infecting dose of bacteria and ranges 3 to 56 days (average 10 to 20 days). In untreated cases of average severity, the duration of sickness averages about 4 weeks[1].

In the first week the features are non specific and include rising remittent fever, headache, malaise, constipation and non productive cough. In the second week, the patient looks apathetic and toxic with sustained high temperature, the abdomen is distended and splenomegaly is common. There is relative bradycardia. Pink papules (rose spots) can be seen especially on the lower chest wall and upper abdomen[1].

In the third week, fever persists and patients are more ill and a confusional "Typhoid" state sets in. Abdominal distension is more pronounced and diarrhea is common. Tachypnea, weak pulse and basal lung crackles can occur. Death can occur due to

overwhelming toxaemia, myocarditis, intestinal hemorrhage or perforation. Considerable weight loss is common. Intestinal complications may occur in the fourth week[1].

A study in Indonesia concluded that no symptom and sign combination could adequately be used clinically define a case of Typhoid[20]. A different study in Turkey showed that some clinical features and laboratory findings could be predictive of typhoid fever in absence of microbiological confirmation. These are hepatomegaly, splenomegaly, relative bradycardia, rose spots, thrombocytopenia and elevated aspartate aminotransferase[21]. All the same, clinical algorithms have not generally been validated[2].

Chronic or recurrent infections can occur in association with schistosomiasis infections as the bacteria are carried within the parasites protected by the body's immune defences[1].

Relapse and re infection

Between 10% and 20% of patients treated with antibiotics suffer a relapse after the initial recovery. This typically occurs a week or so after stopping therapy. A relapse is generally milder and shorter than the initial illness[1].The *Salmonella enterica* serovar Typhi isolate from a patient in relapse usually has the same antibiotic susceptibility pattern as the isolate obtained during the original episode. If re infection occurs, it can be distinguished from the original infection by molecular typing[22,23].

Carrier states

Fecal carriers

After clinical recovery, fecal cultures remain positive in a high proportion of the patients during the immediate convalescent period, but stools rapidly become negative. By the end of the third month,3% of patients will still be positive[1] . Between 1% and 3 % will continue to excrete organisms in their stools for more than a year-chronic carriers- and they are likely to remain so for the rest of their lives[1]. The incidence of chronic carriage is higher in women and the elderly and in patients with cholelithiasis[24].

Urinary carriers

Persisting urinary carriage is rare after the third month in the absence of urinary tract pathology. However in areas endemic with urinary schistosomiasis, it is common[1].

Laboratory diagnosis

There are various laboratory diagnostic tests for Typhoid. These include Widal and cultures of various specimens like blood, urine, stool, bone marrow and rose-spots. Newer tests are urinary Vi antigen assays, typhidot, tubex and polymerase chain rection[1].

Cultures

Blood culture is the gold standard but its utility in early diagnosis is limited in early phase of the illness making isolation difficult. Blood cultures are not done due to long waiting time and unavailability of microbiologic facilities[4]. They are also positive only in 40-60% of cases and when done early in the course of the illness (1st one week)[25]. Low sensitivity of the blood cultures especially in developing countries is due to widespread antibiotic availability and prescribing[9]. Sensitivity increases with amount of blood cultured and ratio of blood to broth[2].

Bone marrow cultures are 80 to 95 % sensitive [2].They are positive even when patients have been on antibiotic treatment for several days[26]. A high yield (60%) has been reported in rose spot cultures[1]. Stool cultures are 30 percent positive in patients with acute typhoid fever[2].They are positive within the first week and the positivity rises steadily thereafter[1,2] .Urine cultures are positive less often[1].

Widal test

Widal test was developed in 1896 by Georges Fernand Isidore Widal[27]. The Widal test measures antibodies against the flagellar (H) and somatic (O) antigen of the causative organism. In acute infection, the O antibody appears first, rising progressively, later falling and often disappearing after a few months. H antibody appears a little later but persists for longer. Rising or high O antibody titre generally indicates acute infection, whereas raised H helps to identify the type of enteric fever[1].

