A COMPARISON OF THIRD GENERATION CEPHALOSPORINS, AZITHROMYCIN AND COMBINED THERAPY FOR THE TREATMENT OF UNCOMPLICATED ENTERIC FEVER IN DHULIKHEL HOSPITAL

THESIS SUBMITTED FOR THE PARTIAL FULFILLMENT OF THE

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DHULIKHEL, NEPAL

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DEDICATED

TO

ALL THE PATIENTS WHO WERE THE PARTICIPANTS

AND ALSO THE PURPOSE OF THIS STUDY

DECLARATION

I, Dr Sudeep Shrestha, registered with Kathmandu University, Batch April 2012- 2015 hereby declare that the present thesis entitled "**A COMPARISON OF THIRD GENERATION CEPHALOSPORINS, AZITHROMYCIN AND COMBINED THERAPY FOR THE TREATMENT OF UNCOMPLICATED ENTERIC FEVER IN DHULIKHEL HOSPITAL**" has not been submitted in candidature of any other degrees.

I also hereby declare that Kathmandu University shall have the rights to preserve, use and disseminate this thesis in print or electronic for academic/ research purposes.

Dr Sudeep Shrestha

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Date:

CERTIFICATE

This is to certify that Dr. Sudeep Shrestha has done this thesis entitled "**A COMPARISON OF THIRD GENERATION CEPHALOSPORINS, AZITHROMYCIN AND COMBINED THERAPY FOR THE TREATMENT OF UNCOMPLICATED ENTERIC FEVER IN DHULIKHEL HOSPITAL**" under my direct supervision and guidance in partial fulfilment of the regulation for MD degree (Internal Medicine) examination of Kathmandu University to be held in 2015.

As an Associate Professor of the Department, I have immense pleasure in forwarding it to Kathmandu University, Dhulikhel.

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This is to certify that Dr Sudeep Shrestha has done this thesis entitled "**A COMPARISON OF THIRD GENERATION CEPHALOSPORINS, AZITHROMYCIN AND COMBINED THERAPY FOR THE TREATMENT OF UNCOMPLICATED ENTERIC FEVER IN DHULIKHEL HOSPITAL**" under the direct supervision and guidance of Dr. Rajendra Koju, Associate Professor, Department of Internal Medicine, Dhulikhel Hospital, Kathmandu University Hospital. This study is in partial fulfilment of the regulation for MD degree (Internal Medicine) examination of Kathmandu University to be held in 2015.

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Date: Dr Sudeep Shrestha

ABSTRACT

"A COMPARISON OF THIRD GENERATION CEPHALOSPORINS, AZITHROMYCIN AND COMBINED THERAPY FOR THE TREATMENT OF UNCOMPLICATED ENTERIC FEVER IN DHULIKHEL HOSPITAL"

Background and objectives: Emerging resistance to antibiotics, including fluoroquinolones, renders therapy of enteric fever increasingly challenging, especially in the Indian Subcontinent, where most cases are concentrated. A single-drug regimen of either Ceftriaxone or Azithromycin exhibits prolonged fever clearance time (FCT) despite in vitro sensitivity.

A recent small-scale study has shown that among returning travellers from Nepal with enteric fever the administration of a combined therapy, which includes Ceftriaxone and Azithromycin, resulted in a shorter time to defervescence, compared to therapy with each of the agents alone. It was suggested that the combination of two antibiotics may have a beneficial effect, as third generation cephalosporin achieve high blood concentrations, whereas Azithromycin penetrates into the intracellular compartment.

The aim of our study was to assess the efficacy of this combination therapy of third generation cephalosporins and Azithromycin on endemic population with enteric fever.

Methods: A prospective randomized controlled trial was conducted on febrile patients who attended Dhulikhel Hospital and were clinically suspected for enteric fever between October 2012 and October 2014. Those with blood cultures positive for *Salmonella typhi* or *paratyphi* were eligible for the study. Exclusion criteria consisted of patients who received antibiotics against *Salmonella* prior to enrolment and patients who experienced major enteric fever-associated complications.

Cohort was randomly allocated into three study arms - Azithromycin or Ceftriaxone or a combination of Ceftriaxone and Azithromycin. Temperature was measured and recorded on 12-hour intervals. Blood cultures were obtained on days 0 and 3 and faecal samples were collected on a follow-up meeting one month after convalescence. The primary outcome measure was Fever Clearance Time (FCT). Secondary outcome measures included treatment failure, Bacteraemia Clearance Time (BCT), development of enteric-related complications and persistent faecal carriage of the bacteria.

Results: Seventy-one culture-positive patients were included in the study, among which 40 (56.3%) were males and 31(43.7%) were females. Fifteen (21.2%) were between 15-19 years of age, 29 (40.8%) between 20- 24 years of age, 10 (14.1%) between 25 to 29 years of age, 5 (7%) between 30 to 34 years of age, 2 (2.8%) between 35 and 39 years of age and 10 (14.1%) were older than 39 years of age. *S. typhi* was the etiology in 39 (54.9%) cases and 32 (45.1%) were caused by *S. paratyphi*- subdivided in to type A (27, 38%) and B (5, 7.1%). Average age for acquiring *S. typhi* infection was 29.9 years, compared to 24.2 years for *S. paratyphi A.*

21.2 years for *S. paratyphi B*. Forty-two (59.2%) subjects were hospitalized and 29 (40.8%) treated as outpatients.
Distribution by study arms: 24 (33.8%) were treated with oral Azithromycin, 22 (31.0%) cases were treated with Ceftriaxone and 25 (35.2%) cases were treated with a combination of Azithromycin and Ceftriaxone. Patients' demographics did not significantly differ among the three study groups.
Mean FCT of combination therapy (85.99 ± 18.24 hrs) is less than that of oral Azithromycin (102.27 ± 20.98 hrs) which is statistically significant (p=0.009). Similarly, the mean FCT of the combination therapy (85.99 ± 18.24 hrs) is less than that of Inj. Ceftriaxone (93.04 ± 24.48 hrs). However, it is not statistically significant (p=0.26). Ceftriaxone group has lower fever clearance time than oral Azithromycin which is not statistically significant (p =0.146).
Mean BCT of combination therapy (2.84 ± 0.47 days) is less than that of oral Azithromycin (3.83 ± 1.20 days) which is statistically significant p <0.001). Similarly the mean BCT of the combination therapy (2.84 ± 0.47 days) is less than that of Inj. Ceftriaxone (3.05 ± 0.49 days). However, it is not statistically significant (p=0.383). The BCT of Inj. Ceftriaxone (3.05 ± 0.49 days) is significantly different (p= 0.001) from that of oral Azithromycin (3.05 ± 0.49 days).
Inj. Ceftriaxone group has lower fever clearance time than oral Azithromycin which is not statistically significant (p =0.146).
Faecal carriage was negative for all 19 stool cultures obtained upon follow-up examination.
No complications were observed in all treatment arms.
Conclusion: Combination therapy of Azithromycin and Ceftriaxone is better in fever clearance and bacteraemia clearance than Azithromycin alone, where as it is not significantly different than Ceftriaxone alone in fever clearance and bacteraemia clearance. Ceftriaxone is better in terms of bacteraemia clearance than Azithromycin. In terms of fever clearance, though Ceftriaxone has better fever clearance time, it is not statistically significant.

Keywords: Enteric fever, antibiotics

GLOSSARY OF ABBREVIATIONS

BCT	Bacteraemia Clearance Time
CIE	Counter immune electrophoresis
DCA	Desoxycholate agar
DD	Divided doses
DIC	Disseminated Intravascular Coagulation
DNA	Deoxyribonucleic acid
ELISA	Enzyme Linked Immunosorbent Assay
FCT	Fever Clearance Time
FQ	Fluoroquinolones
GMS	Grams
HLA	Human leucocyte antigen
IM	Intramuscular
IV	Intravenous
KG	Kilograms
LPS	Lipo-polysaccharides
MDR	Multi-drug resistant
MIC	Minimum Inhibitory Concentration
Mp	MACROPHAGES
NARST	Nalidixic acid-resistant *S. typhi*
OD	ONCE A DAY
PMN	Polymorphonuclear neutrophil
po	Per oral
RES	Reticulo-endothelial system

SSAgar	Salmonella-Shigella Agar
TMP-SMZ	Trimethoprim and sulfamethoxazole or co-trimoxazole
XLD-agar	Xylox-desoxy cholate agar
µg	Micrograms

CONTENTS

INTRODUCTION

Enteric Fever is a food- and water-borne systemic infection caused by *Salmonella enterica* serotype *typhi* (*S. typhi*) and *Salmonella paratyphi* (*S. paratyphi*). The disease remains an important public health problem in developing countries. Current estimates from the World Health Organization (WHO) suggest that the global burden of enteric fever is approximately 21 million cases annually with more than 210,000 deaths and that of paratyphoid Fever causes an additional 5 million cases.[1]

Enteric fever is a highly prevalent infection in Nepal. A recent study done in a Nepalese hospital showed that Salmonella accounted for the majority (74.5%) of positive blood cultures.[2]This finding can be attributed to lack of adequate sanitary conditions in Nepal and recurrent contamination of water supplies, especially during the rainy season (monsoon). To cite an example, an outbreak of *S. typhi* infecting 5,936 people in Bharatpur in 2002 was traced to the municipal water supply.[3]

Enteric fever is most common in pre-school and school age children. A previous study conducted at Dhulikhel Hospital exhibited 71.4% of patients were of less than 30 years of age. The most common presentations were fever with temperature above 38˚C (axillary temperature) for more than 5 days, headache and chills.[4]

While fluoroquinolones are the mainstay treatment for enteric fever in many endemic regions, treatment has become more challenging in South Asia, as multidrug-resistant strains emerged. In these areas, third generation cephalosporin and Azithromycin are the treatment of choice. However, both agents are not ideal for the treatment of typhoid fever and exhibit a slow response rate. According to a study conducted in Vietnam, patients with nalidixic acid-resistant

Salmonella typhi infection treated with Ceftriaxone or Azithromycin have a mean fever clearance time of longer than a week, a high rate of stool carriage and a high transmission potential, with failure rates greater than 20%.[5]

Most beta-lactam antibiotics have been considered ineffective against organisms which grow inside mammalian cells, such as *S. typhi* and *S. paratyphi* because their concentrations inside mammalian cells are much lower than outside. Nevertheless, it has been proven that a fair amount of third generation cephalosporins can enter cells and inhibit the growth of these organisms. Poor penetration capability of the drug into cells results in difficulty to eradicate the bacteria from the intracellular niche, thus it can account for a relatively long mean time to defervescence.[6] In randomized controlled trials of third-generation cephalosporins the fever-clearance times averaged one week and the rates of treatment failure were 5 to 10 percent. The relapse rates were 3 to 6 percent, and the fecal carriage rates were less than 3 percent.[7]

Azithromycin, a member of the macrolide class of antibiotics, possesses many characteristics for effective and convenient treatment of enteric fever, including in vitro activity against many enteric pathogens, excellent penetration into most tissues, and achievement of concentrations in macrophages and neutrophils that are more than 100-fold higher than concentrations in serum.[8] However, this tendency of the drug to concentrate intracelullarly rather than in the serum has been demonstrated to cause persistent extra-cellular bacteremia.[9] Previous studies have shown cure rates of 95 percent were achieved with five to seven days of treatment with Azithromycin.[7]

In the current study we intend to compare the efficacy of third generation cephalosporins vs. Azithromycin vs. combined therapy of both agents for the treatment of uncomplicated enteric

fever in terms of time to defervescence. The rationale of introducing this dual regimen is its pharmacokinetic profile, which suggests a complimentary action of the two agents – Ceftriaxone or cefixime on the extracellular compartment and Azithromycin on the intracellular compartment.

In case one of the aforementioned regimens will be proven significantly more effective than the others, this may provide a potential benefit in decreasing the disease transmission and carriage rates as well as reducing the disease burden on health economy.

REVIEW OF LITERATURE

History and Nomenclature

Around 430–424 BC, a devastating plague, which some believe to have been typhoid fever, killed one third of the population of Athens, including their leader Pericles. The balance of power shifted from Athens to Sparta, ending the Golden Age of Pericles that had marked Athenian dominance in the Greek ancient world. Ancient historian Thucydides also contracted the disease, but he survived to write about the plague. His writings are the primary source on this outbreak and modern academics and medical scientists consider epidemic typhus the most likely cause. In 2006 a study detected DNA sequences similar to those of the bacterium responsible for typhoid fever.[9]

The most notorious carrier of typhoid fever, but by no means the most destructive, was Mary Mallon, also known as Typhoid Mary. In 1907, she became the first American carrier to be identified and traced. She was a cook in New York. She was closely associated with fifty-three cases and three deaths. Public health authorities told Mary to give up working as a cook or have her gall bladder removed. Mary quit her job initially but returned later under a false name. She was detained and quarantined after another typhoid outbreak. She died after 26 years in quarantine due to pneumonia.[10]

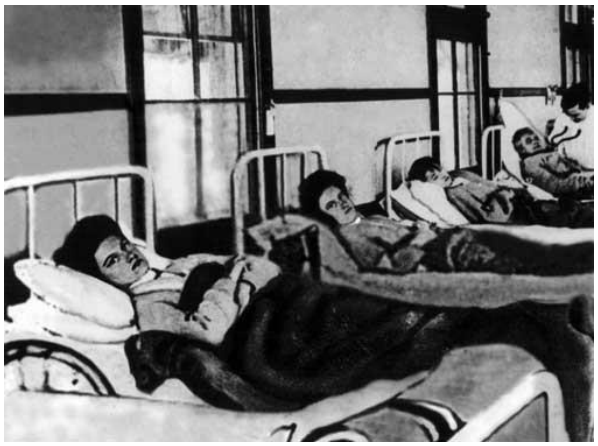


Figure 1 Mary Mallon (foreground) in a hospital bed during her first quarantine

The typhoid bacillus was first observed by Eberth (1880) in the mesenteric nodes and spleen of fatal cases of typhoid fever and was isolated by Graffky (1884). It came to be known as Eberth Graffky bacillus or *Eberthella typhi*. Salmon and Smith described a bacillus which was believed to cause hog-cholera (mistakenly, as it is a viral disease). This bacillus, later called *S. cholerae suis*, was the first of a series of similar organisms to be isolated from animals and human beings- the genus Salmonella. It was subsequently realized that the typhoid bacillus also belonged to this group in spite of minor biochemical differences, and it was re-designated *S. typhi*.[11]

The name Typhoid is derived from the ancient Greek typhos, an ethereal smoke or cloud that was believed to cause disease and madness. In the advanced stages of typhoid fever, the patient's level of consciousness is truly clouded.

