INCIDENCE OF SALMONELLA BACTERAEMIA AND ANTIBIOTIC RESISTANCE OF SALMONELLA IN PAEDIATRIC PATIENTS IN KUMASI

by

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A Thesis submitted to the Department of Clinical Microbiology,

Kwame Nkrumah University of Science and

Technology

in partial fulfillment of the requirements for the degree

of

MASTER OF PHILOSOPHY

Department of Clinical Microbiology

College of Health Science

September 2007

CERTIFICATION

I, Nana Yaa Awua-Boateng, hereby declare that this submission is my own work towards the MPhil and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in text.

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ABSTRACT

Salmonella bloodstream infection, especially due to non-typhoidal strains, is a potential health problem for Ghanaian children and may be complicated by resistance to commonly available antibiotics. This study was undertaken to establish the incidence of Salmonella bacteraemia and antibiotic resistance of Salmonella isolates obtained from paediatric patients presenting at the Komfo Anokye Teaching Hospital (KATH) from January to December, 2005.

Blood specimens were taken from children below the age of twelve years with suspected bacteraemia. The blood was inoculated in Brain Heart Infusion broth and cultured on Blood and MacConkey agar. The bacterial isolates were identified by the Standard Manual Method and the Analytical Profile Index (API) machine. Susceptibility testing was also done.

Out of the 372 bacteriologically confirmed Salmonella bacteraemia cases, 79.6% (296/372) were in children up to 12 years of age. Of the 296 Salmonella isolates, 87.2% (258) were *Salmonella spp* and 12.8% (38) were *Salmonella typhi*. From above, the incidence of *Salmonella typhi* was 1% (38/3908) and *Salmonella spp* 6.6% (258/3908).
Bacteraemia due to *Salmonella spp* was highest in children between the ages of 0-3 years (88.5%) followed by 4-7 years (9.1%) with children between 8-12 years having the lowest (2.4%).
The antibiotic sensitivity patterns for both *Salmonella spp* and *Salmonella typhi* showed that the least sensitivity was towards cotrimoxazole, ampicillin and chloramphenicol. *Salmonella spp* had sensitivities of 20.7%, 12.2% and 22% whilst *Salmonella typhi* had sensitivities of 2.7%, 7.6% and 16.9% towards cotrimoxazole, ampicillin and chloramphenicol respectively.

The Minimum Inhibitory Concentrations (MIC_{90}) for ampicillin (0.156 – 2560ug/ml), chloramphenicol (2.5 – 2560ug/ml) and ciprofloxacin (0.0195 – 40ug/ml) for *Salmonella spp* isolates were >2560ug/ml, >2560ug/ml and 0.078 ug/ml respectively. *Salmonella typhi* isolates had MIC_{90}'s of >2560ug/ml, >2560ug/ml and 0.156ug/ml to ampicillin (0.063 – 2560ug/ml), chloramphenicol (0.063 – 2560ug/ml) and ciprofloxacin (0.039 – 40ug/ml).

The investigation undertaken established the current incidence in Kumasi to be 7.6% (296/3908). It also suggested an increasing resistance to antibiotics commonly used.There is therefore the need to do regular screening of antibiotic sensitivities and periodic MIC check on important pathogens.

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ACKNOWLEDGEMENT

Great is the Lord and most worthy of praise; His greatness no one can fathom.

To my parents, Dr. and Mrs. Awua-Boateng, I did it! Thank you for your immense support, prayers and faith in me.

I am extremely grateful to my supervisor, Prof. E.H. Frimpong, for his guidance, patience and advice to the end.

To Mr. R.A. Lartey, thank you will never be enough.

I am immensely indebted to the staff of the Microbiology Department of the Komfo Anokye Teaching Hospital. Your contribution will be remembered forever.

I am also thankful to the staff of the Department of Clinical Microbiology, College of Health Sciences.

My heartfelt gratitude goes to Nana K. Danso Akuamoah Boateng and Daniel Boakye Barnie. Thank you for the love and for always being there for me. Surely your hearts are made of gold.

CHAPTER ONE

1.1 Introduction

Salmonellae are Gram negative bacilli which normally inhabit the gastro-intestinal tract. Dr. Daniel Salmon, an American pathologist, in 1886 first identified one of these organisms in pigs (Arnold, 1989). The name “Salmonella” was thus given to this group of bacteria. The genus Salmonella belongs to the group *Escherichiae* in the family *Enterobacteriaceae*. This genus has more than 2300 serotypes described by the Kauffman-White schema.

It has been found that all *Salmonella* serovars form a single DNA hybridization group, i.e. a single species composed of seven subspecies. To avoid confusion with the familiar names of serovars, the species name *Salmonella enterica* was proposed with the following names for the subspecies (Todar, 2005) :

i) enterica I

ii) salamae II

iii) arizonae IIIa

iv) diarizonae IIIb

v) houtenae IV

vi) bongori V

vii) indica VI

Each subspecies contains various serovars defined by a characteristic antigenic formula. Since this formal Latin nomenclature may not be clearly understood by physicians and epidemiologists, who are the most familiar with the names given to the most common serovars, the common serovars names are kept for subspecies I strains, which represent more than 99.5% of the *Salmonella* strains isolated from humans and other warm-blooded animals (Todar, 2005).

There are several techniques used to detect bacteria. They include selective broths agar, cell separation by immuno-magnetic capture, chromogenic agars, and molecular methods (Balows et al, 1991).

Following isolation on selective media, identification can be carried out using latex agglutination, biochemical profiles which identify organisms phenotypically and molecular methods such as PCR (www.rapidmicrobiology.com).

Salmonella are commonly classified according to serology using Kauffman-White classification scheme. Specialists laboratories do serotyping and phage typing (www.rapidmicrobiology.com).

Medically, Salmonella can be classified into two major groups. These are the invasive and the non-invasive salmonellae. The invasive salmonellae which cause enteric fevers can also be called typhoidal salmonellae. They are made up of *Salmonella typhi* and *paratyphi A, B* and *C*. The non-invasive ones which normally cause gastroenteritis can also be called non-typhoidal salmonellae (NTS). They are made up of all the other serotypes such as *Salmonella typhimurium, S. enteriditis, S. panama* and *S. schwarzengrund*.

In recent times, the non-typhoidal Salmonella (NTS) serotypes are becoming increasingly invasive and thus an increase in the incidence of bacteraemia with NTS has become evident (Kupeli et al, 1995; Yagupsky et al, 2002). In this case they cause infections at sites outside the gastro-intestinal tract, most of the time invading the blood stream. These invasive complications are most likely when there is a predisposing host factor present, such as extremes of age and impaired host immunity.