The Widal test is the one most commonly used diagnostic test in the developing countries[3].The test has been preferred due to unavailability of microbiologic facilities and long waiting time for culture results .Over reliance of the Widal test has led to the underutilization of cultures[4] and created an impression of high typhoid prevalence[28]. The role of Widal test has been used to increase the index of suspicion for the presence of

typhoid fever. This is by demonstrating a four fold increase in titres between the acute and convalescent phases of the infection, 7 to 10 days apart[29,30].

However, a fourfold increase in titers is not always demonstrable even in culture proven Typhoid[31,32]. A one to two or two to three fold increase is common[33,34]. This low titer increase can be due to lack of antibody response in immunosuppressed patients, early treatment by antibiotics, delay in obtaining an acute phase sample in the natural history of the disease and presence of high levels of background antibodies in a region of endemicity[25,31,32].

A Widal test interpreted as positive may be seen in apparently healthy persons in endemic areas as a result of a previous sub clinical infection[35]. In such areas, there is high prevalence of anti *Salmonella* antibodies in healthy adolescents and adults and a single Widal test will virtually not be of diagnostic assistance. High O and H titers in previously unvaccinated patients in non endemic areas are suggestive of typhoid fever. The same is true for children less than 10 years of age in an endemic area[36].

A positive Widal test can also mean non typhoid infections. Significant *Salmonella typhi* agglutinins have been detected in the sera of healthy individuals and patients infected with beta haemolytic *Streptococci, Helicobacter pylori, Treponema pallidum*, *Brucella* and *Toxoplasma* [5]. *Salmonella enterica* serovar Typhi shares O and H antigens with other Salmonella serotypes and also shares cross reacting epitopes with other enterobacteriaceae[2]. Vaccinations with typhoid vaccine may lead to persistently elevated H agglutinins and transient elevation of O agglutinins. All these can result in false positive results.

A negative Widal test can be associated with a positive culture. This occurs especially when the Widal test is done early in the course of the disease. Therefore the predictive value of the negative Widal test can be low[4].Some patients with typhoid may mount no detectable antibody response or have no demonstrable rise in antibody titre[2]. Effective treatment early in the disease may not necessarily be associated with reduced titer as high widal titers may persist and hence form biased basis of further treatment[4,37].

Sensitivity, specificity and predictive values of widal test varies greatly among geographic areas[2].The results of a single test tube dilution or slide agglutination are virtually not interpretable unless the sensitivity and specificity of the test for the specific laboratory and patient population are known[38]. Locally determined cut offs are important for proper interpretation of the widal test[31,40]. When a typhoid epidemic area becomes endemic over several years, it is important to re-set the widal cut off points again after re evaluating the community[31].

A study in 4 % of randomly selected groups of healthy individuals in Nairobi and Naivasha in Kenya showed at least 1:160 titres of both O and H antibodies[39]. The significant titres therefore in these areas will be O titres more than 1:160.

Polymerase chain reaction

Polymerase chain reaction (PCR) and probes have been developed and can detect *Salmonella enterica* serovar Typhi directly in the blood[41]. The serogroup and flagella antigens can be detected with 100% sensitivity and specificity[42]. However they are not widely used and are not practical in areas where typhoid is common[2].

Blood culture - PCR test

In this test, the level of *Salmonella enterica* serovar Typhi bacteria in a blood sample can be increased to a level where regular PCR can detect. This is by using 3 hour enrichment of *S typhi* in tryptone soya broth containing 2.4 per cent of ox bile. The whole blood culture - PCR assay takes 8 hours compared to several days of conventional blood culture[43]. This assay is superior in speed and sensitivity to both conventional blood culture and PCR tests. Its use allows early detection of causative organism and facilitates initiation of prompt treatment among patients with typhoid fever[43].

Vi Agglutination reaction

This is used to screen carriers and has 70 to 80 percent sensitivity and 80 to 95 percent specificity[2].

Antimicrobial treatment

Various antimicrobial agents have been used in the treatment of typhoid. These include the first line drugs like chloramphenicol, ampicillin, cotrimoxazole and amoxicillin.