Epidemiology

Compared to older patients, typhoid fever is more common in children and young adults.[12] Worldwide, typhoid fever is most prevalent in impoverished areas that are overcrowded with poor access to sanitation. Non-epidemic incidence estimates suggest that south-central Asia, Southeast Asia, and southern Africa are regions with high incidence of *S. typhi* infection (more than 100 cases per 100,000 person years.[1] Other regions of Asia and Africa, Latin America, the Caribbean, and Oceania have a medium incidence of 10 to 100 cases per 100,000 person years. These estimates, though, are limited by lack of consistent reporting from all areas of the world and are based on extrapolation of data across regions and age groups. As an example, the incidence estimates within Africa are based upon reports from Egypt and South Africa only and thus may not be accurately defined.

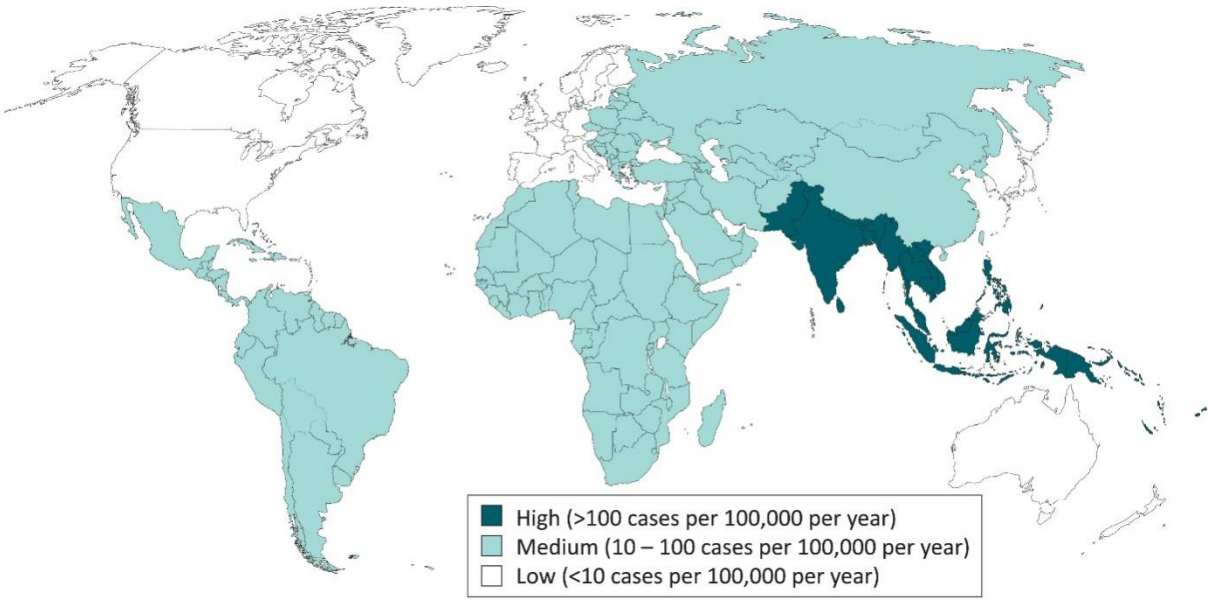


Figure 2 World Enteric ever Map CDC

Because humans are the only reservoir for *S. enterica* serotype Typhi, a history of travel to settings in which sanitation is poor or contact with a known typhoid case or carrier is useful for identifying people at risk of infection outside of endemic areas, although a specific source or contact is identified in a minority of cases.

Approximately 200 to 300 cases of *S. typhi* are reported in the United States each year.[13] About 80 percent of these cases occur among travellers to countries where typhoid fever is endemic; in many circumstances, such travellers have not received appropriate vaccination despite guideline recommendations. Among 580 cases of vaccine-preventable diseases among returned international travellers reported to the multinational GeoSentinel Surveillance Network between 1997 and 2007, confirmed or probable enteric fever (due mainly to *S. typhi* but also *S. paratyphi*) was the most common, particularly in travellers to south-central Asia.[14] Only 38 percent of those with enteric fever had a pre-travel clinical encounter. However, the possibility of *S. typhi* infection in returning travellers with a history of vaccine receipt should not be discounted, since the vaccine is not completely effective.

Patients who acquire infection abroad are usually older than those who acquire disease in US outbreaks and are more likely to have drug-resistant infection. *S. typhi* outbreaks in the United States are most often food borne; they are generally limited in size but can cause substantial morbidity.[15,16] The risk factors for the development of enteric fever due to typhoid or paratyphoid may differ. In an Indonesian study, transmission of paratyphoid fever was more frequently observed outside the home (e.g., via consumption of food purchased from street vendors); transmission of typhoid fever was more frequently observed within the household (e.g., via sharing utensils, presence of a patient with typhoid, lack of soap or adequate toilet facilities).[17]*S. paratyphi* also appears to be an increasing cause of enteric fever among vaccinated travellers, as the typhoid vaccine is ineffective against most *S. paratyphi* infections.[18, 19]

Microbiology

Typhoid fever and paratyphoid fever (also known as enteric fever, but collectively referred to as typhoid fever here) belongs to genus Salmonella.[7]

Salmonellae currently comprise above 2000 serotypes or species, all of them potentially pathogenic. For practical purposes, they are divided into two groups: (1) the enteric fever group consisting of the typhoid and paratyphoid bacilli that are exclusively or primarily human parasites; and (2) the food poisoning group, which are essentially animal parasites but which can also infect human beings, producing gastroenteritis, septicemia or localized infection.[20]

The genus Salmonella consists of two species, *Salmonella enterica* and *Salmonella bongori*; the former is further divided into six different subspecies. Based upon high levels of DNA similarity, most clinically important Salmonellae are formally classified within a single

subspecies, *Salmonella enterica*, subspecies enteric.[21] Familiar organisms such as *Salmonella typhi, Salmonella choleraesuis,* and *Salmonella enteritidis,* previously believed to represent separate species based upon antigenic structures, host range, and biochemical characteristics, are now individual serotypes of this single subspecies. Many laboratories will continue to report names recognizable to clinicians such as: *Salmonella typhi* murium or *Salmonella enterica* serovar Typhi. Serotype and serovar are synonymous.

Salmonella are relatively easy to identify in the clinical microbiology laboratory[22]. Salmonellae grow under both aerobic and anaerobic conditions. Salmonella are Gram stain negative oxidase negative and virtually all are lactose negative (white on MacConkey agar plates); most Salmonellae produce hydrogen sulfide, which is easily detected on selective indicator plates such as Hektoen, or Salmonella-Shigella agar, which are used for plating stool specimens.

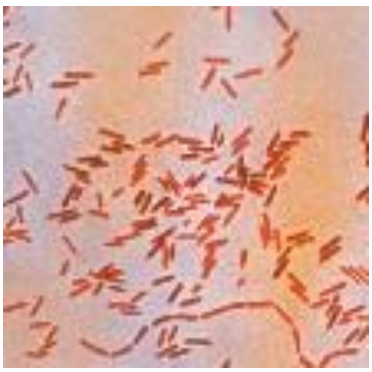


Figure 3 Salmonella typhi, the agent of typhoid. Gram stain

Most laboratories identify Salmonellae by a combination of antigenic and biochemical reactions. Suspicious colonies are agglutinated using antisera directed against specific O (lipopolysaccharide) and H (flagellar) antigens that allow identification of the serogroup. Only *S. typhi, S. paratyphi* C, and some strains of *Salmonella dublin* and *Citrobacter freundii* possess the Vi capsular polysaccharide antigen[23], which can be rapidly detected by slide agglutination studies.

Although serogrouping may provide a clue as to the specific organism, this may not always be useful clinically. As an example, both *S. enteritidis* (which most frequently causes gastroenteritis) and *S. typhi* (which causes enteric fever) belong to group D; *S. enteritidis* may occasionally cause a systemic "typhoidal" illness with bacteremia. Formal serotyping is more specific than serogrouping and usually is only performed at state or reference laboratories.

Some have advocated the use of typing techniques such as pulsed-field gel electrophoresis on strains of *S. enterica* serotype *typhi murium* to detect outbreaks that might otherwise be missed. The Minnesota Department of Health adopted such an approach and identified 16 outbreaks accounting for 154 of 958 isolates between 1994 and 1998.[24] Twenty-seven percent of isolates were resistant to at least five antibiotics when sensitivity testing was performed; the multidrug resistant strains all had unique pulsed-field gel electrophoresis patterns.

Colony Characteristics

Blood agar

On blood agar, *S. typhi* and *S. paratyphi* usually produce non-haemolytic smooth white colonies. (Fig. 4)

• MacConkey agar

On MacConkey agar, Salmonellae produce lactose non-fermenting smooth Colonies. (Fig. 5)

• SS agar

On SS agar, salmonellae usually produce lactose non-fermenting colonies with black centers (except *S. paratyphi* A, whose colonies do not have black centers).

• Desoxycholate agar (DCA)

On desoxycholate agar, salmonellae produce lactose non-fermenting colonies with black centers (except *S. paratyphi* A, whose colonies do not have black centers). (Fig 6)

• Xylose-lysine-desoxycholate agar (XLD)

On xylose-desoxycholate agar, salmonellae produce transparent red colonies with black centers (except *S. paratyphi* A, whose colonies do not have black centers). (Fig 7)

• Hektoen enteric agar

On Hektoen enteric agar, salmonellae produce transparent green colonies with black centers (except *S. paratyphi* A, whose colonies do not have black centers).

• Bismuth sulfite agar

On this medium, salmonellae produce black colonies.

Figure 4 Salmonella typhi, shown on blood agar

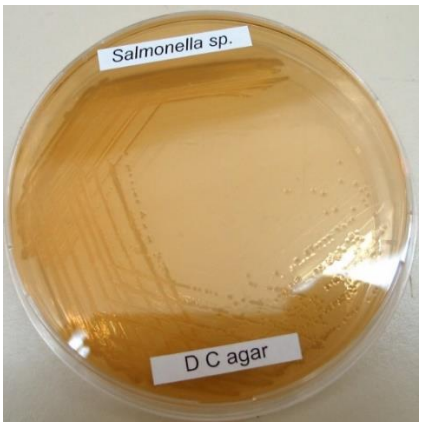


Figure 5 Salmonella typhi, shown on MacConkey agar subculture

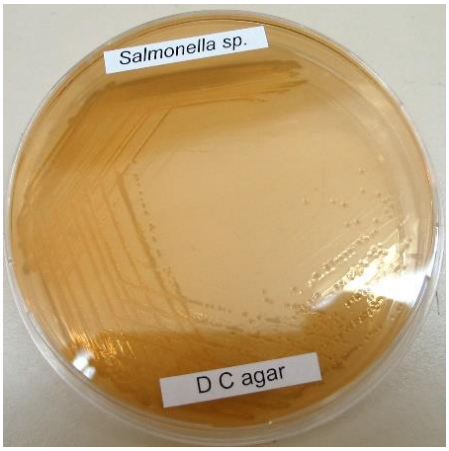


Figure 6 A microbiological culture of Salmonella sp. bacteria on DC agar culture medium

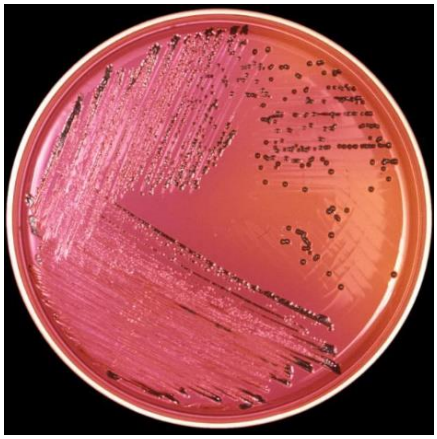


Figure 7 Salmonella sp. after 24 hours growth on XLD agar

Antigenic Structures

Salmonellae possess the following antigens based on which they are classified and identified: (1) flagellar antigen H, (2) somatic antigen O, and (3) a surface antigen Vi, found in some species. Several strains carry fimbriae. Fimbrial antigens are not important in identification but may cause confusion due to their nonspecific nature and widespread sharing among enterobacteriae.

Somatic (O) or Cell Wall Antigens

Somatic antigens are heat stable and alcohol resistant. Cross-absorption studies individualize a large number of antigenic factors, 67 of which are used for serological identification. O factors labelled with the same number are closely related, although not always antigenically identical.

Surface (Envelope) Antigens

Surface antigens, commonly observed in other genera of enteric bacteria (e.g., *Escherichia coli* and *Klebsiella*) may be found in some Salmonella serovars. Surface antigens in Salmonella may mask O antigens, and the bacteria will not be agglutinated with O antisera. One specific surface antigen is well known: the Vi antigen. The Vi antigen occurs in only three Salmonella serovars (out of about 2,200): *typhi*, *Paratyphi* C, and Dublin. Strains of these three serovars may or may not have the Vi antigen.

Flagellar (H) Antigens

Flagellar antigens are heat-labile proteins. Mixing salmonella cells with flagella-specific antisera gives a characteristic pattern of agglutination (bacteria are loosely attached to each other by their flagella and can be dissociated by shaking). Also, antiflagellar antibodies can immobilize bacteria with corresponding H antigens.

A few *Salmonella enterica* serovars (e.g., Enteritidis, *typhi*) produce flagella which always have the same antigenic specificity. Such an H antigen is then called monophasic. Most Salmonella serovars, however, can alternatively produce flagella with two different H antigenic specificities. The H antigen is then called diphasic. For example, *Typhi* murium cells can produce flagella with either antigen i or antigen 1, 2. If a clone is derived from a bacterial cell with H antigen i, it will consist of bacteria with i flagellar antigen. However, at a frequency of 10^{-3}- 10^{-5}, bacterial cells with 1, 2 flagellar antigen pattern will appear in this clone.