Increasing NTS bacteraemia has been reported all over the world. Yagupsky et al (2002), in Israel found the incidence of non-typhi Salmonella bacteraemia among children to increase from 9.3 per 100,000 inhabitants in 1990-1992 to 26.8 per 100,000 in the 1993-1995 periods. This increment was attributed to *Salmonella virchow* and *enteriditis*.

In another study in Kenya, Kariuki et al (2005) demonstrated that the prevalence of NTS, multiple resistant to all the commonly used drugs such as ampicillin, cotrimoxazole and chloramphenicol rose from 31% in 1994 to 42% in 2003.

Brooks et al (2002) confirmed a typhoid incidence of 3.9 episodes per 1,000 persons during fever surveillance in a Dakar urban slum. In the same study, the relative risk for pre-school children was 8.9 episodes per 1,000 persons.

Again, there are increasing reports of NTS bacteraemia in HIV infected patients. Chierakul et al (2004) conducted a survey of bloodstream infections in a regional hospital in northeastern Thailand during the onset of the HIV epidemic. They discovered that NTS bloodstream infections rose coincidentally with the increase in the HIV sero-prevalence and preceded the increase in other HIV associated infections. They concluded that this increase could be an early signal of an impending rise in AIDS.

In Ghana, co-infection of NTS and malaria has been reported in Kumasi. A study by Evans et al in 2004 showed that bacteraemia in children often had malaria like symptoms. Bacteraemia should be considered in cases where malaria like symptoms are presented.

Many studies have shown that the increase in incidence in NTS is most prominent in children (Chui et al, 1994; Diez et al, 2004). In Taiwan, paediatric patients less than three years of age had a high probability of extra-intestinal infections with NTS (Chui et al, 1994). According to Diez et al (2004), the incidence of NTS infections increased the most among children younger than 4 years in Spain. Unpublished data at the Microbiology department of the Komfo Anokye Teaching Hospital (KATH) show that 79.6% of all positive blood cultures with Salmonella bacteraemia are in children.

The law in Ghana defines a child as anyone under the age of eighteen. However, for the purposes of this study, children are defined as those from birth to twelve years of age. This age range is being settled on because studies of published works have revealed that children within this age group are mostly affected by similar diseases. A study by Bar-Meir et al (2005) revealed that the mean patient age in a twelve-year study of children with Salmonella gastroenteritis was age group 2-8 years, of which there was no significant gender bias.

However, Broide et al (2005) found out in a study that Salmonella infection has two peaks of incidence at ages 1 – 5 and 15 – 65 years. Here, bacteraemia was seen to be prominent in the extreme ages. Diez et al (2004) realized that the mean age of the children used in their study was 11.1 months and the range was 3 days to 11 years. In a study conducted by Udgaonkar et al (1995), the paediatric age group accounted for 93% of the 28 cases which yielded NTS from their clinical material.

Infections with NTS usually cause a self limiting illness but increasingly, bacteraemia is seen as a complication. According to Smith et al (1990), several factors are known to increase the propensity

to invasive disease with its related sequelae. These factors include extremes of age groups, underlying illness and impaired host immunity.

The clinical spectrum of extra intestinal salmonella infection, comprising enteric fever and invasive infections owing to invasive Salmonellae, is known. It ranges from no symptoms to fatal Gram negative shock (Shanson, 1989).

Two major changes in the epidemiology of NTS have occurred during the second half of the twentieth century. First, *Salmonella typhimurium* strains resistant to multiple antibiotics have emerged and spread within the population of animals used for food (Rabsch et al, 2001). Second, *S enteriditis* has emerged as a major egg associated pathogen (Rabsch et al, 2001). Mensah et al (2001) have also come up with similar results in some studies in Accra.

The injudicious use of chloramphenicol, ampicillin and cotrimoxazole for trivial infections such as gastroenteritis and the common cold has led to the emergence of multi-drug resistance against first line drugs used over the past two decades (Parry, 2003).

The observed increase in bacterial resistance to regularly used antimicrobials has made it more difficult for clinicians to select an appropriate antimicrobial agent for empirical use (Murray et al, 1999). Kariuki et al (2005) reported in their study that the prevalence of NTS multiple resistant to all commonly available drugs including ampicillin and chloramphenicol rose from 31% in 1994 to 42% in 2003 with concomitantly higher Minimum Inhibitory Concentration (MIC) of each drug. This calls for regular studies to detect changing antibiotic sensitivities of organisms. This may involve tests other than that used in routine hospital antimicrobial resistance testing.

A wide range of antimicrobial susceptibility testing methods is in use but three primary methods have been shown to be reproducible and reliable. These are the disk diffusion tests, broth dilution and agar dilution. The Kirby-Bauer and Stoke's methods are the two different kinds of disk diffusion tests commonly used (Bauer et al, 1966). They are simple and straightforward.

Dilution susceptibility testing methods are used to determine the minimal concentration, usually expressed in microgram per milliliter, of an antimicrobial agent required to inhibit or kill a micro-organism. Procedures for determination are carried out by either agar-based or broth-based method. Antimicrobial agents are usually tested at $\log_2$ (twofold) serial dilutions and the lowest concentration that inhibits visible growth of the organism is recorded as the MIC. This method needs a lot more expertise, is not routinely used, and gives more accurate information about the sensitivity of the organism to prescribed antibiotics (Balows et al, 1991).

Flexibility is a major advantage of using the broth-based dilution method. Quantitative results are informative for establishing the relative resistance of certain microorganisms. The MIC values obtained may be used to help establish dosing regimens for optimum therapy. The broth dilution method is necessary for the determination of the Minimum Bactericidal Concentration (MBC). The MIC is the lowest concentration that inhibits macroscopic growth of the organism.

1.2 Statement of the problem

NTS have become increasingly invasive. According to Chiu et al (1994) *Salmonella panama, S. typhimurium* and *S. schwarzengrund* were the commonest causes of extra intestinal infection in

patients with NTS infections. Among Salmonella strains isolated by Wilkens et al (1997) in Accra, *Salmonella enteriditis* predominated over *S. typhi*.

It is estimated that 78% of the rural population in the third world is without clean water supply and 85% is without adequate sewage and other excreta disposal facilities (Hisham, 1983). For infections to occur, the infective dose of 10^{8-9} per ml of Salmonella must be ingested (Shanson, 1989). When such conditions exist, transmission of the bacteria is possible.

There is seasonality in occurrence and frequency of Salmonella infections in Ghana (Marbell et al, 1974). Salmonella typhi infections were seen to follow the rainfall pattern with the peak in July.

Salmonella bloodstream infection, especially due to non-typhoidal strains, is a potential health problem for Ghanaian children and may be complicated by resistance to commonly available drugs (Wilkens et al, 1997). It is desirable from time to time to characterize the strains involved and determine accurately their current resistance to commonly used antibiotics.