Examples of quinolones used are ofloxacin, pefloxacin, ciprofloxacin and norfloxacin. Also useful are third generation cephalosporins like ceftriaxone, cefotaxime and cefoperazone. Azithromycin is also used.[1]

Increasing resistance to antibiotics treatment

Since the introduction of Chloramphenicol in 1948, antimicrobial resistance has progressively included many drugs. In the 1980s, strains resistant to chloramphenicol, ampicillin and cotrimoxazole (multidrug resistant, MDR) emerged. In the 1990s strains resistant to nalidixic acid and ciprofloxacin were detected and thereafter to extended spectrum cephalosporins.[6,7,8] .The resistance to quinolones, in most strains of serovar Typhi, has been attributed to point mutations in the quinolone resistance determining region (*QRDR*) of the chromosomes which codes for the enzymes Deoxy ribonucleic acid (DNA) gyrase A and B and DNA Topoisomerase IV. The single point mutations occurring in the QRDR of the gyr A gene characteristically occurs at position 83 of the DNA gyrase enzyme (changing serine to phenylalanine) and at position 87 (changing aspartate to tyrosine or glycine) [44,45]. Resistance to chloramphenicol, ampicillin and cotrimoxazole is due to plasmids.[47]

A plasmid transmissible among the Enterobacteriaceae has been characterized and is responsible for determining the phenotype of cephalosporin resistance. This plasmid encodes an extended spectrum beta lactamase[46].The prevalence of MDR *Salmonella enterica serovar Typhi* has been increasing in Kenyatta National Hospital from 50 -65% in 1997 -1999 to 70 -78% in 2001 -2002[47]. In August 2011, nearly 75 per cent of all isolates of *Salmonella enterica* serovar Typhi in urban Nairobi were MDR [13]. The resistant strains have been spreading within Kenya replacing the sensitive type. These MDR strains will need rational use of more expensive drugs. This has also been noted in south East Asia[47,48].

High level ciprofloxacin resistance in *Salmonella enterica* serotype Typhi has been noted in India[49]. Also, there is no much evidence to support the use of fluoroquinolones to all cases of Typhoid as satisfactory cure rates can be obtained in drug sensitive cases with first line agents like chloramphenicol[50].

To prevent further development of resistant strains, indiscriminate use of antibiotics should be discouraged. After culture and sensitivity tests, appropriate drugs should be given[51].This is important especially where prolonged courses of antibiotics are inappropriately given to patients due to persistently positive widal tests [4].Treatment regimens should restrict further second and third line antibiotic usage in Primary Health care settings[52].These measures will help reduce the pressure on public health systems in developing countries as treatment options are few with for example, the increasing quinolone resistance[10].They will also improve the knowledge of healthcare providers and hence have basis for better preventive and treatment strategies[53].

Fatality

Average case fatality is less than 1 per cent but varies greatly in different regions of the world. In hospitalized patients, the case fatality varies from less than 2 per cent in Pakistan[54] and Vietnam [55] to 30 to 50 percent in some areas in Papua New Guinea [56] and Indonesia[57,58]. Highest rates are in children less than one year and the elderly[54,59]. A major contributor to poor outcome is probably a delay in diagnosis[1] and in instituting effective antibiotic treatment[1,2].

Statement of the problem

Typhoid fever is a systemic illness that causes significant morbidity and mortality with a global estimate 12 to 33 million cases and 200,000 to 600,000 deaths annually. In the developing countries, laboratory diagnosis of typhoid poses a great challenge. The gold standard of diagnosis is microbiological isolation of the causative organism. However due to poor resources in many health care facilities, this is not usually done. A single Widal test done in an acute phase serum is the most commonly used diagnostic test. This test has a limited sensitivity (81%) and specificity (93%) when compared to blood cultures.

There are many confounders when Widal test is done. These include the time when the acute phase sample is collected, level of immunity in the patient, previous vaccination to typhoid fever, previous use of antibiotics, previous history of typhoid and presence of high level of antibodies to the bacteria in patients from endemic areas.

Poor interpretation of the Widal test leads to inappropriate prescribing of antibiotics which predisposes to antimicrobial drug resistance. This is worsened by the wide availability of over the counter antibiotics in the developing countries.