Pathogenesis

All pathogenic Salmonella species are engulfed by phagocytic cells, which then pass them through the mucosa and present them to the macrophages in the lamina propria. Non-typhoidal

salmonellae are phagocytised throughout the distal ileum and colon. With Toll-like receptor (TLR)–5 and TLR-4/MD2/CD-14 complex, macrophages recognize Pathogen-associated molecular patterns (PAMPs) such as flagella and lipopolysaccharides. Macrophages and intestinal epithelial cells then attract T cells and neutrophils with Interleukin-8 (IL-8), causing inflammation and suppressing the infection.[7, 25]

In contrast to the non-typhoidal salmonellae, *S. typhi* enters the host's system primarily through the distal ileum. *S. typhi* has specialized fimbriae that adhere to the epithelium over clusters of lymphoid tissue in the ileum (Peyer patches), the main relay point for macrophages travelling from the gut into the lymphatic system. *S. typhi* has a Vi capsular antigen that masks PAMPs, avoiding neutrophil based inflammation. The bacteria then induce their host macrophages to attract more macrophages.[25] It co-opts the macrophages cellular machinery for their own reproduction[26] as it is carried through the mesenteric lymph nodes to the thoracic duct and the lymphatic's and then through to the reticuloendothelial tissues of the liver, spleen, bone marrow, and lymph nodes. As bile is a good culture medium for the bacillus, it multiplies abundantly in the gall bladder and is discharged continuously into the intestines. Once there, the *S. typhi* bacteria pause and continue to multiply until some critical density is reached. Afterward, the bacteria induce macrophage apoptosis, breaking out into the bloodstream to invade the rest of the body.[7]

The pathogenesis of enteric fever essentially depends on a number of factors including the infecting species and infectious dose. Ingested organisms survive exposure to gastric acid before gaining access to the small bowel, where they penetrate the epithelium, enter the lymphoid tissue, and disseminate via the lymphatic or hematogenous route. A chronic carrier state is established in an estimated 1 to 5 percent of cases.[7, 27, 28]

The organisms: It has been suggested that *S. typhi* represents a single clone with little intra-species divergence.[29, 30] However, other studies have demonstrated that the *S. typhi* genome has undergone significant evolutionary rearrangement.[31,32] Differences have been found among isolates of *S. typhi* in pulsed field gel electrophoretic (PFGE) patterns, outer membrane protein profiles[33] and ribotypes[34]. Certain flagellar serotypes[35] and PFGE patterns[36] have been associated with more severe clinical illness in endemic areas.

Studies suggest that *S. typhi* evolves in response to environmental pressures, that especially virulent clones may exist, and that further study is needed to understand the specific virulence properties of this pathogen.[36]

Infectious dose: In general, the greater the infectious dose, the higher the attack rate and the shorter the incubation period. In one report, 10^5 colony forming units of the Quailes strain of *S. typhi* was administered orally to 116 adults; 28 percent developed typhoid fever.[37]

Data compiled from 12 outbreaks related to food, water, or occupational laboratory exposures showed "real life" infectious doses are usually low ($<10^3$) with relatively low attack rates (averaging 4 percent) and fairly long incubation periods (12 to 21 days).[38] Smaller numbers of organisms are likely to induce disease in high-risk individuals such as those with achlorhydria or immunosuppressive illnesses such as AIDS.[39]

Incubation Period

The incubation period is 5 to 21 days. In general, lower inocula are associated with longer incubation times. However, both the incubation period and inoculum needed to cause disease vary depending upon host factors such as age, gastric acidity, and immunologic status.

Signs and Symptoms

The majority of patients with typhoid fever present with abdominal pain, fever, and chills.

Classic presentation:

Classic reports described the characteristic stages of typhoid fever in untreated individuals.[40] In the first week of illness, rising ("stepwise") fever and bacteraemia develop.[41] While chills are typical, frank rigors are rare.[18] Relative bradycardia or pulse-temperature dissociation may be observed. In the second week of illness, abdominal pain develops and “rose spots” (faint salmon-coloured macules on the trunk and abdomen) may be seen. During the third week of illness, hepatosplenomegaly, intestinal bleeding, and perforation due to ileocecal lymphatic hyperplasia of the Peyer's patches may occur, together with secondary bacteraemia and peritonitis. Septic shock or an altered level of consciousness may develop; among 300 cases of typhoid fever in Indonesia, these findings were observed in approximately 15 percent of patients.[42] Symptoms gradually resolve over weeks to months in the absence of acute complications or death from overwhelming sepsis.

Effect of antimicrobial therapy:

The clinical features of typhoid fever have changed dramatically in the antibiotic era. When case series from the 1930s were compared with series from the 1970s and 1980s, the prevalence

of splenomegaly fell from 63 to 10 percent, and the prevalence of rose spots fell from 30 to 1.5 percent.[43] Intestinal bleeding was also less frequent.

In the post-antibiotic era, the average mortality rate from typhoid fever is estimated to be less than 1 percent[7], but this varies widely based upon site and resources, and may be 10- to 20-fold higher in the most resource-limited settings. An epidemiological survey of about 1100 cases in Spain (1997-2005) demonstrated a fatality rate of 0.9 percent.[44] A Centers for Disease Control and Prevention (CDC) compilation of 10 hospital-based typhoid fever series reported a mean case-fatality rate of 2 percent (range 0 to 14.8 percent), but noted that these series capture only the most severe and hospitalized cases in those with access to care.[45]

Other clinical manifestation:

The symptoms, signs, and complications of typhoid fever vary widely in different series and may be related to age, geographic area, the causative organism, or the time at which patients seek medical care.

Gastrointestinal manifestations:

Constipation occurring more frequently than diarrhoea was reported in the pre-antibiotic era.[40] Subsequent reports suggest that these symptoms occur with approximately equal frequency or that diarrhoea may be more common, particularly in young children and in adults with HIV infection.[39,46] Specifically, the incidence of diarrhoea in children with culture proven typhoid fever was 78 percent in a series from Australia[47] and 50 percent in a report from Vietnam[48]. Constipation occurs in approximately 30 percent of individuals,[48, 49] perhaps more frequently

in adults. Among 552 patients with culture-confirmed typhoid fever in Bangladesh, abdominal tenderness or distension (57 percent) and rectal bleeding (9 percent) were equally distributed across age groups.[50]

Intestinal perforation generally occurs more frequently among adults than children and is associated with high mortality rates. Among 105 adults with typhoid fever in India, this complication was observed in 10 percent of patients.[51] In the Bangladesh study, intestinal perforation was observed in three percent of patients overall, but in 25 percent of patients over 31 years old.[50] An outbreak of typhoid fever in Uganda was detected specifically because of a high incidence of intestinal perforation, seen in patients of all ages.[52] Over an 18-month period, 249 cases with a median age of 16 years were identified and 18 percent of them died.

Neurological manifestations:

Although headache is a frequent symptom reported in 44 to 94 percent of cases,[48,49, 52,53] other neurological manifestations including disordered sleep patterns, acute psychosis, myelitis, and rigidity have been observed but are uncommon,[54] as are meningitis and focal central nervous infections with *S. typhi.*[55] An outbreak of typhoid fever at the Malawi-Mozambique border was notable for a relatively high incidence of associated neurological findings, found in 40 of 303 cases (13 percent).[53] These included signs of upper motor neuron disease (e.g., hyper-reflexia, spasticity and sustained clonus), ataxia, and Parkinsonism.

Patients with severe typhoid fever may develop "typhoid encephalopathy," with altered consciousness, delirium, and confusion. This has been observed in up to 17 percent of patients,

with no clear frequency difference between children and adults.[50] In one study of 38 patients in Indonesia with typhoid fever, delirium, obtundation and stupor were grave prognostic signs, with mortality rate as high as 55 percent.[42] In this study, intravenous dexamethasone was administered in a randomized placebo-controlled fashion as an adjunctive to antibiotic therapy; a reduction in mortality from 55 to 10 percent was observed. In another series of 23 cases of typhoid encephalopathy from Bangladesh, the mortality rate was 13 percent; in a retrospective analysis of this series, survivors were more likely to have received IV dexamethasone.[56]

Other extra-intestinal manifestations:

Other protean symptoms have been reported to varying degrees. Cough is not rare and has been observed in approximately 20 to 45 percent; arthralgias and myalgias occur in about 20 percent.[48,49,52,53] Focal extra-intestinal manifestations including involvement of the hepatobiliary, cardiovascular, respiratory, genitourinary, musculoskeletal, and central nervous systems have been described as a result of bacteremic seeding, but are observed infrequently.[57]

Special populations

Children:

Certain clinical manifestations associated with typhoid fever occur with different frequency in children compared with adults; age differences were specifically examined in a review of 552 culture-confirmed cases in Bangladesh.[50] Pneumonia and febrile seizures were overall infrequent but occurred more commonly in children, whereas intestinal perforation was not seen in patients under five years old. In the study, younger patients tended to have higher WBC counts; 14 of the 15 patients with a WBC count >20 x10 ^{3}/mm ^{3} were younger than five years old.

Even among infants, there is variability in the severity of the disease. In a series from Chile, febrile infants with typhoid fever had relatively mild illnesses not requiring hospitalization,[58] while a study from Bangladesh noted a fatality rate of 11 percent.[50]

HIV-infected patients:

The severity of enteric fever does not appear to be markedly increased in the setting of HIV infection, although nontyphoidal salmonellosis is known to be more complicated in HIV infection. However, there is some evidence that immunocompromised patients fare poorly with typhoidal infections. One study of four individuals with AIDS in Peru described atypically severe diarrhea or colitis.[39] In a Tanzanian series of 104 cases of intestinal perforations due to typhoid fever treated surgically at a university hospital, mortality was associated with HIV-positivity and low CD4 count at admission, among other factors.[59] Other case reports have documented unusual manifestations of *S. typhi* infection such as arteritis[60] or chorioamnionitis[61] in HIV-infected patients.

Chronic carriers:

In general, chronic carriers do not develop recurrent symptomatic disease. They appear to reach an immunologic equilibrium in which they are chronically colonized and may excrete large numbers of organisms, but have a high level of immunity and do not develop clinical disease.[28,62-64] Chronic carriers frequently have high serum antibody titers against the Vi antigen, which is a clinically useful test for rapid identification of such patients.[65, 66]

Transmission

S. typhi has no non-human vectors. The following are modes of transmission:

- Oral transmission via food or beverages handled by an individual who chronically sheds the bacteria through stool or, less commonly, urine.
- Hand-to-mouth transmission after using a contaminated toilet and neglecting hand hygiene.

Laboratory Studies

The reported sensitivities of tests for *S. typhi* vary greatly in the literature, even among the most recent articles and respected journals. The diagnosis of typhoid fever (enteric fever) is primarily clinical.

Blood Counts

The results of various lab investigations differ in different age groups e.g. blood leukocytes are frequently high in relation to the fever and toxicity and may reach 15,000-20,000/cmm. Thrombocytopenia may be a marker of severe illness and accompany DIC. The hemoglobin is normal in the initial stages but drops with progressing illness and severe anemia is unusual.

Leucopenia, eosinopenia, thrombocytopenia and anemia in enteric can be attributed to the myeloid maturation arrest, decrease in the number of erythroblasts and megakaryocytes and increased phagocytic activity of histiocytes in the bone marrow.[67]

Hemophagocytosis is an important mechanism in producing neutropenia, anemia and thrombocytopenia in several infectious and noninfectious disorders.[68]

Culture

Blood cultures are positive in 40 to 80 percent of patients, depending upon the series and culture techniques used. Blood cultures may require several days of incubation. The diagnosis can also

be made by culture of stool, urine, rose spots, or duodenal contents (via string capsule)[69] Stool culture is positive in up to 30 to 40 percent of cases, but is often negative by the time that systemic symptoms bring patients to medical attention.[58]

Duthie et al[70] obtained an isolation rate of 92% for blood culture with the Bactec 460 system using a blood: broth ratio of 1:6. This yield is comparable with those reported by other workers using 10% Oxgall.[71] The Bactec 460 media contain liquid which probably contributes to the good recovery rates of the inoculum.[72] The yield is higher for Bactec system compared to the yield on clotted blood and streptokinase added blood.[73]

Bone marrow culture is the most sensitive routinely available diagnostic tool.[74] This may be particularly important in complicated cases or when antimicrobial therapy has already been initiated and the diagnosis remains uncertain. Bone marrow cultures may be positive in as many as 50 percent of patients after as many as five days of antibiotics.[46] In one series of 44 patients with typhoid fever, *S. typhi* was isolated from 98 percent of bone marrow cultures compared with 70 percent of blood cultures.[75]

Serology

Serologic tests such as the Widal test are of limited clinical utility in endemic areas because positive results may represent previous infection. The Widal test detects anti-*S. typhi* antibodies, and the minimal titers defined as positive for the O (surface polysaccharide) antigens and H (flagellar) antigens must be determined for individual geographic areas; they are higher in developing regions than in the United States.[72] When paired acute and convalescent samples are studied, a fourfold or greater increase is considered positive. Positive results have been reported in 46 to 94 percent of cases.[76] In a study of healthy blood donors

performed in central India, seropositivity for typhoid fever using the *S. typhi* O antigen or *S. typhi* H antigen was observed in 8 and 14 percent, respectively.[76]

Newer serologic assays using enzyme-linked immunosorbent assay (ELISA) and dipstick techniques perform somewhat better than the Widal test, but sensitivity and specificity are not adequate for routine diagnostic use.[77] An ELISA for antibodies to the capsular polysaccharide Vi antigen is useful for detection of carriers, but not for the diagnosis of acute illness.[65,66]

Polymerase chain reaction (PCR)

PCR has been used for the diagnosis of typhoid fever with varying success[78]. Nested PCR, which involves two rounds of PCR using two primers with different sequences within the H1d flagellin gene of *S. typhi*, offers the best sensitivity and specificity. Combining assays of blood and urine, this technique has achieved a sensitivity of 82.7% and reported specificity of 100%. However, no type of PCR is widely available for the clinical diagnosis of typhoid fever.