1.3 Research Objectives

1.3.1 Main Objectives

This research seeks to

- Provide data on the incidence and antibiotic sensitivity of Salmonella causing bacteraemia in children attending Komfo Anokye Teaching Hospital (KATH), Kumasi.

1.3.2 Specific Objectives

- To establish the incidence of Salmonella bacteraemia in children presenting at KATH within one year.
- To characterize Salmonella species in blood cultures using both the manual and API method.
- To determine the antibiotic sensitivity of Salmonella using the Kirby-Bauer disk diffusion method to the following drugs: ampicillin (10 ug), cotrimoxazole (25 ug), chloramphenicol (30 ug), gentamicin (10 ug), ciprofloxacin (5 ug), cefuroxime (30 ug), ceftriaxone (30 ug), ceftazidime (30 ug), and amikacin (10 ug).
- To determine the MIC and MBC of ciprofloxacin, chloramphenicol and ampicillin using the broth dilution method.

1.4 Limitations of the study

Certain factors which may affect the quality of the study include:-

- Failure to isolate organisms due to incorrect profile reading using the standard manual method.
- Fifty three (53) out of the two hundred and ninety six (296) isolates tested using API due to inadequate reagents.

1.5 Importance of the study

Infections due to Salmonella *spp* in children remain an important public health problem in many tropical and sub-tropical countries where clean water supply and sanitation are poor. The multi-system febrile illness has the gastrointestinal system as the portal of entry for the causative organisms.

The treatment of these infections has become increasingly difficult due to limited choice of antibiotics. This means that more expensive drugs with more potency will be needed to treat the diseases.

Even though existing literature on the treatment is voluminous and expanding certain issues, such as current incidences of infection and currently effective antibiotics, still need to be addressed. It is hoped that in a series of relevant research, these issues will be adequately addressed in paediatric patients with bacteriologically confirmed cases who were admitted to KATH.

The current incidence of Salmonella bacteraemia in children at KATH is not known. The Minimum Inhibitory Concentration of the commonly used drugs which is not routinely done gives a better understanding of the amount of effective drug needed. This work is therefore aimed at providing the current incidence in Kumasi, Ghana.

CHAPTER TWO

REVIEW OF RELATED LITERATURE

2.1 The organism

Salmonella is a genus of rod-shaped Gram negative enterobacteria that causes typhoid fever, paratyphoid fever and food borne illness (Ryan and Ray, 2004). It is motile in nature and produces hydrogen sulfide.

2.1.1 History

Salmonella was named after Daniel Elmer Salmon, an American veterinary pathologist who, together with Theobald Smith (better known for his work on anaphylaxis), first discovered the *Salmonella* bacterium from pigs (Arnold, 1989; Ryan and Ray, 2004; (www.whonamedit.com). Most cases involve undercooked meat, particularly poultry (Atlas, 1995); other sources have been implicated in *Salmonella enterica* infections. Blood culture was undertaken in cases where enteric fever, caused by *Salmonella typhi* or *Salmonella paratyphi*, was suspected.

2.1.2 Microbiology

Salmonella are Gram-negative bacteria. In a clinical laboratory, they are usually isolated on MacConkey agar, Xylose Lysine Deoxycholate (XLD) agar or Desoxycholate-citrate agar (DCA) agar. Because they cause intestinal infections and are greatly outnumbered by the bacteria normally found in the healthy bowel, primary isolation requires the use of a selective medium; thus, use of a relatively non-selective medium such as Cysteine Lactose Electrolyte Deficient (CLED) agar is not often practiced. Numbers of salmonella may be so low in clinical samples that stools are routinely also subjected to "enrichment culture" where a small

volume of stool is incubated in a selective broth medium, such as selenite broth or Rappaport Vassiliadis Soya peptone broth overnight. These media are inhibitory to the growth of the microbes normally found in the healthy human bowel, while allowing salmonellae to become enriched in numbers. Salmonellae may then be recovered by inoculating the enrichment broth on one or more of the primary selective media. On blood agar, they form moist colonies about 2 to 3 mm in diameter. They do not ferment lactose (Ryan and Ray, 2004; Tortora et al, 2001).

2.1.3 Classification

Salmonella taxonomy is complicated (Tindall et al, 2005). As of 7th December 2005, there were two species within the genus: *S. bongori* (previously subspecies V) and *S. enterica* (formerly called *S. choleraesuis*), which is divided into six subspecies (Todar, 2005)

- I—*enterica*
- II—*salamae*
- IIIa—*arizonae*
- IIIb—*diarizonae*
- IV—*houtenae*
- V—*obsolete* (now designated *S. bongori*)
- VI—*indica*

There are also numerous (totaling over 2500) serovars within both species, which are found in a disparate variety of environments and which are associated with many different diseases. The vast majority of human isolates (>99.5%) are subspecies of *S. enterica*. For the sake of simplicity, the Centre for Disease Control (CDC) recommend that Salmonella species be

referred to only by their genus and serovar:

e.g. *Salmonella typhi* instead of the more correct designation, *Salmonella enterica* subspecies *enterica* serovar typhi.

Salmonella isolates are most usually classified according to serology (Kauffman-White classification).The main division is first by the somatic O antigen, then by flagellar H antigens. H antigens are further divided into phase 1 and phase 2. The full description of a salmonella isolate is given as (O antigens, Vi: H antigen phase 1: H antigen phase 2) (Cheesbrough, 2000).

In a clinical laboratory, only a small number of serovars are looked for (the remainder being rare or not clinically significant). The Health Protection Agency (UK) recommends the testing for the following antigens routinely (www.hpa.org.uk, Cheesbrough, 2000):

- O antigens: 2 4 6.7 8 9 and 3.10
- phase 1 H antigens: 1 2 3 4 5 6 7
- phase 2 H antigens: a b c d E G i r

Isolates that cannot be identified using this panel are sent to the reference laboratory for identification (Cheesbrough, 2000).

2.2 Review of empirical studies

2.2.1 Incidence of Salmonella Infection in Children

In India, Udgaonkar *et al* (1995), conducted a two year study and found out that the pediatric age group predominated in the study, accounting for 93% (26) of cases. *Salmonella*

typhimurium (86%) was the main isolate, the other being *Salmonella Newport* (14%). Septicaemia was seen with 100% mortality in infants below one month of age. Two cases of meningitis were also seen.

Wilkins *et al* (1997), in Ghana, found out that salmonella bloodstream infections especially due to non-typhoidal strains, is a potential health problem for Ghanaian children. Out of the 21.6% (24) children with Salmonella bacteraemia, 59% (14) was due to *Salmonella spp* and 25% (6) was due to *Salmonella typhi*.