Multidrug resistant (MDR) *Salmonella typhi* (resistance of Salmonella typhi to the first line drugs used that is, chloramphenicol, cotrimoxazole and ampicillin) was discovered since the early 1980s.The prevalence of this resistant strain has been increasing to date. In Kenyatta National Hospital for instance, it increased from 50 - 65% in 1997 to 1999 to 70 -78 % in 2001 to 2002 period. In August 2011, nearly 75% of all Salmonella typhi isolates in urban Nairobi were MDR and the high incidence rate (adj. inc.>2000 per 100,000py) was comparable to the rates in Asia.

Justfication

There needs to be a timely intervention to help stem this high incidence of typhoid and worsening MDR levels. The need for increasing use of cultures and antimicrobial sensitivity testing, where resources are available, needs to be greatly emphasized. This will prevent progressive use of more expensive drugs and hence reduce pressure on public health systems in developing countries as antibiotic treatment options will be limited with the worsening of MDR. This intervention will be at the level of policy

formulation. This will target health care providers in improving typhoid laboratory diagnostic services and subsequent antimicrobial treatment. The University of Nairobi Health Services laboratory offers both Widal and culture services and serves patients from all over the country of Kenya. The relative contributions of the diagnostic tests to the antimicrobial treatment will be assessed. The study will form a baseline for further studies with multiple health care facilities.

Research question

Is typhoid being appropriately diagnosed and treated at the University of Nairobi Health Services?

General objective

To assess the laboratory diagnostic tests and antimicrobial treatment of typhoid at the University of Nairobi Health Services.

Specific objectives

1. To assess the utilization of Widal test in the diagnosis of typhoid at the University of Nairobi Health Services.
2. To assess the utilization of other Laboratory diagnostic tests of typhoid at the University of Nairobi Health Services.
3. To assess the Widal test titres at which antimicrobial treatment for typhoid is given to patients at the University of Nairobi of Nairobi Health Services.
4. To assess the antibiotics given for treatment of typhoid at the University of Nairobi Health Services

CHAPTER 3: MATERIALS AND METHODS

Study design

This was a Retrospective study that looked at data collected between January and December 2010.

Study area description

The University Health Services' Laboratory is located at the main campus of the University of Nairobi, off State House Road. This laboratory serves patients seen from the Main Campus and those referred by clinicians from the other Campuses namely, Chiromo, Upper and Lower Kabete, Kenyatta National Hospital, Parklands, Kenya Science and Kikuyu Campus.

The clinic serves University staff, their dependants and students. The laboratory staff works 24 hours a day throughout the year.

Study population

The target population was all patients sent to the laboratory for typhoid laboratory investigations.

Inclusion criteria

Patients who had both typhoid laboratory requisition tests and subsequent clinicians' treatment records.

Exclusion criteria

Patients who lacked either typhoid laboratory requisition tests or subsequent clinicians treatment records or both.

Sample size

Since there were no studies that have the prevalence of typhoid using cultures in Kenya, we used a prevalence of 50%. Using the Fisher's formula:

$N=z^2(p)(1-p)/m^2$

Where,

N is sample size, z =1.96, p is prevalence 50% and m is margin of error of 5%

P=50/100, 1-p=50/100

N is therefore;

$\frac{1.96x1.96(50/100)(50/100)}{(5/100)(5/100)}$ = 384patients.

Sampling method

A total of 4565 patients were eligible in the study period. These made the sampling frame. The sample size needed was 384.To get the number of patients to be selected for every month, 384 was divided by 12 to get 32 patients. This was to take care of the possible seasonal or monthly variations that would occur in the year.

Systematic random sampling was used to select the patients from the total number of each month. The first patient in every month was selected at random by use of computer generated random numbers. This first patient had to be selected within the value of k (total number of patients in a month divided by 32) .Subsequently, every k^{th} patient was selected in an orderly manner to reach the required number as shown in Table 1 below;

Table 1: Selection of patients

Month	Number in sampling frame	Number of the first random Patient (computer generated)	Value of K (total number divided by 32)
January	499	7	15
February	414	12	12
March	409	11	12
April	500	12	15
May	285	5	9
June	320	1	10
July	415	8	12
August	342	7	10
September	298	2	9
October	300	1	9
November	402	9	12
December	361	3	11

Consenting procedures

The KNH/UON Research and Ethics Committee approved the study in November 2011.Authority for the study was sought from the Vice Chancellor, University of Nairobi through the Chief Medical Officer, University Health services (UHS), Nairobi. This was granted in January 2012.