Other Laboratory abnormalities

Abnormal liver function tests are frequently observed.[43,79] In an outbreak in 34 patients, abnormal liver function tests were observed in all but one patient.[43] In some patients, the clinical and laboratory picture may be suggestive of acute viral hepatitis.[80] In one study comparing 27 patients with Salmonella hepatitis to 27 cases of viral hepatitis, Salmonella hepatitis was more frequently associated with bradycardia (42 versus 4 percent) and fever >40ºC (44 versus 4 percent); serum aminotransferase also tended to be lower (peak serum ALT 296 versus 3234 IU/L). A potential diagnostic challenge in patients presenting with abnormal liver function tests is that the two infections may be present at once.

Cerebrospinal fluid studies are usually normal or reveal a mild pleocytosis (<35 cells/mm ^{3}), even in patients with neuropsychiatric symptoms.[55]

Staging

The proper treatment approach to typhoid fever depends on whether the illness is complicated or uncomplicated. Complicated typhoid fever is characterized by melena (3% of all hospitalized patients with typhoid fever), serious abdominal discomfort, intestinal perforation, marked neuropsychiatric symptoms, or other severe manifestations. Depending on the adequacy of diagnosis and treatment, complicated disease may develop in up to 10% of treated patients. Delirium, obtundation, stupor, coma, or shock demands a particularly aggressive approach.[57]

Diagnosis of Carriers

Chronic Salmonella carriage is defined as excretion of the organism in stool or urine >12 months after acute infection. Rates of chronic carriage after *S. typhi* infection range from 1 to 6 percent.[7,62,65] Chronic carriage occurs more frequently in women and in patients with cholelithiasis or other biliary tract abnormalities.[81,82] Chronic carriage in the urine is almost always associated with a defect in the urinary tract (e.g., urolithiasis, prostatic hyperplasia) or concurrent bladder infection with Schistosoma.[83]

Chronic carriers represent an infectious risk to others, particularly in the setting of food preparation as evidenced from the story of "Typhoid Mary".[10] For this reason, eradication of carriage when identified should be attempted.

The *S. typhi* carrier state may be an independent risk factor for carcinoma of the gallbladder as well as other cancers.[84]

Treatment

Treatment of typhoid fever has been complicated by the development and rapid dissemination of typhoidal organisms resistant to ampicillin, trimethoprim-sulfamethoxazole, and chloramphenicol. In recent years, development of creeping resistance to fluoroquinolones has resulted in more challenges.

Multidrug-resistant strains:

Multidrug-resistant (MDR) strains have caused numerous outbreaks in the Indian subcontinent, Southeast Asia, Mexico, the Arabian Gulf, and Africa.[85,86] These patterns of resistance are reflected in travellers returning to the United Kingdom and the United States. In 1999, 26 percent of *S. typhi* strains characterized at a United Kingdom reference lab were MDR strains[87], and 17 percent of 293 strains evaluated by the Centres for Disease Control and Prevention (CDC) from 1996 to 1997 were resistant to five drugs.[88] In contrast, a study from India has reported the "reemergence" of sensitivity to older drugs: 67 percent of 60 *S. typhi* blood isolates and 80 percent of 20 *S. paratyphi* blood isolates from 2001 through 2004 were sensitive to chloramphenicol.[89]

Resistance patterns have led to a shift toward the third generation cephalosporins, Azithromycin and fluoroquinolones as empiric therapy for typhoid fever while awaiting the results of antimicrobial susceptibilities.

A report of 113 *S. typhi* strains collected in India from 1987-2006 did not demonstrate frank resistance but revealed “MIC creep” for Ceftriaxone. A gradual increase in Ceftriaxone MIC has been observed in five-year increments: 0.047mcg/mL,0.098mcg/mL, 0.211 mcg/mL and 0.365 mcg/mL for Ceftriaxone MIC.[90]

Two isolates of *S. paratyphi* B expressing extended-spectrum beta-lactamases have been reported from Turkey.[91]

Fluoroquinolone-resistant organisms:

Nalidixic acid-resistant organisms with decreased susceptibility to the clinically important fluoroquinolones have become a major problem worldwide. High-level resistance to Ciprofloxacin have been reported from India in *S. paratyphi* and *S. typhi* (MICs of 8 mcg/mL to 16 mcg/mL).[92-94]

Nalidixic acid-resistant *S. typhi* (NARST, NaR) have decreased Ciprofloxacin sensitivity and are less effectively treated with fluoroquinolones, especially with the use of empirical "short course" of three to five days, which is very effective against susceptible organisms. The clinical impact of NaR organisms is illustrated by the following studies:

- A retrospective review of 150 patients from Vietnam showed that patients infected with NaR *S. typhi* defervesced more slowly (256 versus 84 hours) and more frequently required retreatment (33 versus 0.8 percent) than those infected with nalidixic acid-sensitive organisms.[95]
- Pooled data from several studies of almost 500 bacteremic patients treated with two, three, or five days of Ofloxacin, found that those patients with NaR strains who got Ofloxacin had greater rates of clinical treatment failure than those treated with comparator drugs (e.g., Ceftriaxone, Azithromycin, or cefixime).[96]

This situation has been further complicated by the emergence of newer mechanisms of resistance to fluoroquinolones. Some isolates may have decreased sensitivity to clinically

important fluoroquinolones, but appear to be sensitive to nalidixic acid, calling into question the reliability of using NaR as a marker of FQ resistance.[97]

Antimicrobial regimens:

Typhoid fever is usually treated with a single antibacterial drug. The optimal choice of drug and duration of therapy are uncertain.[95,98,99] Antibiotic selection depends upon local resistance patterns, patient age, whether oral medications are feasible, the clinical setting, and available resources. In some circumstances, older agents such as chloramphenicol, ampicillin, or trimethoprim-sulfamethoxazole may be appropriate, but these drugs are generally not used widely because of high levels of resistance. Oral chloramphenicol is no longer available in the United States but is still used in other parts of the world. Successful treatment in uncomplicated cases usually results in clinical improvement within three to five days. It is reasonable to begin with a parenteral agent and then complete therapy with an oral drug once symptoms improve.

Drugs of choice for the treatment of typhoid fever in adults include:[7]

- A fluoroquinolone such as Ciprofloxacin (500 mg twice daily) or Ofloxacin (400 mg twice daily), either orally or parentally for 7 to 10 days. The fluoroquinolones should not be used as a first-line treatment for typhoid fever in patients from South Asia or other regions with high rates of fluoroquinolone resistance unless antibiotic susceptibility data demonstrate fluoroquinolone or nalidixic acid sensitivity.[100,101]
- A beta-lactam such as Ceftriaxone (2 to 3 g once daily) parentally or cefixime (20 to 30 mg/kg per day orally in two divided doses) for 7 to 14 days.

Alternative agents for adult patients who cannot be treated with the above antimicrobials and for fluoroquinolone resistant isolates include:

- Azithromycin (1 g orally once followed by 500 mg once daily for five to seven days, or 1 g orally once daily for five days).[102,103]

Fluoroquinolones appear to have therapeutic advantages over beta-lactams for the treatment of uncomplicated typhoid fever and are now considered by many experts to be the drug of choice for fully susceptible organisms in patients who can take these drugs. Quinolones are bactericidal and concentrated intracellularly and in the bile. Ciprofloxacin and Ofloxacin are widely available and efficacious: Norfloxacin is very poorly absorbed and should not be used. When treating fully susceptible organisms, the quinolones may result in more rapid defervescence than beta-lactam agents or chloramphenicol because of more rapid elimination of intracellular bacteria.[104]

Azithromycin is capable of achieving excellent intracellular concentrations, and its use for treatment of typhoid fever has been increasing as a result of rising fluoroquinolone resistance. There are no standard microbiological breakpoints for determining Salmonellae MICs in routine clinical practice; in research studies MICs are typically 4 to 32 mcg/mL. These values typically exceed serum drug levels; Azithromycin is concentrated intracellularly at levels 50 to 100 greater than serum levels.[105] The first report of Azithromycin resistance in *S. paratyphi* A resulting in treatment failure was reported in a traveller returning to Great Britain from Pakistan. The patient was successfully treated with a two week course of IV Ceftriaxone, 2 g daily.[106]

A large open label randomized controlled trial of Gatifloxacin (10 mg/kg once daily for seven days) versus chloramphenicol (75 mg/kg/day in four divided doses for 10 days) for uncomplicated, culture-proven typhoid fever showed equivalent cure and relapse rates in children and adults in Patan Hospital, Lalitpur, Nepal. The authors concluded that Gatifloxacin

should be the preferred treatment for enteric fever in developing countries because of its shorter treatment duration and fewer adverse events (14 versus 24 percent of patients).[107]

Pandit A. et al[108] from Patan Hospital, Lalitpur, Nepal, compared the effect of Gatifloxacin with a third-generation cephalosporin. In their 390-patient study, median fever clearance time was 92 hours (84-114 hours) for Gatifloxacin recipients and 138 hours (105-164 hours) for Cefixime treated patients ($p < 0.0001$). 37.6% of patients who completed the 7-day Cefixime trial experienced clinical failure, as compared to 3.5% in the Gatifloxacin group. Due to these findings enrollment for the study was suspended by independent data safety monitoring board.

Another study conducted in Vietnam by Dolecek Cet al[109] has compared Gatifloxacin versus Azithromycin. Three hundred and fifty eight subjects with culture-confirmed typhoid fever were randomized into two groups. The median fever clearance time was 106 hours for both treatment arms ($p = 0.984$) and failure rate was similar as well (9% patients in Gatifloxacin group and 9.3% in Azithromycin group, $p = 0.854$).

Similarly, a meta-analysis of 7 trials by Trivedi NA et al[110] suggested that Azithromycin is marginally better than Ceftriaxone in reducing the chance of clinical failure [RR 0.46 (95% CI 0.25‑0.82)], while in comparison to Ceftriaxone, it significantly reduced the chance of relapse [RR 0.1 (95% CI 0.01‑ 0.76)]. A meta-analysis of 20 prospective trials that enrolled more than 1,600 culture-positive patients has also concluded that Azithromycin to be better than Ceftriaxone in cure rate and relapse rate.[111]

Conversely, a study conducted on 149 children and adolescents with culture-confirmed *Salmonella typhi* and *paratyphi* in Egypt, which compared Ceftriaxone and Azithromycin

treatment, has suggested no significant differences in cure rates between the two regiments (94% in the Azithromycin group and 97% in the Ceftriaxone group). However, mean time to clearance of bacteremia was longer in the Azithromycin group and there were more cases of relapse in the Ceftriaxone group.[8]

A recent study by Dong-Min Kim et al[9] was conducted in order to identify in-vitro synergistic combinations of antibiotics against *S. typhi*. The combinations of Ciprofloxacin and Cefotaxime vs. the combination of Ciprofloxacin and Azithromycin vs. the combination of Cefotaxime and Azithromycin were evaluated. It was found that Ciprofloxacin plus Cefotaxime was the best in vitro combination against *S. typhi*, and this combination may have greater efficacy than a fluoroquinolone alone. The efficacy of the combination of Cefotaxime and Azithromycin was not consistent and was insufficient to show an in vitro synergistic effect.

Other Treatment Considerations

Corticosteroids:

Early studies with chloramphenicol suggested that concomitant corticosteroid therapy might be beneficial in patients with typhoid fever. In a randomized, prospective, double blind study performed in Indonesia in the early 1980s, the administration of 3 mg/kg of dexamethasone as an initial dose with chloramphenicol was associated with a substantially lower mortality in critically ill patients (shock, obtundation) with typhoid fever compared with those who received chloramphenicol alone (10 versus 55 percent).[42] Whether these findings will be confirmed in the "post-chloramphenicol era" and in different clinical settings is uncertain, but severe typhoid fever remains one of the few indications among acute bacterial infections for corticosteroid therapy infections.[112] Dose in adults and children with severe disease (delirium, obtundation,

stupor, coma, or shock) consists of an initial dose of 3 mg/kg followed by 1 mg/kg every six hours for a total of 48 hours.

Ileal perforation:

Typhoid ileal perforation usually occurs in the third week of febrile illness and is due to necrosis of the Peyer's patches in the anti-mesenteric bowel wall.[113] Affected patients present with increasing abdominal pain, distension, peritonitis, and sometimes secondary bacteraemia with enteric aerobic and anaerobic microorganisms.

Prompt surgical intervention is usually indicated, as is wider antimicrobial coverage to cover faecal peritonitis. The extent of surgical intervention remains controversial; the best surgical procedure appears to be segmental resection of the involved intestine, when possible.[114, 115]

In a retrospective review from West Africa including 112 patients undergoing laparotomy for typhoid perforation, most of the perforations were single (80 percent) and in the terminal ileum.[116] Primary repair was successful in 84 percent of cases, although re-operative management was required in some patients who did not respond immediately. Even with surgery, mortality rates of 14, 16, and 34 percent have been reported in series from Nigeria, Togo, and the Ivory Coast.[116-118]

Relapse:

Relapse of typhoid fever after clinical cure can occur in immunocompetent individuals; in such cases, it typically occurs two to three weeks after resolution of fever. Earlier studies in which the bacteriostatic agent chloramphenicol was the standard drug used noted relapse rates of 10

to 25 percent.[119] Most recent studies, which include multidrug resistant *S. typhi* infections and newer antibiotics, have noted lower relapse rates of 1 to 6 percent.[42, 120] Thus, the fluoroquinolones may reduce relapse rates when the organisms are fully sensitive. An additional course of therapy with a drug to which the organism is clearly sensitive is indicated for relapsing illness. Longer treatment courses with third generation cephalosporins are also reasonable.

Management of Carriers

Chronic carriers do not develop recurrent symptomatic disease. They appear to have reached an immunologic equilibrium in which they are chronically colonized, and may excrete large numbers of organisms, but have high levels of systemic immunity and do not develop clinical disease. However, chronic carriers represent an infectious risk to others, particularly if involved in food preparation. For this reason, eradication of carriage is usually attempted once such individuals are identified.

The fluoroquinolones appear to be much more effective and better tolerated than ampicillin for eradication of chronic carriage. In one study of 23 carriers, for example, the cure rate with norfloxacin (400 mg orally twice daily for 28 days) was 86 percent in those with normal gallbladders and 75 percent in those with gallstones.[121] Several smaller studies, evaluating 10 to 12 patients each, have found that Ciprofloxacin (500 or 750 mg orally twice daily) for 14 to 28 days eliminated carriage in 90 to 93 percent of cases.[122]

Thus, attempted eradication with four weeks of fluoroquinolone therapy is a reasonable approach, with subsequent consideration of additional therapy and cholecystectomy, if needed and appropriate.