In Taiwan, Chui et al (1994) saw in their study that children under 3 years had a high probability of extra-intestinal infection with NTS. In a study in Spain by Diez et al (2004), the mean age of the 27 patients used in their study was 11.1 months and the range was 3 days to 11 years. Yagupsky et al (2002) noted a significant rise in the incidence of NTS bacteraemia in children less than 4 years. In Senegal, Broide et al (2005) found out in a study that Salmonella infection has two peaks of incidence at ages 1-5 and 15-65 years; whilst in Nigeria, Ogunyele et al (2005) found one which was 5-11 years.

In Israel, Yagupsky *et al* (2002) demonstrated that the incidence of children's bacteraemia has experienced a significant increase, associated with *S. virchow* and *enteriditis*. In this study, the overall incidence of Salmonella infections was 123.5 per 100 000 inhabitants. However, for children under four years of age the incidence of bacteraemia increased from 9.3 per 100 000 in 1990 – 1992 period to 26.8 per 100 000 in 1993 – 1995 period.

2.2.2 Seasonality of Occurrence

Marbell et al (1974) stated that there is seasonality in the occurrence and frequency of Salmonella infections in Ghana. They also said Salmonella typhi infections were seen to follow the rainfall pattern with the peak in July.

Kariuki et al in two different studies (2005 and 2006) also noticed that a higher number of samples were obtained in May and June (it mostly rains during these months). They suggested that it might be due to reduced sanitary conditions in homes and the environment in which children live and play.

2.2.3 History of antimicrobial resistance

Since its introduction in 1948, chloramphenicol has been the treatment choice for typhoid fever and remained the standard against which newer antimicrobials were compared (Mandal et al, 2004). Treatment with chloramphenicol reduced mortality from 20% to 1%. Despite this, chloramphenicol treatment is associated with high rate of continued and chronic carriage and high mortality rate in some recent series reported from developing countries (Miller et al, 2000; Mandal et al, 2004). Ampicillin and cotrimoxazole were found to be effective alternate drugs (Parry, 2004).

The use of chloramphenicol and ampicillin, not only for Salmonella but for other infections too, has gradually led to increased MICs of the drugs, threatening their therapeutic efficacy. Kariuki et al (2005) in Kenya noted the large increase in MICs of all commonly used drugs including chloramphenicol and ampicillin over the last decade. On the contrary, withdrawal

of selection pressure resulted in the re-emergence of ampicillin susceptible isolates, with very low MICs.

Cephalosporins, in the recent past, have gained importance for the treatment of enteric infections (Gautam et al, 2002). Parenterally administered 3rd generation cephalosporins are effective in the treatment of typhoid fever. Results of 92–100% sensitivity to 3rd and 4th generation cephalosporins were observed by Gautam et al in 2002 and Nath et al in 2003. Notwithstanding this, some authors have reported treatment failure with these cephalosporins in recent years (Mandal et al, 2004).

Quinolones are highly effective against salmonellae *in vitro* (Mandal, 2004). Ciprofloxacin is considered the drug of choice for the treatment of multidrug resistant typhoid, replacing chloramphenicol (Mandal, 2004). Hemalatha et al in India reported a sensitivity of 95% to ciprofloxacin in 1999.

However, due to the widespread use of ciprofloxacin since its introduction onto the market in the early1900s, resistance and treatment failures were being increasingly observed and reported. Gautam et al reported a decrease in sensitivity of *S. typhi* to ciprofloxacin from 89% to 81% (1997-2001). Threlfall et al (2001), noted that 23% of *S. typhi* in 1999 in the UK showed decreased susceptibility to ciprofloxacin.

Generally, a margin of safety 10 times the MIC is desirable to ensure successful treatment of the disease (Atlas, 1995). For ampicillin, MIC interpretive standards for dilution susceptibility testing states a dilution value of less than 8ug/ml as susceptible and 16ug/ml as

moderately susceptible. Thirty two micrograms per milliliter (32ug/ml) is stated as the interpretive standard for resistance (Balows et al, 1991).

Parry (2003) has reported that there is growing resistance to ciprofloxacin. The MIC interpretive standard for ciprofloxacin is given as < 1ug/ml for susceptible and > 8ug/ml for resistant strains. Madhulika et al (2004) in India found the MICs of ciprofloxacin to be less than 0.5mg/l.

2.2.4 Antimicrobial resistance

In Ghana, Wilkins *et al* (1997) found out that the non-typhoidal strains may be complicated by resistance to the commonly available antibiotics and that there was resistance to several antibiotics.

In India, Udgaonkar *et al* (1995), conducted a two year study and found out that twenty eight (28) patients admitted at a government hospital yielded non-typhoidal salmonellae which were multi-drug resistant from their clinical material.

In Spain Guerra et al (2000) analyzed the resistance profile for 15 antimicrobial agents of 333 Salmonella strains and reported that though all the strains were susceptible to amikacin, ceftazidime and ciprofloxacin, resistance to ampicillin and chloramphenicol ranged between 22-46%.

In a study in South Africa by the Water Research Commission (www.wrc.org.za) enteric pathogens such as Salmonella showed marked sensitivity to ciprofloxacin (96.6%), amikacin (100%) and ceftriaxone (93%). In South Africa Yurdakok et al, 1997 and Wasfy et al, 2000 had similar results.

In Kuwait, Dobardzic (1996) found the resistance of chloramphenicol, ampicillin and cotrimoxazole to vary between 18% and 50%. Kariuki et al (2005) in their study in Kenya reported that the prevalence of NTS which are multiple resistant to commonly used drugs including ampicillin and chloramphenicol rose from 31% in 1994 to 42% in 2003.

Parry (2003) conducted a study that showed that antimicrobial resistance in Salmonella to chloramphenicol, ampicillin and cotrimoxazole is common in Africa. He summarized his work by saying that "the resistance is increasing to several critical antimicrobials used to treat invasive salmonellosis including cephalosporins and quinolones and that in resource poor countries, such drug resistant Salmonella infections may become effectively untreatable".

2.3 Pathogenesis

Salmonella infection includes several syndromes. These are gastroenteritis, enteric fevers, septicaemia, focal infections and an asymptomatic state. Particular serovars show a strong tendency to produce a particular syndrome, for example *S. typhi, paratyphi – A, B* and *C* produce enteric fevers (Gianella et al, 1979).

On occasion any serotype can produce any of the syndromes. In general, more serious infections occur in infants, in adults over 50 and in debilitating illnesses (Shanson, 1989).

2.3.1 Pathogenesis of Salmonella infection in Enteric fever

After entering the ileal mucosa layer, the Salmonella bacterium enters through the lymphatic system to the lymph nodes and after a period of multiplication invades the blood stream.