Data collection procedures

For each patient selected, the relevant information to answer the above four specific objectives was abstracted from the patients laboratory and file records. This information was documented on pre tested serialized data collection forms (see appendix 2)

Confounders

Potential confounders were;

1. Previous use of antibiotics which could be associated with negative cultures and low widal titres.
2. Previous vaccination against typhoid which could be associated with high O titres.
3. Patients with immunosuppresion could be associated with poor antibody response and hence low widal titres.
4. Patients from Typhoid endemic areas could have high antibody titres and be misinterpreted as possible infection.
5. The acute phase serum sample for widal could be obtained early before antibody response has occurred

There was difficulty in controlling for the confounding factors because the data used was secondary data.

Training

Three research assistants were trained in one day. The training was face to face and targeted how to get all the relevant files and records. This was important especially for records that weren't computerized.

Good working relations with the laboratory and record staff was also emphasized.

Data collection instruments

The information from the patients' files and records was documented in the serialized data collection forms which had previously been pretested.(appendix 1)

Data management

Hard copies of serialized data collection forms were stored under lock and key. Limited access was under the Principal Investigators' charge. Soft copies were saved in computers with password access. Back up information was saved in flash disks. The data from the serialized data collection forms was stored in an Excel data base. It was cleaned for errors, missing and duplicate entries to ensure good quality of the records.

Statistical analysis

Statistical package for social sciences (SPSS 17) was used for statistical analysis. Descriptive statistics on socio-demographic aspects like age and sex was done to characterize the patients in the study. Appropriate summary statistics were used to describe the distribution of variables. This included for example, mean for continous variables like age and proportions for categorical variables like sex. Description of various variables was done by bar graphs and frequency distribution tables.

Study limitations

1. Incomplete information in the patients' files and laboratory records. This included for example Age of patient, date of visit and antibiotics given.
2. Some patients' files and / or laboratory records were lost or misplaced.
3. Some patients adviced for review with results may not come back- lost for follow up.
4. Some patients treated for a clinical diagnosis of typhoid infection were not sent to the laboratory.
5. For patients having more than one diagnosis (typhoid inclusive), the choice of antibiotics given was influenced by the other diagnoses for example pregnancy.
6. Time and financial constrains contributed to the choice of the study site.

CHAPTER 4: RESULTS

Demographic characteristics of patients selected

A total of 384 patients were selected. Of these, 200 were males (52.1%) and 184 were females (47.9%).The age range was 2 to 66 years with a mean of 29.7 years.

Utilization of Widal test in the diagnosis of typhoid

A total of 4545 widal tests were requested during the study period (January to December 2010) as shown in table 2 below.

Table 2: Utilization of the widal test

Month	Number of Widal tests done
January	499
February	414
March	409
April	500
May	285
June	320
July	415
August	342
September	298
October	300
November	402
December	361
Total	**4545**

Of the 384 study patients who had Widal test done for the diagnosis of Typhoid, the distribution of results was as shown in table 3 below.

Table 3: Distribution of Widal test results

Widal test titre	Total number of patients	Percentage
O neg H neg	290	75.52
O neg H1:160	32	8.33
O neg H 1:320	10	2.60
O1:160 H neg	27	7.03
O1:160 H1:160	7	1.82
O1:160 H1:320	2	0.52
O1:320 H neg	9	2.34
O1:320 H1:160	2	0.52
O1:320 H1:320	5	1.30
Total	**384**	**100**

Patients who had O titre negative results were 86.45% while 9.37% had O titres of 1:160. These two groups combined made up 95.82 % of the total and were below the recommended cut-off of O 1:320. Only 4.16 % of patients had O titres above the cut-off of at least 1:320.

Utilization of other laboratory diagnostic tests of typhoid

During the study period, other tests utilized for typhoid diagnosis included Stool culture, Blood culture and Salmonella stool antigen. The total number requested is as outlined in table 4 below.