Prevention

Typhoid fever results from the ingestion of contaminated food or water. For travelers, the main mechanism of transmission is ingestion of the local cuisine in areas where sanitation and personal hygiene may be poor. The inoculum in food is likely higher than that in contaminated water.

Typhoid vaccines

There are two vaccines available for protection against *S. typhi*: live oral *S. typhi* vaccine strain TY21a and parenteral Vi polysaccharide vaccine. Neither is completely effective against *S. typhi* and neither provides protection against paratyphoid fever.

For travellers to high-risk areas such as the Indian subcontinent, typhoid vaccination may provide protection at very little risk. In a review of laboratory-confirmed cases reported to the Centres for Disease Control and Prevention (CDC) between 1994 and 1999, 75 percent of cases were associated with travel; only 4 percent of these travellers had been vaccinated.[123]

In endemic areas, prevention of enteric fever would require implementing immunization for young children. In a study including more than 37,000 children two to five years of age, the parenteral Vi polysaccharide vaccine was useful for inducing both direct and indirect protection (overall protection was 57 percent).[124] These data suggest that further information is required regarding vaccination in the prevention of typhoid in endemic areas. Natural infection does not provide complete protection against recurrent illness (which is not the same as relapsed infection). One study suggests early treatment of natural infection may blunt humoral responses to capsular antigens.[125] Vaccination may be considered even after clinical illness, particularly

in those not living in endemic areas, if re-exposure is expected. The optimal timing for vaccination following clinical illness is not known.

Experimental Vaccines

Research is ongoing for alternative vaccines for the prevention of typhoid due to the inconvenience of the oral vaccine and the frequent need for re-immunization with both the oral and injectable formulations.[126]

One vaccine far along in development follows the model of other polysaccharide-protein conjugate vaccines (e.g., *Haemophilus influenza* and *Streptococcus pneumoniae* vaccines) by conjugating the Vi capsular polysaccharide of *S. typhi* to a nontoxic recombinant *Pseudomonas aeruginosa* exotoxin A. The vaccine was tested for safety, immunogenicity, and efficacy in a randomized controlled trial in Vietnamese children ages two to five years.[127] *S. typhi* was isolated from 4 of 5525 children receiving two doses of vaccine compared with 47 of 5566 injected with placebo for a 91.5 percent efficacy over the subsequent 27 months. This efficacy persisted at 89 percent over 19 additional months of passive surveillance.[128] Although not yet available, this vaccine holds promise for the immunization of individuals ≥2 years of age.

AIMS AND OBJECTIVES

General objective

To assess the efficacy of third generation Cephalosporin vs. Azithromycin, third generation Cephalosporin vs. combined therapy of both agents and Azithromycin vs. combined therapy of both agents, for the treatment of uncomplicated Enteric Fever in endemic population.

Specific objectives

Primary outcome

- Fever clearance time (an axillary temperature $<37.5^0$C)
- Time to clearance of bacteremia

Secondary outcome

- Treatment failure (defined as the need to switch antibiotic treatment according to physician's decision)
- Development of typhoid-related complications
- Late relapse (within one month of presentation)
- Fecal carriage
- Adverse drug reactions (if any)

MATERIALS AND METHODS

Study Design	Hospital-based non-blinded, randomized, prospective interventional trial
Type of study	Experimental Study (patients were randomly assigned into three treatment arms)
Study variables	Independent variable – Type of antibiotic regimen Dependent variable – Fever clearance time, clearance of bacteremia, development of typhoid-related complications, late relapse, fecal carriage and adverse drug reactions
Period of Study	The study was conducted during the period from October 2012 to October 2014
Study Site and its Justification	Dhulikhel Hospital, Dhulikhel, Nepal. Dhulikhel Hospital resides in a region where enteric fever is highly endemic. The antibiotic susceptibility profile of the pathogen in that area coincides with the multidrug resistant strain common in the Indian subcontinent.
Target Population	Eligible subjects were those who fulfill the following inclusion criteria, irrespective of age
Inclusion criteria	All patients with blood culture-proven Enteric Fever (*S. typhi* or *S. paratyphi* A) at a single hospital in Dhulikhel, Nepal, who have given their informed consent to participate in the study
Exclusion criteria	• Allergy to cephalosporins or macrolides

	• Patients already on antibiotics • Major Enteric Fever-associated complications • Significant underlying illness • Pregnancy and / or lactation
Sampling Methods	Non-probability simple random sampling. Sample was selected solely from patients with culture-positive Enteric Fever who willingly attended Dhulikhel Hospital. Randomization was performed according to order of arrival (first patient was assigned to first arm; second patient was assigned to second arm, etc.)
Sample Size	Power analysis indicated minimum sample size of 69 patients (sub-divided into 3 treatment groups of 23 each), assuming a 36 hour difference in time to defervescence among treatment groups and given alpha error probability of 0.05 and power of 0.80.
Ethical Consideration	Prior to the commencement of the study, the research protocol was approved by the Academic Committee and Institutional Review Committee of Kathmandu University School of Medical Sciences. The aims and objectives of the study were well explained to the patients or their legal guardians in easily understandable local language and written consent (see Annex II) was taken from each patient or their legal guardians. It was assured that all information and records would be kept strictly confidential. Participants

were informed that they could withdraw from the study at any time without giving reason and without fear. The cost of the investigations was borne by Dhulikhel Hospital.

Data Collection Procedure

Patients clinically suspected for enteric fever but underwent medical assessment and blood tests were obtained.

Cohort was randomly allocated into three treatment arms as follows:

Three arms of the study were:

- Inj. Ceftriaxone 2 grams IV OD
- Tab. Azithromycin 500 mg PO OD
- Inj. Ceftriaxone 2 grams IV OD + Azithromycin 500 mg PO OD

Injection Ceftriaxone which is a third generation cephalosporin was used in the study.

Subjects who were found to have blood cultures positive for *S. typhi* or *S. paratyphi* were eligible for enrollment in the study.

Fever duration before antibiotic treatment was recorded.

During hospitalization vital signs, physical examination, ECG, laboratory tests (CBC and quantitative CRP), urinalysis and repeated blood cultures were collected. Stool cultures were taken on follow-up evaluation to assess carriage.

For those who were not hospitalized, the patient or the patient party would record the axillary temperature at least twice a day and provide the record to the physicians on follow up.

Testing of the organism for susceptibility to various antibiotics was performed, and in case of resistance to current regimen, treatment was changed accordingly and patient excluded from the study.

Patients were discharged from hospital 24-48 hours following defervescence or upon their request and required to complete a 7-day antibiotic course (in case of persistence of fever, treatment was longer).

For the study workflow chat, see Annex I.

Tools and Techniques for Data Collection

Demographic data, fever duration and antibiotic treatment prior to hospitalization were collected through a questionnaire written in Nepali language.

Vital signs and ECG were recorded using designated devices.

Physical examination was performed by the ward's physicians.

Laboratory tests, including urinalysis and stool cultures were processed in the hospital's laboratory.

Patients' blood samples were cultured in the hospital's microbiological laboratory using BACTEC radiometric blood cultures. Cultures were subsequently tested for susceptibility to various antibiotics.

All the aforementioned variables were collected and recorded using a pre-designed proforma (see Annex II).

Data management and analysis

Data including subject's demographics, questionnaire, arm of treatment, hospitalization and follow-up were recorded in Proforma (see Annex II) and later managed by MS Excel and analysis done using SPSS version 16.0. Descriptive statistics was used to calculate frequency, percentage. For analytical study, ANOVA was applied for numerical variable and chi square test was used for categorical variable. The findings were summarized by using tables, bar

diagrams and pie charts. Results were expressed as mean ± standard deviation. Statistical significance was defined as $P<0.05$.

Study definitions

Fever – Axillary temperature of 38°C and above.

Time to defervescence – time taken from the beginning of administration of antibiotic regimen until first measurement of temperature persistently lower than 37.5°C.

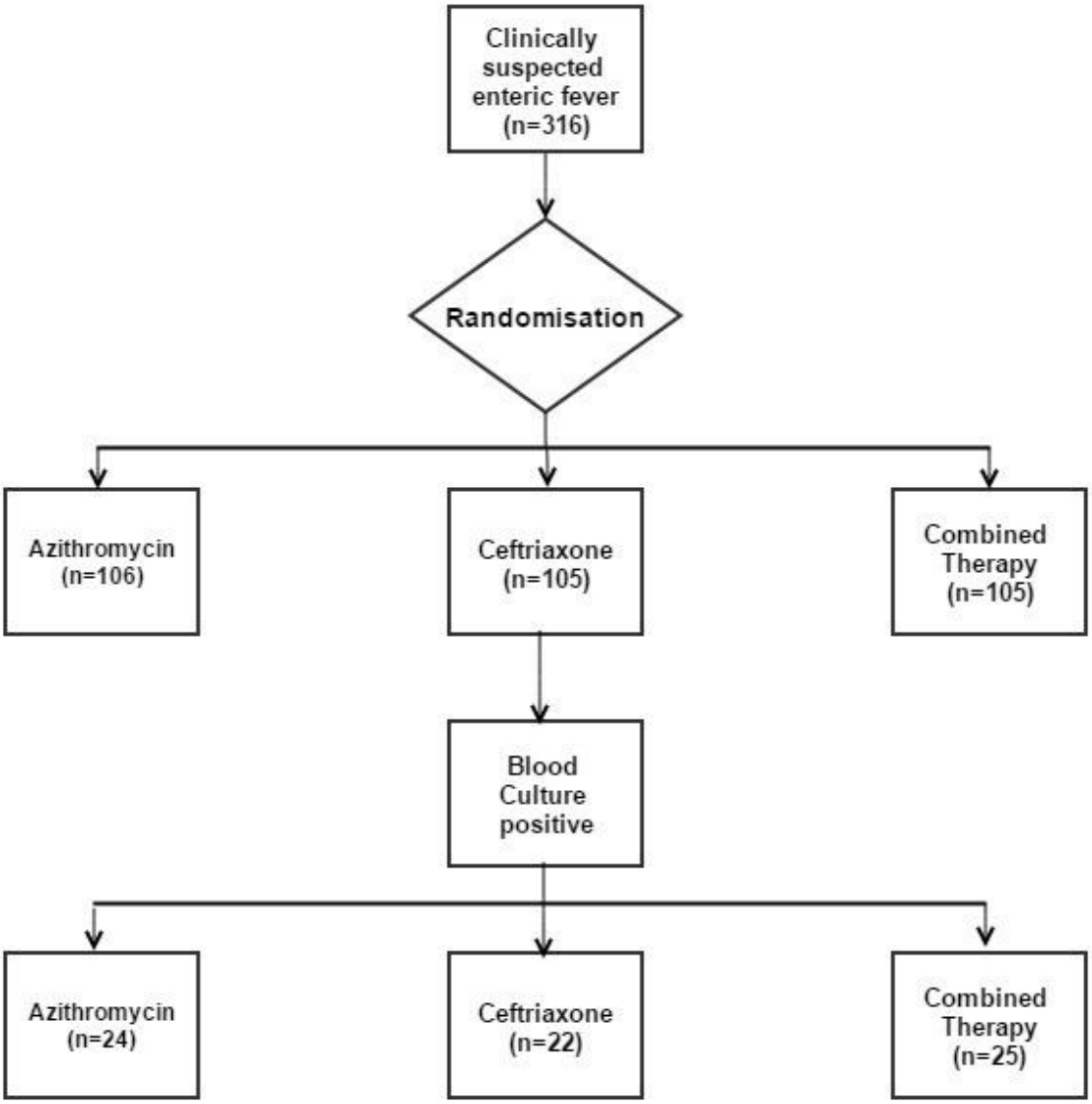
Confirmed case of Enteric Fever – A patient with fever, which has lasted for at least three days, with a laboratory- confirmed positive blood culture of either *S. typhi* or *S. paratyphi*.

Chronic carrier of Enteric Fever - Excretion of *S. typhi* in stool one month or more after relapse of acute Enteric Fever.

OBSERVATIONS AND RESULTS

Participant flow and recruitment

Between October 2012 and October 2014, 316 patients raised a clinical suspicion of enteric fever and were assessed for eligibility, of which 71 had positive blood cultures for either *S. typhi* or *S. paratyphi*.



Flowchart 1: Showing Participant flow and recruitment

As shown in Flowchart 1, the cases were randomized into three treatment arms- 24 (33.8%) were treated with Azithromycin, 22 (31.0%) cases were treated with Ceftriaxone and 25 (35.2%) cases were treated with a combination of the aforementioned antibiotics.

Among culture-positive cases, 49 patients (69%) were presented to OPD and 22 patients (31%) presented to ER.

42 (59.2%) subjects were hospitalized and 29 (40.8%) treated as outpatients.

Average time for hospitalization among in-patients was 5.48 ± 1.49 days (2-9 days).

All patients who were admitted were seen daily by at least one physician and those who were treated on outpatient basis were seen on third day.

For those who were not hospitalized, the patient or the patient party recorded the axillary temperature at least twice a day and provided the record to the physicians on follow up.

All patients included in the study were seen by a physician on the seventh day following their presentation for follow-up examination.

52(73%) patients had provided their phone number and an follow-up was attempted at least one month after recovery. 26 subjects were contacted successfully, of which none reported relapse of symptoms and all agreed to provide a repeated stool sample. No samples were found to be positive for *S. typhi* or *S. paratyphi*, thus indicating carriage of the pathogen.

Demographics

Among subjects participating in the study among which 40 (56.3%) were males and 31(43.7%) were females.