After the primary bacteraemia the organisms enter the liver, spleen, kidney and bone marrow where they multiply and cause infection of these organs followed by re-invasion of the blood stream causing secondary bacteraemia. The secondary illness is responsible for causing fever and clinical illness characterized by severe headache, fever, chills, dry cough, rose spots, abdominal tenderness, malaise, epitasis and fluctuating mood (Gianella et al, 1979; Nester et al, 2001).

Complications associated with enteric fever include profuse bleeding in the intestine (intestinal haemorrhage) and intestinal perforation (Finlay et al, 1989).

2.3.2 Salmonella Gastroenteritis

Ingestion of food contaminated with Salmonella leads to bacterial food poisoning causing gastroenteritis. The pathogenesis is similar to that for enteric fever but the incubation period is shorter and it is limited to the gastrointestinal system. *Salmonellae* are a common source of food poisoning worldwide. Symptoms of *Salmonella* gastroenteritis are watery green offensive loose stools, vomiting, fever, abdominal pain, dehydration, cramps and renal failure (Gianella et al, 1979; Nester et al, 2001).

2.3.3 Bacteraemia with focal infection

Bacteraemia is common in infection with *Salmonella spp*. Due to bacteraemia, *Salmonellae* becomes present throughout the body resulting in persistent and focal or metastatic infection to many organs in the body. Focal infections caused by Salmonella bacteraemia include Salmonella meningitis, arthritis, damaged heart valves and atherosclerotic plaques within large arteries (Gianella et al, 1979; Nester et al, 2001).

2.3.4 Asymptomatic carriers

Even after complete recovery from Salmonella infection, some carriers continue to secrete bacilli in their stools for weeks. Chronic carriers can spread Salmonella for more than a year. A classical case is “Typhoid Mary” who caused an uproar in the United States. A cook called Mary Mallon over a ten year period cooked for eight different families. Denying she ever had the disease, “Typhoid Mary” is known to have infected fifty-four people; three of whom died (Arnold, 1989; Shanson, 1989; Atlas, 1995).

2.4 Diagnosis of Salmonella Infection

According to Froome and Whitehead (1955), diagnosis can be done bacteriologically and/or serologically. The bacteriological method deals with cultures whilst serology involves the response of the immune system to pathogens or introduced substances.

Cultures of blood, stool, and urine should be obtained. Blood cultures are usually positive only during the first 2nd week of illness, but stool cultures are usually positive during the 3rd to 5th week. If these cultures are negative and typhoid fever is strongly suspected, culture from a bone marrow biopsy specimen may reveal the organism (http://www.merck.com).

Serological methods have increasingly been used for the detection of invasive Salmonella serotypes including *enteritidis*.

Typhoid bacilli contain antigens (O and H) that stimulate the host to form corresponding antibodies. A 4-fold rise in O and H antibody titers in paired specimens obtained 2 wk apart suggests S. typhi infection. However, this test is only moderately (70%) sensitive and lacks

specificity; many nontyphoidal Salmonella strains cross-react, and liver cirrhosis causes false positives (http://www.merck.com).

Sera can be tested for Salmonella agglutinins. In a study by Barsoum and Awad (1972), dilutions of 1:40 to 1:5,120 in were done for comparative tube and microtitre plate agglutination tests. Each serum was tested with commercially obtained Salmonella antigens (febrile antigens, Lederle Laboratories, Pearl River, N.Y.): Salmonella group A (somatic, 1, 2, 12), Salmonella group B (somatic 1, 4, 5, 12), Salmonella group D (typhoid 0; somatic, 1, 9, 12, vi), paratyphoid A (flagellar a), paratyphoid B (flagellar b, 1, 2), and typhoid H (flagellar d). Known positive sera and 0.9% saline were used as controls.

Different types of ELISA, particularly indirect or double antibody-blocking assays using a variety of antigens such as lipopolysaccharide, flagella and SEF14 fimbrial antigen are used as part of control programmes in a number of countries (Barrow, 1994). There are many advantages to using such assays for preliminary screening of flocks prior to using bacteriological culture methods (Barrow, 1994).

An enzyme immunoassay (EIA) for the detection and measurement of serum IgM, IgG, and IgA antibodies to salmonella has been developed with commercially available lipopolysaccharides (LPSs) of Salmonella typhimurium and S enteritidis combined as antigen. This EIA method offers a substantial advance in the serological diagnosis of acute salmonella infections; it detects antibodies to the salmonellae of groups B and D, which constitute about 70% of culture-positive cases of human salmonellosis. Antibodies to other salmonellae are also detected. This EIA is particularly valuable for the detection of

salmonella antibodies during post-infectious complications when isolation of the organism is often no longer possible (Isomäki O et al, 1989).

2.5 Antimicrobial susceptibility methodology

Determining the antimicrobial susceptibility of a pathogen is important in aiding the clinician to select the most appropriate agent for treating that disease (Atlas, 1995).

A wide range of antimicrobial susceptibility testing methods is used but three primary methods have been shown to be accurate and reliable. These are disk diffusion, broth and agar dilution susceptibility tests.

Disk diffusion test refers to the diffusion of an antimicrobial agent of a specified concentration impregnated into disks, tablets or strips and placed on solid culture media seeded with a bacterial inoculum (Atlas, 1995). The diffusion of the antimicrobial agent into the seeded culture media results in an antimicrobial gradient. When the concentration of the antimicrobial becomes so dilute that it can no longer inhibit the growth of the test bacterium, a zone of inhibition is formed. The larger the zone, the lower the concentration of the drug needed to inhibit the growth of the organism.

Broth dilution is a technique in which a standardized microbial inoculum is tested against varying concentrations of an antimicrobial agent (usually doubling dilutions) in a standardized liquid medium (Atlas, 1995). It is most often referred to as the “gold standard”. The broth dilution method can be performed either in tubes containing a minimum volume of 2ml (macro dilution) or in smaller volumes in micro titration plates (Balows et al, 1991; Craig, 1993). The lowest concentration that completely inhibits visible growth as detected by

the unaided eye is recorded as the MIC (Balows et al, 1991). The MBC is defined as the lowest concentration of the antibiotic that reduced the inoculum by 99.9% within 24 hours (Balows et al, 1991, Nester et al, 2001).

Agar dilution is similar to the broth dilution but in this case the varying concentrations of the antimicrobial agent are incorporated into the agar and a standardized suspension of the bacteria is added to it.

Apart from these three methods, there are newer methods which have been proven to be equally accurate. A good example is the E test.