Table 4: Utilization of other laboratory tests for diagnosis of typhoid

Month	Stool Culture	Blood Culture	Salmonella Stool antigen
January	18	0	4
February	12	2	0
March	13	1	0
April	11	0	1
May	9	1	0
June	12	2	0
July	16	3	0
August	19	3	0
September	10	1	0
October	24	2	0
November	13	2	0
December	4	7	0
Total	**161**	**24**	**5**

Of all the cultures done during the study period, stool cultures were more 161/185 (87.03%) compared to blood culture 24/185 (12.97%). Of the 384 patients selected, only 6 patients had cultures done. A total of 5 stool cultures and 2 blood cultures were done for these patients. All these stool and blood cultures had no bacteria isolated; hence no patient was treated on the basis of a positive culture.

The Salmonella Stool Antigen was done for a few patients with positive O and H titres of at least 1:160.

Other tests that can be used in diagnosis of typhoid fever include Typhidot, Tubex , PCR, Blood culture-PCR, Urinary Vi antigen and cultures of bone marrow, rose-spots and urine. All these tests were not done during the study period. Resources for Typhidot, Tubex, PCR, Blood Culture – PCR and urinary Vi antigen were not available while there were no specific requests for bone marrow, urine and rose spots culture.

The Widal test was the most highly utilized for diagnosis of Typhoid (for all the tests done during the study period) at (95.99%) followed by Stool Culture (3.40%), Blood Culture (0.50%) and finally Salmonella Stool Antigen (0.10%).

Widal test titre at which antimicrobial treatment is given

Antimicrobials were given at all titres documented (from O negative H negative to O 1:320 H 1:320) as shown in Table 5 below.

Table 5: Widal titres at which antimicrobial treatment was given

Widal titre result	Antimicrobial given	% given	Antimicrobial not given	Total
O neg H neg	17	5.86	273	290
O neg H1:160	11	34.38	21	32
O neg H 1:320	5	50	5	10
O1:160 H neg	17	63	10	27
O1:160 H1:160	4	57.14	3	7
O1:160 H1:320	2	100	0	2
O1:320 H neg	8	88.89	1	9
O1:320 H1:160	2	100	0	2
O1:320 H1:320	5	100	0	5
Total	**71**	**18.49**	**313**	**384**

The kit used at study site would give a positive result only if the O or H titre is positive at least 1:160 dilution.

The proportion of patients at each widal titre who received antimicrobial treatment is shown in the column (% given).

Of all the patients who received antimicrobial treatment, 46.48% had O negative Widal titres while 32.39% had O positive titre at 1:160.These two groups constituted 78.87% of all treated. The rest, 21.12%, were treated at O titres of 1:320. Of all the patients treated, only two had cultures done (2.81%; one a stool culture and the other a stool and blood culture). Both cultures had no growth.

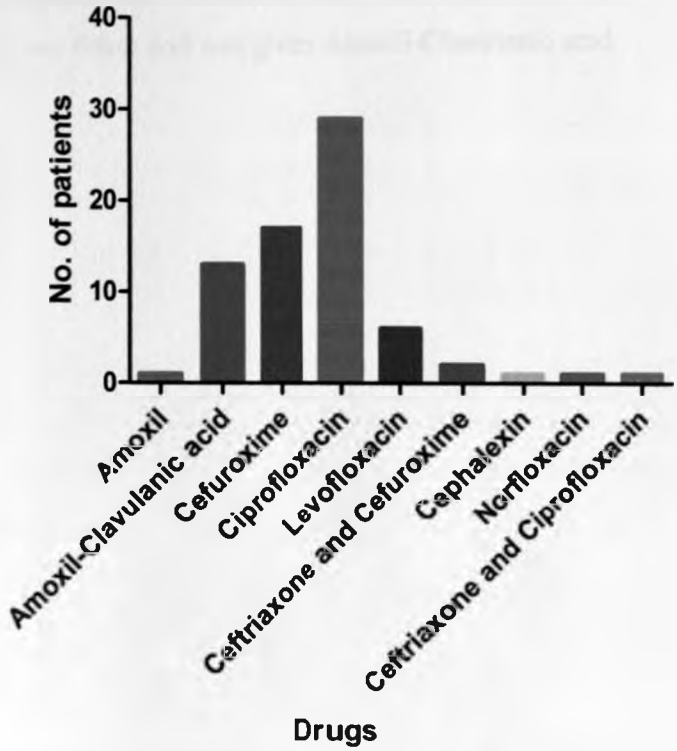
Of all the patients who did not receive antimicrobial treatment, 99.67% had Widal O neg and Widal O positive at 1:160 dilution. In this group only four patients had cultures done (1.28%; 3 stool cultures and one blood culture all of which had no growth).