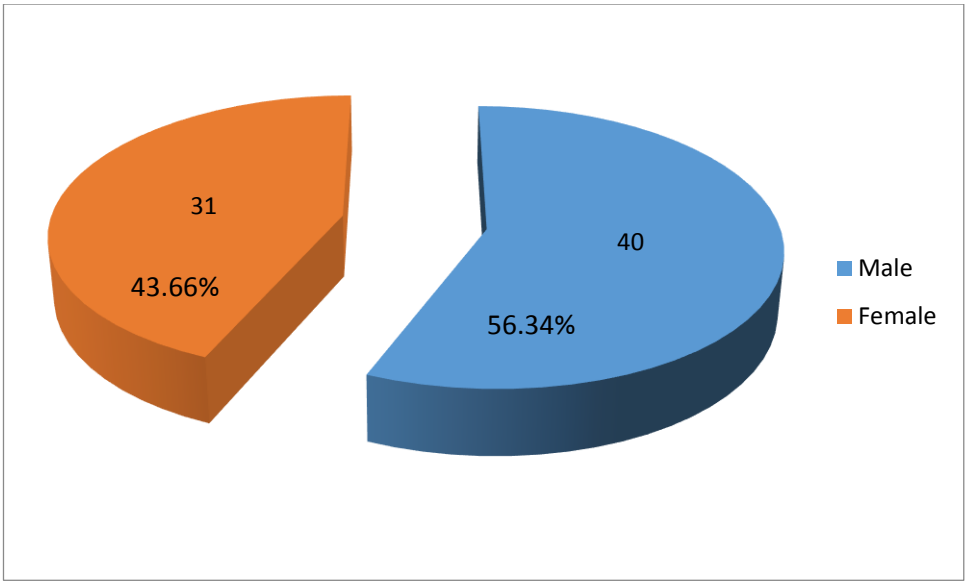


Figure 8 Pie chart showing sex distribution of enteric fever cases

Fifteen (21.2%) were between 15-19 years of age, 29 (40.8%) between 20- 24 years of age, 10 (14.1%) between 25 to 29 years of age, 5 (7%) between 30 to 34 years of age, 2 (2.8%) between 35 and 39 years of age and 10 (14.1%) were older than 39 years of age. See Fig. 9

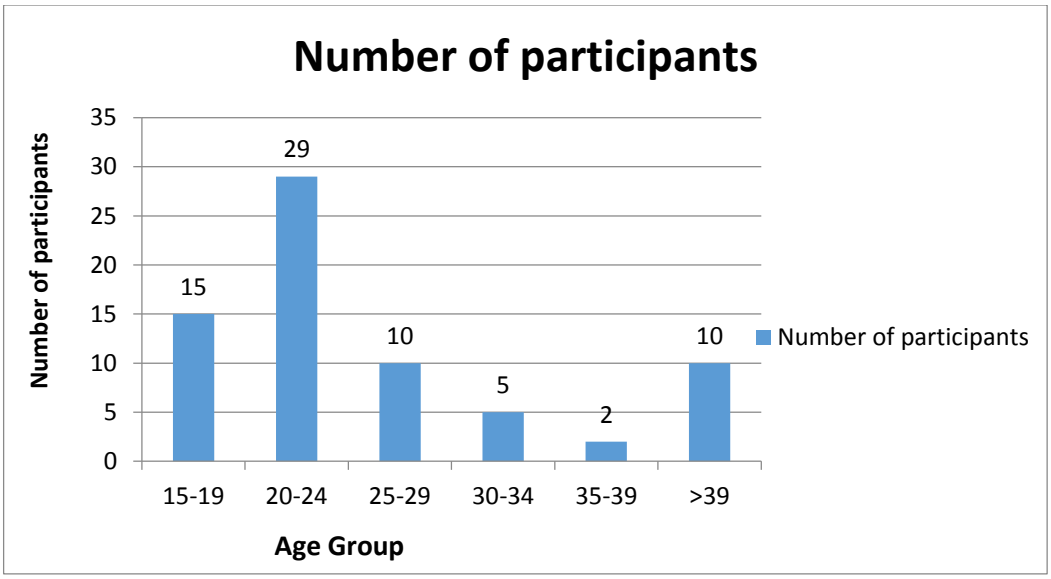


Figure 9: Bar diagram showing distribution of age groups of the patient enrolled in the study

Thirty-one of the subjects were students, 8 were businessmen, 14 were housewives, 9 were farmers and 4 were service holders, 3 were laborers and 2 were health professionals. The rest did not specify an occupation. (See Table 1)

Thirty-one were married, 38 were unmarried and 2 widows.

Four patients reported underlying illnesses of hypertension, one had COPD, one had asthma and 2 had diabetes mellitus. The rest of the subjects did not suffer from any known chronic diseases. (See table 1)

Table 1 Socio-demographic characteristics of patients (n=71)

	Variables	Number	Percentage (%)
Age	15-19	15	21.1
	20-24	29	40.8
	25-29	10	14.1
	30-34	5	7.0
	35-39	2	2.8
	Above 39	10	14.1
Gender	Male	40	56.3
	Female	31	43.7
Occupation	Agriculture	9	12.7
	Business	8	11.3
	Health professional	2	2.8
	Housewife	14	19.7
	Laborer	3	4.2
	Service	4	5.6
	Student	31	43.7
Marital status	Married	31	43.7
	Unmarried	38	53.5
	Widow	2	2.8

Baseline Characteristics

The baseline age and the number of days of fever did not vary significantly in the three groups. Table 2 shows the baseline age and fever before the presentation was comparable among the three groups.

Table 2 Baseline age and number of days of fever in three arms

Characteristics	Azithromycin arm (1)	Ceftriaxone arm (2)	Azithro + Ceftriaxone arm (3)	P- value
Age	29.50 ±16.93	24.12 ± 7.69	27.52 ± 10.21	0.337
Fever before presentation (days)	5.62 ±1.81	5.59 ± 2.3	6.76 ± 2.59	0.130

Presentation

The average duration of fever prior to hospitalization was 6.01 days and was not significantly different among the three arms of study. Apart from fever, 58 (81.7%) presented with headache, 51 (71.8%) with chills, 41 (57.7%) with malaise, 36 (50.7%) with nausea, 31 (43.7%) with sweating, 30 (42.3%) with anorexia, 20 (28.2%) with diarrhea, 11 (15.5%) with myalgia, 9 (12.7%) with arthralgia, 9 (12.7%) with constipation, 8 (11.3%) with cough, 7 (9.9%) with abdominal pain, 6 (8.5%) with vomiting, 3 (4.2%) with sore throat and 1 (1.4%) with conjunctival injection. (See table 3)

Table 3 Distribution according to the presenting complaints (n=71)

Presenting complaints	Number of cases	Percentage (%)
Fever	71	100
Headache	58	81.7
Chills	51	71.8
Malaise	41	57.7
Nausea	36	50.7
Sweating	31	43.7
Anorexia	30	42.3
Diarrhoea	20	28.2
Myalgia	11	15.5
Arthalgia	9	12.7
Constipation	9	12.7
Cough	8	11.3
Abdominal pain	7	9.9
Vomiting	6	8.5
Others		
Sore throat	3	4.2
Conjunctival injection	1	1.4

Upon presentation 23 (32.4%) exhibited non-tender hepatosplenomegaly,5 (7%) subjects had rash and in 2 (2.8%) abdominal tenderness was elicited. The study did not attempt to find isolated hepatomegaly and splenomegaly but rather hepatosplenomegaly. See Table 4

Table 4 Physical findings elicited at the time of presentation (n=71)

Physical Examination	Number of cases (%)	Azithromycin arm	Ceftriaxone arm	Combination therapy arm
Hepatosplenomegaly	23 (32.4%)	6 (25%)	10 (45.4%)	7 (25%)
Rash	5 (7%)	2 (8.33%)	2 (9.1%)	1 (4%)
Abdominal tenderness	2 (2.8%)	2 (8.33%)	0 (0%)	0 (0%)

Blood tests were obtained from all subjects. Thirteen (18.3%) showed leukocytosis (9 in *S. typhi* and 4 in *S. paratyphi* cases) and 5 (7.1%) showed leukopenia.

Bacteriologic Characteristics

Among culture proven Enteric Fever cases, 39 (54.9%) were caused by *S. typhi* and 32 (45.1%) *S. paratyphi*, subdivided in to type A (27, 38%) and B (5, 7.1%). (See Table 5)

Average age for acquiring *S. typhi* infection was 29.9 years, compared to 24.2 years for *S. paratyphi*. 21.2 years for *S. paratyphi*.

Table 5 Bacteriologic Characteristic and their frequency **(n=71)**

Bacteria	Frequency (%)	Azithromycin arm	Ceftriaxone arm	Combination therapy arm
Salmonella typhi	39(54.9%)	13 (54.2%)	13 (59.1%)	13 (52.0%)
Salmonella paratyphi A	27(38.0%)	8 (33.3%)	8 (36.4%)	11 (44%)
Salmonella paratyphi B	5(7.0%)	3 (12.5%)	1 (4.5%)	1 (4.0%)
Total	71(100.0%)	24	22	25

S. typhi was found to be resistant to nalidixic acid in 37 (94.87%) cases and partially sensitive to ciprofloxacin in 5 (12.8%) and resistant in 5 (12.8%) cases. Two cases (5.13%) of *S. typhi* were partially sensitive to Azithromycin. All cases were sensitive to Ceftriaxone.

S. paratyphi was found to be resistant to nalidixic acid in 31 (96.8%) cases and partially sensitive or resistant to ciprofloxacin in 32 (100%) cases. 5 cases (15.6%) of *S. paratyphi* were

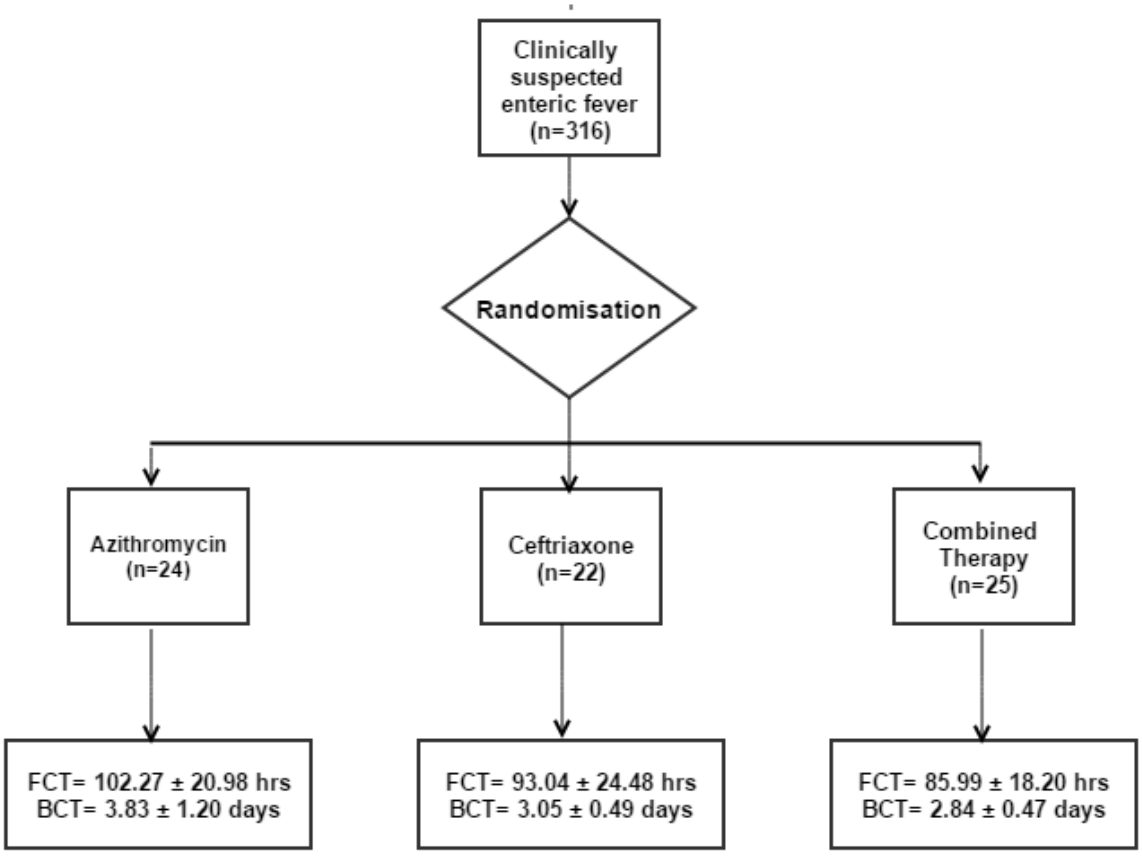
resistant or partially sensitive to Azithromycin. One (3.7%) case was resistant to Ceftriaxone. (For a complete list of antibiotic resistances see table 6)

Table 6: Antibiotic susceptibility according to the organisms

		Bacteria			Total
Drugs		*S. typhi*	*S. paratyphi* A	*S. paratyphi* B	
Ceftriaxone	s	39	26	5	70
	r	0	1	0	1
	ps	0	0	0	0
Azithromycin	s	37	23	4	64
	r	0	1	1	2
	ps	2	3	0	5
Nalidixic acid	s	2	1	1	3
	r	36	26	5	67
	ps	0	1	0	3
Ciprofloxacin	s	5	0	0	5
	r	5	0	1	6
	ps	29	27	4	60
TMP/SMX	s	39	27	5	71
	r	0	0	0	0
	ps	0	0	0	0
Chloramphenicol	s	39	25	5	69
	r	0	2	0	2
	ps	0	0	0	0
Ampicillin	s	38	17	3	58
	r	0	5	1	6
	ps	0	5	1	6
Gentamycin	s	39	27	5	71
	r	0	0	0	0
	ps	0	0	0	0

Abbreviations: s: sensitive, r: resistance, ps: partially sensitive

Fever Clearance Time and Bacteremia Clearance Time



Flowchart 2 Showing Fever Clearance Time (FCT) and Bacteremia Clearance Time (BCT) in three different arms

Fever clearance time was 102.27 ± 20.98 hrs. for subjects treated with Azithromycin, 93.04 ± 24.48 hrs. for subjects treated with Ceftriaxone and 85.99± 18.24 hrs. for those receiving a combined regimen. (See Table 7)

Table 7: Fever Clearance Time in the three treatment arms

Treatment Arm	Azithromycin	Ceftriaxone	Combined Therapy
Fever Clearance Time (FCT) in hrs.	102.27±20.98	93.04±24.48	85.99±85.99

A significant difference between the three groups is found using ANOVA test for fever clearance time. (See Table 8)

Table 8 ANOVA Table for the three treatment arms for fever clearance time

Fever clearance	Sum of Squares	df	Mean Square	F	P-value
Between Groups	3258.519	2	1629.259	3.610	0.32
Within Groups	30688.754	68	451.305		
Total	33947.273	70			

* The mean difference is significant at the 0.05 level.