The E Test (AB Biodisk, Solna, Sweden) is a new method for performing antimicrobial susceptibility test. It consists of an impervious carrier (5- by 50-mm strip) with a predefined antimicrobial gradient which is placed on an inoculated agar plate and processed like a disk diffusion test. Results are generated directly as MICs from a continuous concentration gradient covering 15 twofold dilutions, and MICs are read where the edge of the inhibition zone intersects the strip (Baker et al, 1991).

In their study, they compared the E Test with disk diffusion, broth micro-dilution, and agar dilution tests by using a challenge set of 195 gram-positive and gram-negative bacteria for 14 antimicrobial agents. Also, disk diffusion, broth micro-dilution, and agar dilution tests were compared with each other. All test method comparisons gave >94% agreement for the category of susceptibility. The E Test category agreement with disk diffusion and broth micro-dilution was 95.1%, and with agar dilution it was 95.2%. The E Test results were as

reliable as the results obtained by the standard antimicrobial susceptibility testing methods.

A number of guidelines are available for antimicrobial susceptibility testing and subsequent interpretive criteria. These include the National Committee for Clinical Laboratory Standards (NCCLS), Japan Society for Chemotherapy (JSC), British Society for Antimicrobial Therapy (BSAC), Comite de l'Antibiogramme de la Societe Francaise de Microbiologie (SASFM) and others (Bager, 2000; Craig, 1993).

2.6 Epidemiology and Control

2.6.1 Epidemiology

Salmonella serotypes are important zoonotic pathogens in humans and animals (Winokur et al, 2000). Contaminated food then becomes a major mode of transmission for non-typhoidal salmonellae because there is an enormous animal reservoir. The most common animal reservoirs are chickens, turkeys, pigs and cows; dozens of other domestic and wild animals also harbour these organisms (Carli et al, 2001).

Because of the ability of salmonellae to survive in meats and animal products that are not thoroughly cooked, animal products are the main vehicles of transmission (Atlas, 1995). The magnitude of the problem is demonstrated by the following yields of salmonellae: 41% of turkeys examined in California, 50% of chickens cultured in Massachusetts and 21% of frozen egg whites examined in Spokane, W.A. (Mishu et al, 1994).

Salmonellae cause a wide range of human diseases such as enteric fever, gastroenteritis and bacteraemia (Bennassar et al, 2000). The epidemiology of typhoid fever and other enteric

fevers primarily involves person-to-person spread because these organisms lack a significant animal reservoir (Shanson, 1989). Contamination with human faeces is the major source of spread, and the usual vehicle is contaminated water. Occasionally, contaminated food usually handled by an individual who harbours *Salmonella typhi* may be the vehicle.

In typhoid fever and non-typhoidal Salmonellosis, two other factors have epidemiologic significance. First, an asymptomatic human carrier state exists for the etiological agents of either form of the disease. A classical case of disease transmission by an asymptomatic carrier occurred in the early 1900s when a cook, Mary Mallon, spread 50 cases of typhoid fever in ten years to seven different households (Atlas, 1995). Approximately 3% of the persons infected with S. typhi and 0.1% of those infected with NTS become chronic carriers (Mishu et al, 1994). The carrier state may last from many weeks to years. Thus, human and animal reservoirs exist. It is interesting to note that children rarely become chronic typhoid carriers.

Second, the use of antibiotics in animal feed and the indiscriminate use of antibiotics in humans create selection pressure that favours the increased resistance to antibiotics by the organisms.

2.6.2 Salmonella associated diseases

Other salmonellae are frequent causes of food borne illness, and can especially be caught from poultry and raw eggs and more generally from food that has been cooked or frozen, and not eaten straight away. It can also be caught by handling reptiles, such as iguanas or terrapins, which commonly host the Salmonella bacteria. In March 2006, The New York

Times reported that the US government said that 16.3% of all chickens were contaminated with salmonella (Mishu et al, 1994; Atlas, 1995). In the mid to late 20th century, *Salmonella enterica* serovar Enteriditis was a common contaminant of eggs. This is much less common now with the advent of hygiene measures in egg production and the vaccination of laying hens to prevent salmonella colonization. Many different salmonella serovars also cause severe diseases in animals other than human beings.

After bacterial infections, Reiter's Syndrome can develop (Ryan and Ray, 2004).

2.5.3 Control

Salmonellae are difficult to eradicate from the environment. However, as the major reservoir for human infection is poultry and livestock, to significantly reduce human exposure would mean a reduction in the number of salmonellae harboured in the live stock.

In Denmark for example, all animal feed are treated to kill salmonellae before distribution. This resulted in a marked reduction in salmonellosis (Wegener et al; 2003).
Recently, the U.S. Department of Agriculture has approved the radiation of poultry to reduce contamination by pathogenic bacteria such as Salmonella and Campylobacter. However, this method has not been widely accepted though the technology would greatly reduce the magnitude of the salmonella problem (www.usda.gov).

Gastroenteritis treatment measures include replacing fluid loss by oral and intravenous routes, and controlling pain, nausea and vomiting (www.nhs.uk). Specific therapy consists of antibiotic administration. Enteric fevers should be treated with antibiotics. Antibiotic therapy of non-typhoidal salmonellosis should be reserved for septicemic and focal infection

syndromes. Antibiotics should not be used in uncomplicated Salmonella gastroenteritis because they significantly prolong the faecal excretion of the organism and increase the number of antibiotic resistant strain.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

The study sites were the Microbiology department and the Paediatric wards of KATH made up of the Paediatric Emergency Unit (PEU), wards B4, B5 AND C5. KATH is a tertiary referral centre in Kumasi. According to the KATH statistics in 2002, there are more than 7,500 admissions per year and the wards are run at well over 150% bed occupancy (KATH annual report,2002).

Children from the Out Patients Department (OPD) were also included in this study.

3.2 Subjects

All paediatric in-patients and out-patients suspected of septicaemia, aged 1 day to 12 years, presenting from January 1 to December 31, 2005 and satisfied the inclusion criteria were screened after obtaining parental consent.

The inclusion criteria were as follows:

- Patients between 1 day and 12 years of age
- Patients suspected of septicaemia
- Patients with bacteriologically confirmed Salmonella infection

3.3 Sample

In the calculation of the sample size an assumption of 10 isolates per month was made. This determined that a sample size of 100 or more would be representative. However, going by the

annual isolation rate of Salmonella in the microbiology laboratory which is 14 to 41 per month, a sample size of 200 or more would give a true representation.

3.4 Media used for isolation of Salmonella

The following media were used in the isolation of the Salmonella. Their composition and mode of preparation can be found in Appendix I.

- Brain Heart Infusion broth
- Blood agar
- MacConkey agar
- Cysteine Lactose Electrolyte Deficient (CLED) agar
- Nutrient agar

The most common isolates of *Enterobacteriaceae* have a characteristic appearance on blood and MacConkey agar which is useful in preliminary identification (Balows *et al*, 1991).