Only two patients below the cut off had antimicrobial treatment for typhoid together with other concurrent diagnoses. These were Upper respiratory tract infection and Urinary tract infection. Majority of patients treated 69/71(97.18%) had no concurrent diagnoses hence treatment was purely based on diagnosis of typhoid.

Antimicrobial agents prescribed for treatment of typhoid

A total of 71 patients were given antibiotics. Quinolones constituted 52.11% of the total, Cephalosporins 28.16% and Penicillins 19.71%.The distribution of specific agents given is as shown in Figure 1 below.

Figure 1: Antimicrobial agents prescribed



Three patients were given a combination of antibiotics. Two patients received ceftriaxone injections followed by oral cefuroxime and one had ceftriaxone injection followed by ciprofloxacin tablets.

The Kenya National treatment guidelines 2009 indicate that the following drugs can be used in treatment of typhoid- Chloramphenicol, cotrimoxazole, Amoxicillin, Ciprofloxacin, Ofloxacin, Norfloxacin, and Ceftriaxone.

Cefuroxime, amoxicillin- clavulanic acid, cephalexin were used in the treatment though not in the treatment guidelines.

None of the antibiotics given was based on any positive culture. One patient with a pregnancy of 3 months (with no culture done and with widal titre of O neg H 1:320) was given Amoxil-Clavulanic acid as opposed to quinolones possibly due to their contraindication in pregnancy.

Treatment for typhoid and other concurrent infections were made for two patients .One had Urinary tract infection (widal O 1:160 H neg, no culture done) and was given ciprofloxacin. The other had Upper respiratory tract infection (widal O neg H 1:160, no culture done) and was given Amoxil-Clavulanic acid.

CHAPTER 5: DISCUSSION

Typhoid is a major public health problem in developing countries. Timely diagnosis and institution of effective antimicrobial treatment is important to reduce poor outcomes[1,2] This can be achieved by a review of the diagnostic and treatment strategies used and upgrading them so that appropriate interventions can be made.

The results (Table 2) revealed that Widal test is the most commonly used test in diagnosis of Typhoid fever at the University Health Services (95.99%).This concurs with a report that it is the most utilized test in diagnosis of typhoid in developing countries [4].

Many patients (95.82%) presenting with a clinical diagnosis of typhoid actually turned out to be below the cut off of at least O 1:320.This means that other differential diagnoses should be considered so as to get the underlying illnesses when such patients present to the clinicians.

Cultures, though recommended as the confirmatory test of typhoid[1], were rarely done. Stool cultures were requested 3.4% of the time and blood culture 0.5%.Though stool cultures were done more than blood cultures, they can be associated with false positives due to long term fecal carriage[1].The low usage of cultures may be due to the long duration it takes to get results [4], overreliance on the use of the widal test[4] and their low sensitivity associated with previous antibiotic use[9].Cultures are also not usually done in most settings due to unavailability of microbiologic facilities[4], but where these resources are accessible like in the UHS, more cultures should be done.

The Widal test is used to increase index of suspicion for the diagnosis of Typhoid fever. When used as a basis of treatment, then it should be interprated against locally determined cut-offs [60,61,62]. In Nairobi, this is an O titre of at least 1:320 [39].

The antimicrobial treatment was given at all titres reported. This was from O negative H negative to O 1:320 H 1:320 titres. Of all patients treated, 78.87% had at most a titre of O 1:160.This was below the recommended cut off [39]. Furthermore, 97.19% of all patients treated had no cultures done. This means that most of the treatment given was based on Widal test alone and was below the cut off.

However, treatment given would have been in order assuming the below cut off titres were false negatives (meaning if cultures were done, they would have resulted in growth of *Salmonella enterica* serovar Typhi). Also, the blood samples for Widal tests could have been taken early in the course of illness before antibodies develop.