Post hoc analysis using LSD (Least Significant Difference) revealed a statistically significantly longer fever clearance time with Azithromycin (p=0.009) in comparison to combination of Ceftriaxone and Azithromycin, but not with Ceftriaxone alone (p =0.146), whereas the fever clearance time between Ceftriaxone alone and combination of Ceftriaxone and Azithromycin is not statistically significant (p=0.260). (See Table 9)

Table 9: Post hoc analysis using LSD for the three treatment arms for fever clearance time

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.
Azithromycin	Ceftriaxone	9.2254	6.2704	.146
	Azithromycin+Ceftriaxone	16.2808*	6.0710	.009
Ceftriaxone	Azithromycin	-9.2254	6.2704	.146
	Azithromycin+Ceftriaxone	7.0555	6.2102	.260
Azithromycin+ Ceftriaxone	Azithromycin	-16.2808*	6.0710	.009
	Ceftriaxone	-7.0555	6.2102	.260

* The mean difference is significant at the 0.05 level.

For all of the subjects a second blood culture was obtained on the 3rd day after enrollment to the study in order to establish elimination of bacteremia. Nine cases were found to exhibit persistent bacteremia on the second blood culture, of which 8 received Azithromycin, 1 received Ceftriaxone, none received the combination of the aforementioned regimens. For all these patients a third culture was obtained and was found to be negative. The average time to clearance of bacteremia was 3.24 days – 3.83± 1.20 days for subjects under Azithromycin treatment, 3.05±0.49 days for subjects under Ceftriaxone treatment and 2.84±0.47 days for the combination of the two. (See Table 10)

Table 10: Bacteremia Clearance Time in the three treatment arms

Treatment Arm	**Azithromycin**	**Ceftriaxone**	**Combined Therapy**
Bacteremia Clearance Time (BCT) in days	3.83 ± 1.20	3.05 ± 0.49	2.84 ± 0.47

A significant difference between the three groups is found using ANOVA test for bacteremia clearance time. (See Table 11)

Table 11: ANOVA Table for the three treatment arms for bacteremia clearance time

Days to neg CS	**Sum of Squares**	**df**	**Mean Square**	**F**	**P-value**
Between Groups	13.282	2	6.641	10.346	.000
Within Groups	43.648	68	.642		
Total	56.930	70			

* The mean difference is significant at the 0.05 level.

Post hoc analysis using LSD (Least Significant Difference) revealed a statistically significantly longer bacteremia clearance time with Azithromycin as compared to with Ceftriaxone (p-value =0.001) and combination of Ceftriaxone plus Azithromycin (p= <0.001), whereas the bacteremia clearance time between Ceftriaxone alone and combination of Ceftriaxone and Azithromycin is not statistically significant (p= 0.383). (See Table 12)

Table 12: Post hoc analysis using LSD for the three treatment arms for bacteremia clearance time

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.
Azithromycin	Ceftriaxone	.788*	.236	.001
	Azithromycin+Ceftriaxone	.993*	.229	.000
Ceftriaxone	Azithromycin	-.788*	.236	.001
	Azithromycin+Ceftriaxone	.205	.234	.383
Azithromycin+ Ceftriaxone	Azithromycin	-.993*	.229	.000
	Ceftriaxone	-.205	.234	.383

* The mean difference is significant at the 0.05 level.

Urine culture was obtained from 26 patients and was found negative in all cases. Forty (56.34%) patients have provided their phone number and were contacted at least one month after recovery.

None of the cases in the three arms reported relapse.

Repeated stool cultures were obtained from 19 (26.8%) cases and showed no growth of the organism.

There were no reported cases of complications or adverse drug reactions.

DISCUSSION

The present study compares the efficacy of Ceftriaxone vs. Azithromycin, Ceftriaxone vs. combined therapy of both agents, and Azithromycin vs. combined therapy of both agents in the treatment of uncomplicated enteric fever cases aged 16 to 81 years, who presented to Dhulikhel Hospital, Kathmandu University Hospital, Dhulikhel, Kavre between October 2012 and October 2014.

During the period, 316 patients raised a clinical suspicion of enteric fever and were assessed for eligibility. The cases were randomized in the three arms- Tab. Azithromycin 500 mg PO OD arm, Inj. Ceftriaxone 2 grams IV OD arm and Combination of Inj. Ceftriaxone 2 grams IV OD and Azithromycin 500 mg PO OD therapy arm. A total of 71 subjects had positive blood cultures- 24 (33.8%) in Azithromycin arm, 22 (31.0%) cases in Ceftriaxone arm and 25 (35.2%) in combination therapy arms.

The three groups were compared in terms of distribution by sex and days of fever before the presentation. Severity of signs was equally distributed in the three groups. The presenting duration of fever in this study was within the time frames (1-14 days) as reported in previous trials on the treatment of typhoid fever.[8,129,130]

Male (56.3%) population was found to be predominantly affected as compared to females (43.7%) in the study. Similar observations were made by N Sharma et al[4] from Kavre, Nepal, Zailani et al[131] from Nigeria, Butler et al[50] from Bangladesh. In all these countries, the male population is more exposed for working and other purposes than females, which may explain the higher infection rates obtained for the males in the population.[132] Other factors such as greater mobility and social behavioral attitudes may attribute to the disproportionate number

of cases in the male population.[133] Further study is therefore needed to determine the underlying risk factors, to prevent certain gender and age groups being infected by enteric fever.

In this study, 54.9% of the patients were in their second decade of life which is comparable with studies done by Zailani et al[131] in Nigeria, and N Sharma et al[4] at Dhulikhel Hospital about a decade back. Therefore early diagnosis and management of enteric fever cases is economically beneficial.

Stuart et al[40] reported that in the pre-antibiotic era constipation occurred more frequently than diarrhea. Subsequent reports by Gasem et al[46] suggested that these symptoms occur with approximately equal frequency or that diarrhea may be more common. In this study the frequency of diarrhea was 28.2% while that of constipation was 12.7%. The reasons for the variability in enteritis among Salmonella strains are not certain. In a cell culture model of gastroenteritis, Salmonella strains associated with enteritis induced IL-8-mediated neutrophil transmigration across the epithelial cell; these observations were not seen with *S. typhi* or other strains not associated with enteritis.[134] The mutated IL-8 response and relative absence of neutrophil infiltration seen with *S. typhi* may be responsible for this shift in symptom from constipation to diarrhea.

In this study, apart from fever, 58 (81.7%) presented with headache, 51 (71.8%) with chills, 41 (57.7%) with malaise, 36 (50.7%) with nausea, 31 (43.7%) with sweating, 30 (42.3%) with anorexia, 20 (28.2%) with diarrhea, 11 (15.5%) with myalgia, 9 (12.7%) with arthralgia, 9 (12.7%) with constipation, 8 (11.3%) with cough, 7 (9.9%) with abdominal pain, 6 (8.5%) with vomiting, 3 (4.2%) with sore throat and 1 (1.4%) with conjunctival injection. In a systematic literature review on clinical presentations of enteric fever over a 30 year period (1977-2007), Jombo et al[135] compiled that apart from fever, headache was present in 45%, chills in 21.9%,

anorexia in 19.5%, fatigue in 12.8%, nausea in 10.8% and cough in 3.1%. The frequency of presentation varies in the sense that headache, chills, cough and anorexia were found to be in higher frequency in this study. This could be due to the geographical and ethnic variation of the study population. The clinical presentations are comparable to that reported by Maskey et al[136] from Kathmandu, Nepal.

In this study, 32.4% subjects exhibited hepatosplenomegaly and 7% rash. Jombo et al[135] had found splenomegaly in 7.8%, hepatomegaly in 3.2% and rash in 3.1%. The study did not attempt to find isolated hepatomegaly and splenomegaly but rather hepatosplenomegaly.

This study revealed that 18.3% subjects had leukocytosis and 7.1% had leukopenia. Similar finding were noted in a systematic literature review by Jombo et al[135] with 14.5% leukocytosis and 9.9% leucopenia.

Infection due to *Salmonella paratyphi A* and *B* is found to be less common (45.1%) than infection due to *S. typhi* (54.9%) in this study; and cases due to *Salmonella paratyphi C* were not reported. A study done by Maskey et al[136] at Patan Hospital in Kathmandu, Nepal revealed *S. typhi* isolates in 67% of cases and *S. paratyphi* isolates in 33% of cases. The increasing proportion of *S. paratyphi* A has previously been reported from Nepal -from 23% in the mid-1990s to 34% of isolates from 1999 to 2003.[2]

Two cases (5.13%) of the *S. typhi* were partially sensitive to Azithromycin. All cases were sensitive to Ceftriaxone. *S. typhi* was found to be resistant to nalidixic acid in 37 (94.87%) cases and partially sensitive to ciprofloxacin in 5 (12.8%) and resistant in 5 (12.8%) cases.

S. paratyphi was found to be resistant to nalidixic acid in 31 (96.8%) cases and partially sensitive or resistant to ciprofloxacin in 32 (100%) cases.5 cases (15.6%) of *S. paratyphi* were resistant or partially sensitive to Azithromycin. One (3.7%) case was resistant to Ceftriaxone. The increase in *S. paratyphi* A parallels the trend of more rapidly increasing flouroquinolone resistance in *S. paratyphi* A compared with *S. typhi*. This helps to explain the poor clinical response to ciprofloxacin and accentuates the need to modify the existing breakpoints. Many clinicians in Nepal and elsewhere do not appreciate the poor relationship between fluoroquinolone disc testing and clinical response, and nalidixic acid disc testing is rarely performed or its significance understood.[2] Consequently, ciprofloxacin is still considered first-line treatment for enteric fever in Nepal, and current laboratory drug susceptibility reports encourage persistent suboptimal treatment, which will continue to fuel the development of further resistance.[2]

The mean FCT observed in the study was 102.27 ± 20.98 hrs. for Azithromycin which is comparable to 106 hrs. observed in a randomized control trial done in Vietnam[109] and 135.0 hrs. seen in a study by Chinh et al[129]. Besides, the FCT was observed to be 93.04 ± 24.48 hrs. for subjects treated with Ceftriaxone. Similar results with FCT of 86.4 ± 38.4 hrs. and 93.6 ± 24 hrs. have been obtained by Franck et al in 2004[8] and from Abbassia Fever Hospital, Cairo in 2000[137] respectively. The FCT between Ceftriaxone and Azithromycin as shown by this study is, however, not significant (p =0.146) as noted by Frenck et al in 2004[8] and from Abbassia Fever Hospital, Cairo in 2000.[137]

Trivedi et al[110] comparing the safety and efficacy of Azithromycin over the alternate drugs and found that the treatment with Azithromycin did not differ significantly from Ceftriaxone for microbiological failure or in fever clearance time but it significantly reduced the chance of

relapse. In a meta-analysis of 20 prospective trials by Butler et al[111] enrolled more than 1,600 culture-positive patients has also concluded that Azithromycin was proven better than Ceftriaxone in cure rate and relapse rate. This observation has been attributed to the fact that Azithromycin is concentrated intracellularly at levels 50 to 100 greater than serum levels.[105] This study, however, showed a statistically significantly longer fever clearance time with Azithromycin (p=0.009) in comparison to combination of Ceftriaxone and Azithromycin, but not with Ceftriaxone alone (p =0.146), whereas the fever clearance time between Ceftriaxone alone and combination of Ceftriaxone and Azithromycin was not statistically significant (p=0.260).

There is no randomized clinical trial till date demonstrating that combination antimicrobial therapy is superior to monotherapy for enteric fever in terms for fever clearance time. In a study of 37 individuals with nalidixic-acid resistant *S. paratyphi* A bacteremia, who were identified as part of an outbreak among Israeli travelers returning from Nepal, all patients improved without complications, but time to defervescence was shorter among those who were treated with Ceftriaxone and Azithromycin compared with Ceftriaxone alone.[138] Given the small size and observational nature of the study and the finding that all patients were infected by a single isolate, an additional study is needed to determine if there is any benefit of using two drugs over one.

The average time to clearance of bacteremia was 3.83 ± 1.20 days for subjects under Azithromycin treatment, 3.05 ± 0.49 days for subjects under Ceftriaxone treatment and 2.84 ± 0.47 days for the combination of the two. Statistical analysis revealed significantly longer bacteremia clearance time with Azithromycin as compared to with Ceftriaxone (p-value

=0.001) and combination therapy (p= <0.001), whereas the bacteremia clearance time between Ceftriaxone alone and combination therapy was not statistically significant. (p= 0.383)

Frenck et al[8] also demonstrated a longer bacteremia clearance time with Azithromycin than with Ceftriaxone. This finding is attributable the fact that Azithromycin is concentrated intracellularly at levels 50 to 100 greater than serum levels.[105] There are no trials done till date demonstrating that the combination antimicrobial therapy is superior to monotherapy for enteric fever in terms for bacteremia clearance time.

Frenck et al in 2004[8] and a study from Abbassia Fever Hospital, Cairo in 2000[137] did not show relapse with Azithromycin when used for the treatment of enteric fever. Similar result without any relapse in the Azithromycin arm was observed in this study. However, both the studies showed relapse with Ceftriaxone in 13.3% of cases[8] and 13.89%[137] of cases in contrast to the absence of relapse in Ceftriaxone arm in this study.

Chronic carriage rates after enteric fever infection range from 1 to 6 percent.[82] However, none of the patients who were on follow up had stool culture reports positive for the pathogen. This could be due to the small number (26.8%) of follow up cases in the study.

There were no reported cases of complications or adverse drug reactions. However, in a study done by Parry et al[103] in Vietnam 1.6% subjects treated with Azithromycin reported adverse drug reaction in the form of joint discomfort. Butler et al[139], however, observed higher incidence of adverse drug reaction in the form of gastrointestinal discomfort. Besides, in a study done by Frenck et al,[137] no subject had a serious adverse event but mild pain at the site of injection being the most common adverse event in the Ceftriaxone group.