3.5 Sample collection

A single blood culture was taken from all the paediatric patients suspected of having bacteraemia. Following cleaning of the skin, 2ml of venous blood was taken and inoculated into 20ml brain heart infusion broth (Shanson, 1989). This was done at the wards for the in-patients. For the out-patients, the same procedure was followed at the microbiology laboratory where the sample was taken.

3.6 Incubation and isolation

The culture bottles of the in-patients were then sent to the microbiology laboratory where they were incubated together with those for outpatients at 37^{o}C for 7 days. The bottles were

examined daily for evidence of bacterial growth, including turbidity and haemolysis. The first subculture was done after 24 hours on Blood and MacConkey agar. If needed, the second and third subcultures were done on the following alternate days. On blood agar, Salmonellae form moist colonies about 2 to 3 mm in diameter whilst on MacConkey agar; they form pale smooth colonies 2-4 mm in diameter and are non lactose fermenters. Such colonies were identified by the use of a series of biochemical tests.

3.7 Procedure for characterization

The Standard Manual Method and an automated processor, the mini Analytical Profile Index (API) machine (bioMerieux, France), were used to characterize the isolates obtained. The mini API was used to confirm 58 of the Salmonella isolates already identified using the standard manual method. This was done to find out if the standard manual method compared favourably with the API diagnosis.

3.7.1 Identification of suspected isolates by the Standard Manual Method

Suspected colonies isolated on MacConkey agar were plated on Nutrient agar to obtain pure culture. Using a sterilized straight wire, two or three colonies were picked and inoculated into a series of biochemical tests. These are Kligler's Iron Agar (KIA) or Triple Sugar Iron (TSI) Agar, Citrate, Urea and Motility tests (MacFaddin, 1980).

3.7.1.1 Kligler's Iron Agar (KIA) /Triple Sugar Iron (TSI) Agar

Five different reactions can be observed (Appendix I) but that suggestive of Salmonella are alkaline slant/acid butt with/without, gas production and alkaline slant/acid butt, gas, H_2S production.

3.7.1.2 Citrate test

This test is performed to determine if the organism is capable of utilizing citrate as a sole source of carbon for metabolism with resulting alkalinity. It mainly aids in the differentiation between genera. Using Simmon's Citrate, a positive test meant growth of the organism with an intense blue colour on the slant. This is the usual reaction for Salmonellae. A negative test gives no growth and no change in the green colour (Appendix I).

3.7.1.3 Urease test

Using Christensen's Urea agar, this test sought to determine the ability of the organism to split urea, forming two molecules of ammonia by the action of the enzyme Urease with resulting alkalinity. A positive test gives an intense pink-red colour on the slant whilst the negative test gives a yellow-orange colour (Appendix I). Salmonellae give a negative test. The composition, mode of preparation and use can be found in Appendix I.

3.7.1.4 Motility

This test is performed to find out if the organism is motile by means of flagella.
Using a sterilized straight wire, two or three colonies are picked from pure culture and stabbed into the centre of the Semisolid Motility Test Medium to a depth of half an inch. After an incubation period of 24-48 hours at 35^{o}C, a positive test which implies motility, exhibits fuzzy streaks of growth. This is because the organism migrates from the stab line and diffuses into the medium. This is a typical reaction for Salmonella. A negative result shows bacterial growth along the stab line leaving the surrounding medium clear. There is no motility. The control shows no growth and the medium remains clear.

3.7.2 Identification of isolates using the mini API

ID 32 E is a standardized system for the identification of *Enterobacteriaceae* and other non-fastidious Gram-negative rods using 32 standardized and miniaturized biochemical tests, as well as a specific database.

The ID 32 E strip is made up of 32 test cupules which contain a dehydrated reactive medium.

To perform the test, the density of an ampoule containing 0.85%NaCl was raised to 0.5 MacFarland by inoculating it with the organism. A densimat was used to check the turbidity. Using an automatic pipette (ATB electronic pipette), 55ul was dispensed into all the 32 cupules. The first six tests (ODC, ADH, LDC, URE, LARL, GAT and 5KG) needed anaerobic conditions, and this was created by putting 2 drops of mineral oil on them before incubation. The lid was placed on the strip and it was incubated aerobically at 37^0C for 18 to 24 hours.

After an incubation period of 24 hours, the reactions are read using the mini API instrument. For the test IND, James reagent was added (to reveal the indole reaction) before it was read. The reader records the colour of each cupule and transmits the information to the computer. Identification is obtained using the identification software to interpret the transmitted results.

The ID 32 E uses 32 miniaturized biochemical tests, as well as specific database. The biochemical tests were:

- ODC- Ornithine DeCarboxylase
- ADH- Arginine DiHydrolase
- LDC- Lysine DeCarboxylase

- URE- UREase
- LARL- L-ARabitoL (acidification)
- GAT- GAlacturonaTe (acidification)
- 5KG- 5-KetoGluconate (acidification)
- LIP- LIPase
- RP- Phenol Red (acidification)
- BGLU-BGLUcosidase
- MAN- MANnitol (acidification)
- MAL- MALtose (acidification)
- ADO- ADOnitol (acidification)
- PLE- PaLatinosE (acidification)
- BGUR-BGlucURonidase
- MNT- MaloNaTe
- IND- INDole production
- BNAG-N- acetyl-B-Glucosaminidase
- BGAL-BGALactosidase
- GLU- GLUcose (acidification)
- SAC- SACcharose (acidification)
- LARA-L-ARAbinose (acidification)
- DARL-D-ARabitoL (acidification)
- GLU- GLUcosidase
- GAL- GALactosidase
- TRE- TREhalose (acidification)

- RHA- RHAmnose (acidification)
- INO- INOsitol (acidification)
- CEL- CELlobiose (acidification)
- SOR- SORbitol (acidification)
- MAL- MALtosidase
- AspA-L-Aspartic acid Arylamidase

3.8 Antibiotic susceptibility testing

Susceptibilities to antimicrobials were determined by controlled disk diffusion and measuring MICs using broth dilution techniques. The Kirby-Bauer disk diffusion method (for SMM) and broth dilution technique (for MIC determination) were used. These were used because they are reproducible and reliable.

Escherichia coli ATCC 25922 and ATCC 35218 (with known MICs) were used as controls for both techniques in each test.

Disk diffusion susceptibilities and MICs were interpreted according to guidelines provided by the National Committee for Clinical Laboratory Standards (2002).