Of all the patients treated, 21.12% had the recommended titre of at least O1:320.This low percentage may have been due to misinterpretation of the Widal test. A study done in Cameroon showed that 48% of doctors and 84% of nurses treated patients who did not require treatment based on the Widal test [63].

In this study patients who would have needed antimicrobial treatment based on Widal test titre of at least O 1:320 were 16/384 (4.16%). In a study in Nigeria of 100 patients with a working diagnosis of Typhoid, the number of treatable patients with the required Widal titre was higher at 55% [66].

Results in figure 1 and 2 show that quinolones are the most prescribed antimicrobials (52.11%).Ciprofloxacin was used more than levofloxacin. More patients were given cephalosporins (28.16 %) compared to penicillins(19.71%).The penicillin mainly used was Amoxil-Clavulanic acid and the cephalosporin mainly used was cefuroxime. However, these two drugs are not in the Kenya National treatment guidelines for management of typhoid[67].

Other studies done showed similar antimicrobial use in the treatment of typhoid fever. A retrospective study of 44 patients done at Kasturba Medical College showed ciprofloxacin was the most commonly used drug (52.27%) followed by cephalosporins (ceftriaxone) 36.36% [51]. Another retrospective study of 100 patients at Kathmandu Medical Teaching Hospital also showed that quinolones were the most highly used drugs[64].

The high and indiscriminate use of quinolones has been associated with antimicrobial resistance[51,64]. However, there is emergence of sensitivity to 1st line drugs like chloramphenicol[65]. This is a very important trend and needs to be monitored by regular local antimicrobial sensitivity surveillance. It will also be more cost effective as 1st line drugs (chloramphenicol, ampicillin and cotrimoxazole) are cheaper compared to the quinolones and cephalosporins.

Conclusion

Widal test is the most commonly utilized test (95.99%) in the University of Nairobi Health Services. Other tests done were stool culture (3.4%) and blood culture (0.5%) and Salmonella stool antigen (0.1%). Tests like PCR, Urinary Vi antigen, typhidot, tubex, bone marrow and rose spot cultures were not done.

Antimicrobial treatment was given at all titres from (O and H) negative to (O and H) positive at 1:320.Most antibiotics (78.87%) were given below the recommended titre of O 1:320. Of all antimicrobial treatment is given, only 2.81% was associated with cultures done. Quinolones were the most prescribed antimicrobials (52.11%) followed by Cephalosporins (28.16%) and then Penicillins (19.71%).

Recommendations

These are necessary so that new policies can be developed on management of typhoid at the UHS Nairobi.

1. Sensitization of the Health care providers at the UHS to improve utilization of the available culture facilities.This will allow more accurate diagnosis of patients with typhoid and improve local surveillance on drug sensitivities especially with the emerging increasing sensitivities on 1st line drugs like chloramphenicol. There is also need to highlight the disadvantages of the Widal test and hence discourage its utilization.
2. Need to explore other tests which currently are not done but have relatively rapid results with high sensitivity like Blood culture –PCR and stool real time PCR.
3. Further research especially a multicenter type to improve generalizability of the results to different health care facilities.

APPENDIX 1: BUDGET

ITEM		Ksh
Salaries	3 Research Assistants @ Ksh 10,000 per month	60,000
	Statistician – one payment for the whole study period	30,000
Materials	Paper, printer, photocoping and binding	60,000
Transport	Investigator @10,000 k sh per month	20,000
	3 Research Assisstants @ Ksh 2,000 K sh each per month	12,000
Fringe benefits	Medical- 3 Research Assistants @5,000 K sh per month for 2 months	30,000
Overheads		30,000
TOTAL		**242,000**

APPENDIX 2: DATA COLLECTION FORM

Serial No. Date seen	Age (years) Sex
WIDAL (Indicate titres)	O H
CULTURE (tick the ones done)	Results obtained – micro organism isolated and sensitivity
Blood	
Stool	
Urine	
Bone marrow	
Rose spots	
Others - PCR - Blood culture - PCR - Salmonella stool antigen - Typhidot - Tubex - Urinary Vi antigen	
Antimicrobial treatment given (write the drug given within 1st week of visit)	
Other concurrent diagnoses eg pregnancy or other clinical conditions that may influence the choice of antibiotic given	

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