SUMMARY

The present non-blinded, randomized, prospective interventional study compares the efficacy of Ceftriaxone vs. Azithromycin, Ceftriaxone vs. combined therapy of both agents, and Azithromycin vs. combined therapy of both agents for the treatment of uncomplicated enteric fever in endemic population cases aged 16 to 81 years, who presented to Dhulikhel Hospital, Kathmandu University Hospital, Dhulikhel, Kavre between October 2012 and October 2014.

During the period, 316 patients raised a clinical suspicion of enteric fever and were assessed for eligibility. The cases were randomized in the three arms- Azithromycin arm, Ceftriaxone arm and Combination therapy arm. A total of 71 subjects had positive blood cultures- 24 (33.8%) in Azithromycin arm, 22 (31.0%) cases in Ceftriaxone arm and 25 (35.2%) in combination therapy arm.

During hospitalization vital signs, physical examination, ECG, laboratory tests (CBC and quantitative CRP), urinalysis and repeated blood cultures were collected. For those who were not hospitalized, the patient or the patient party would record the axillary temperature at least twice a day and provide the record to the physicians on follow up. Blood Culture and sensitivity reports were collected and those who were culture negative for *Salmonella species* were excluded.

Patients were discharged from hospital 24-48 hours following defervescence or upon their request and required to complete a 7-day antibiotic course (in case of persistence of fever, treatment was longer).

Among culture proven Enteric Fever cases, 39 (54.9%) were caused by *S. typhi* and 32 (45.1%) *S. paratyphi*, subdivided in to type A (27, 38%) and B (5, 7.1%).Two cases (5.13%) of the *S. typhi* were partially sensitive to Azithromycin. All cases were sensitive to Ceftriaxone. *S. typhi* was found to be resistant to nalidixic acid in 37 (94.87%) cases and partially sensitive to ciprofloxacin in 5 (12.8%) and resistant in 5 (12.8%) cases. *S. paratyphi* was found to be resistant to nalidixic acid in 31 (96.8%) cases and partially sensitive or resistant to ciprofloxacin in 32 (100%) cases. 5 cases (15.6%) of *S. paratyphi* were resistant or partially sensitive to Azithromycin. One (3.7%) case was resistant to Ceftriaxone.

Fever clearance time was 102.27 ± 20.98 hrs. for subjects treated with Azithromycin, 93.04 ± 24.48 hrs. for subjects treated with Ceftriaxone and 85.99 ± 18.24 hrs. for those receiving a combined regimen.

A significant difference between the three groups is found using ANOVA test for fever clearance time. Post hoc analysis using LSD (Least Significant Difference) revealed a statistically significantly longer fever clearance time with Azithromycin (p=0.009) in comparison to combination of Ceftriaxone and Azithromycin, but not with Ceftriaxone alone (p =0.146), whereas the fever clearance time between Ceftriaxone alone and combination of Ceftriaxone and Azithromycin is not statistically significant (p=0.260).

For all of the subjects a second blood culture was obtained on the 3rd day after enrollment to the study in order to establish elimination of bacteremia. Nine cases were found to exhibit persistent bacteremia on the second blood culture, of which 8 received Azithromycin, 1 received Ceftriaxone, none received the combination of the aforementioned regimens. For all these patients a third culture was obtained and was found to be negative. The average time to

clearance of bacteremia was 3.24 days – 3.83 ± 1.20 days for subjects under Azithromycin treatment, 3.05 ± 0.49 days for subjects under Ceftriaxone treatment and 2.84 ± 0.47 days for the combination of the two.

A significant difference between the three groups is found using ANOVA test for bacteremia clearance time. Post hoc analysis using LSD (Least Significant Difference) revealed a statistically significantly longer bacteremia clearance time with Azithromycin as compared to with Ceftriaxone (p-value = 0.001) and combination of Ceftriaxone plus Azithromycin(p= <0.001), whereas the bacteremia clearance time between Ceftriaxone alone and combination of Ceftriaxone and Azithromycin is not statistically significant(p=0.383).

None of the cases in the three arms reported relapse.

Repeated stool cultures were obtained from 19 (26.8%) cases and showed no growth of the organism.

There were no reported cases of complications or adverse drug reactions.

CONCLUSIONS

Combination therapy of Azithromycin and Ceftriaxone is better in fever clearance and bacteremia clearance than Azithromycin alone, whereas it is not significantly different than Ceftriaxone alone in terms of fever clearance and bacterial clearance.

Ceftriaxone is better in bacteremia clearance than Azithromycin. Regarding fever clearance, though Ceftriaxone appears better than Azithromycin, it is not statistically significant.

Ceftriaxone alone is as effective as combination of Ceftriaxone and Azithromycin for treatment of enteric fever.

RECOMMENDATIONS

Adding Tab. Azithromycin with Inj. Ceftriaxone has no advantage in the treatment of enteric fever.

Ceftriaxone should be used rather than Azithromycin as a monotherapy.

INCIDENTAL FINDINGS AND RECOMMENDATIONS

Resistance to Trimethoprim/sulphamethoxazole was not found in any of the *Salmonella species* probably due to the drug not being used for the treatment of enteric fever for a long time. This drug can hence be used for the treatment of enteric fever.

Very few cases of resistance have been found against Ampicillin and Gentamycin.

LIMITATIONS

Selection bias – Subjects participating in the current study are patients with Enteric Fever, who choose to be treated in Dhulikhel Hospital. This may result in exclusion of patients with a relatively milder course of the disease who can be managed in an outpatient setting, or patients who cannot afford to be admitted to the hospital either financially or due to inaccessibility.

Measurement bias and recall bias – Patients might report inaccurate information during follow up evaluation. The temperature recording of patients not hospitalized, though reliable, may not be very accurate.

Compliance bias – Subjects who were asked to complete the antibiotic course in an outpatient setting may have been reluctant to comply with the instructions given upon discharge, which might have led to relapses, complications and persistent carriage of the disease.

External validity – The study was conducted at a single center, while its results ought to be generalized to the entire Indian subcontinent. One may claim that the study population is insufficiently heterogeneous.

Resolution of measurements – For patients who were not admitted, variables such as clearance of defervescence and bacteremia were assessed on a 12 hour interval, thus decreasing the precision of fever clearance time measurement.

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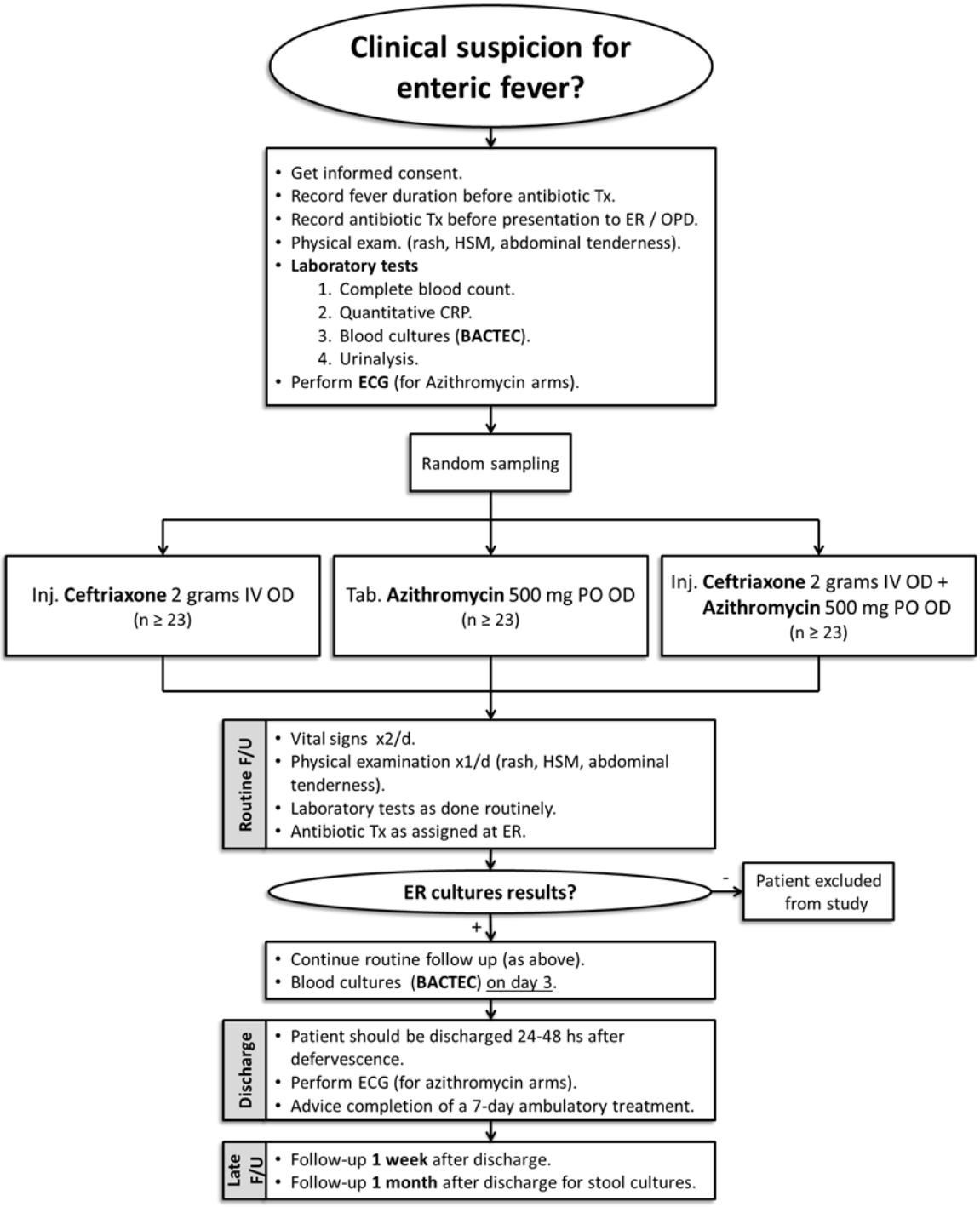
ANNEX

I. Study Workflow chart



II. Performa

Working Proforma

Date:.....

Hospital number:..... Study number:.....

Date of admission:..... Date of discharge:.....

Duration of hospital stay:.....days

Patient admitted from: Emergency☐ OPD☐

I. Identification:
Name:.....
Age:.....years Sex: Male☐Female☐
Occupation:..... Marital status:.....
Address:..... Mobile number: ……

II. Presenting complaints:

- **a.** Fever: Duration:.....days
- **b.** Chills☐
- **c.** Headache☐
- **d.** Cough☐
- **e.** sweating☐
- **f.** Myalgias☐
- **g.** Malaise☐
- **h.** Arthralgia ☐
- **i.** Anorexia☐
- **j.** Nausea☐
- **k.** Vomiting☐
- **l.** Abdominal pain☐
- **m.** Diarrhoea☐
- **n.** Constipation☐
- **o.** Others☐

II. Use of antibiotics prior to admission:.....daysName of antibiotic ……..

III. Past history of typhoid fever: Yes☐No☐

IV. Any significant underlying illness:.....

V. Menstrual and obstretic history: LMP:.....
Pregnant:Yes☐No☐Lactating:Yes☐No☐

VI. Allergy to any drugs:Yes☐No☐if yes specify allergy: .....

VII. Paracetamol intake:.....days

VIII. Vitals:

Date																		
	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm	am	pm
Heart Rate																		
Blood Pressure																		
Respiratoy Rate																		
AxillaryTemperature																		

IX. Physical examination:

Date									
Rash									
Hepatosplenomegaly									
Abdominal tenderness									

X. Investigations:

Date									
Hb									
TLC									
DLC (Neutrophils/Lymphocytes)									
LFT									
ESR/CRP									
ECG									

XI. Culture findings:

	Date	**Sample**	**24 hrs**	**48 hrs**	**72 hrs**	**96 hrs**	**120 hrs**
On admission		Blood					
On admission		Urine					
On Day 3		Blood					
On follow up		Stool					

XII. Sensitivity: (S: sensitive, PS: Partially sensitive, R: Resistant)

Drug	Blood on admission	Blood on Day 3	Urine on admission	StoolOn follow up
Ciprofloxacin				
Ofloxacin				
Ceftriaxone				
Chloramphenicol				
Amoxicillin				
Cotromoxazole				
Nalidixic acid				
Azithromycin				
Cefixime				

XIII. Treatment:

Antibiotics	Dose/Route	Starting date	Stopping date	Duration	Switchover to other antibiotics
Azithromycin					
Ceftriaxone					
Azithromycin+Ceftriaxone					

XIV. Outcome:

- **a.** Cured (free from symptoms and signs) ☐
- **b.** Improved ☐
- **c.** Worsened ☐
- **d.** Unchanged ☐
- **e.** Died ☐

XV. Complications:

- **a.** Gastrointestinal bleeding ☐
- **b.** Gastrointestinal perforation ☐
- **c.** Relapse ☐
- **d.** Adverse drug reactions ☐ (specify reactions: ……)
- **e.** Others ☐

XVI. Condition on discharge:

- **a.** Improved ☐
- **b.** Worsened ☐
- **c.** Unchanged ☐

XVII. Fever clearance time (<37.5C).....hrs

III.Consent

Dhulikhel Hospital

Kathmandu University Hospital

CONSENT FORM

Namaste,

We, Dhulikhel Hospital, Kathmandu University Hospital, are conducting a research on **"A comparison of third generation cephalosporins, Azithromycin and and combined therapy for the treatment of uncomplicated enteric fever"**. The aim of the study is to investigate fever clearance time and determine which regimen is better.

We ask for your voluntary participation in our study.

Participation in this study is not compulsory and you may choose not to answer any individual questions or the entire questionnaire. Whatever information youprovide will be kept strictly confidential.

You will be able to withdraw from the study at any time without giving reason and without fear.

Medications used in the current study are routinely prescribed for the treatment of typhoid fever (and other infectious diseases). Some of their common adverse reactions are: injection site pain, local injection site reactions, diarrhea, abdominal pain, nausea, dyspepsia, flatulence, rash, headache, dizziness.

I have understood the above mentioned explanation and agree to participate in the study.

Name and Signature of the participant: _____

Address: _____

Date: _____

IV. Master Chart