3.8.1 Susceptibility testing of isolates using the Standard Manual Method

The Kirby-Bauer disk diffusion test was performed following the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) (Barrows and Feltham, 1991) with these antibiotics: ampicillin (10ug), cotrimoxazole (25ug), chloramphenicol (10ug), gentamicin (10ug), ciprofloxacin (5ug), ceftriaxone (30ug), ceftazidime (30ug) and amikacin (30ug). These are sold as a multo-disk (Abtek Biologicals Ltd., Britain). Cefuroxime (30ug) was added as a single disk.

A standardized suspension of the Salmonella isolates was prepared in either 0.85% sodium chloride or peptone water. A densimat was used to ensure the turbidity of the resulting solution was 0.5 MacFarland. This was then flooded over a sensitivity plate containing Mueller-Hinton agar. The excess solution was drained off before the surface of the "flooded" agar is allowed to dry. A multodisk impregnated with the antibiotics was then placed on the inoculated agar surface and incubated at 37^0C for 12 to 18 hours. The antibiotics diffuse into the agar, establishing a concentration gradient. Inhibition of microbial growth is indicated by a clear area (zone of inhibition) around the antibiotic disks. The diameter of the zone reflects the concentration gradient established (Atlas, 1995). The zones of inhibition are then compared to a set of standards and the organism is said to be either sensitive or resistant to the antibiotics used (Finegold et al, 1978).

3.8.2 Susceptibility testing of isolates using the mini API

The standardized system for identification of Enterobacteriaceae, ID 32 E, comes along with strip which has 16 antibiotics in 16 cupules used in susceptibility testing.

To perform the test, 10ul was transferred from the ampoule containing 0.85%NaCl and the organism inoculum (at 0.5McFarland) and added to 7ml of ATB medium (this medium comes with the kit). Using an automatic pipette, 135ul was dispensed into all the 16 cupules. It was incubated aerobically at 37^0C for 18 to 24 hours.

After an incubation period of 24 hours, the reactions are read using the mini API instrument. The reader records the colour of each cupule and transmits the information to the computer. Identification is obtained using the identification software to interpret the transmitted results.

3.9 MIC determination using the broth dilution technique

The MICs of ciprofloxacin, chloramphenicol and ampicillin using the traditional broth dilution method was determined for the first 100 *Salmonella spp* isolates and all the 30 *Salmonella typhi* isolates.

3.9.1 Preparation and storage of antimicrobial agents

Stock solutions of 5000ug/ml were prepared for the three antibiotics. Pure powders were obtained from Exir Pharmaceutical Company, Tehran, India.

For ciprofloxacin, this was done by taking 500mg and dissolving it in100ml of sterilized distilled water. A stock concentration of 5,000ug/ml was obtained.

Chloramphenicol and ampicillin were treated in the same manner as ciprofloxacin to obtain a stock concentration of 5,000ug/ml.

The antibiotic stocks were stored at 4-8^{0}C. A dilution scheme that was used to obtain full-range doubling dilutions is outlined in Fig. 3.1.

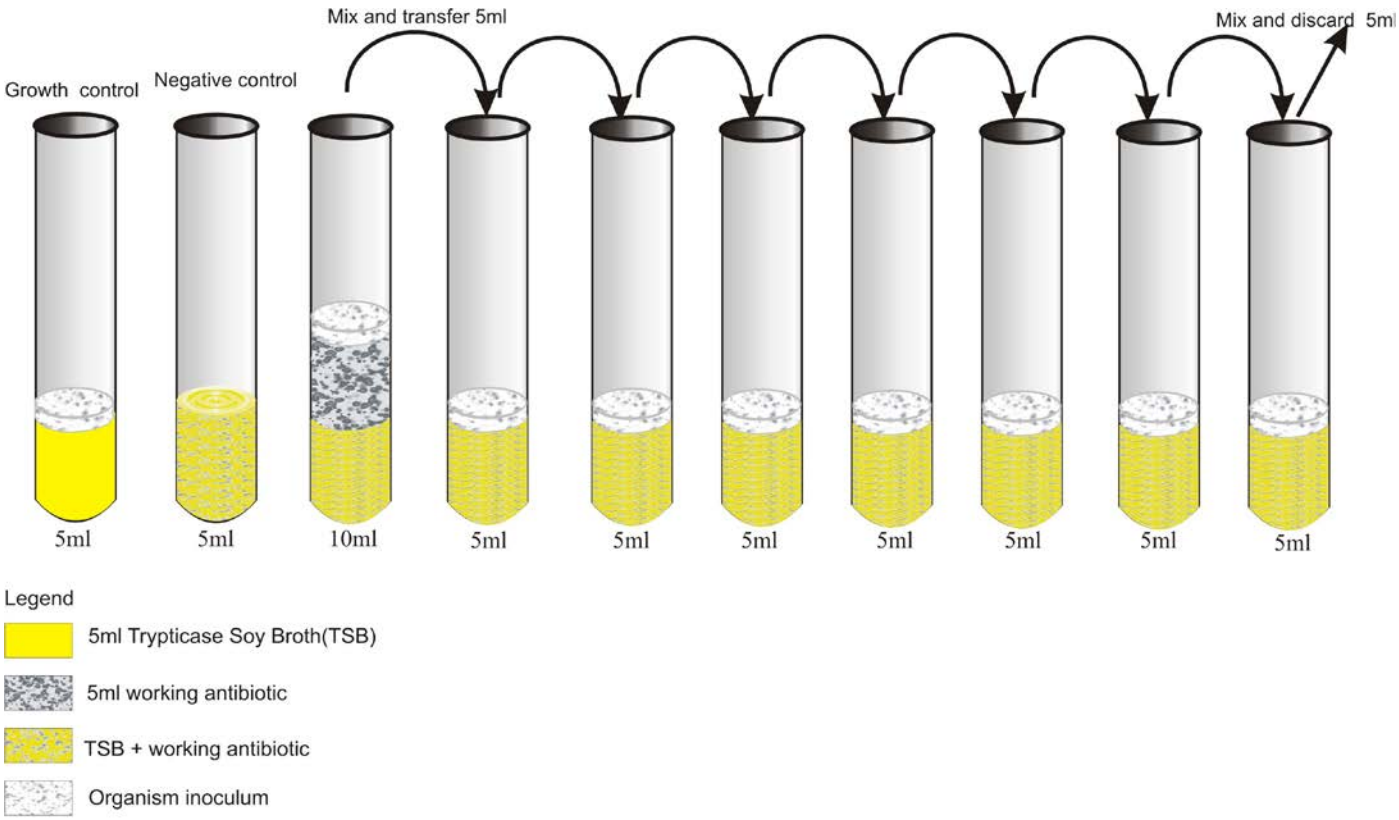

Fig 3.1 MIC determination procedure for 1:2 dilution

3.9.2 Preparation and storage of media

Trypticase Soy Broth with a final pH of between 7.2 and 7.4 was prepared as directed by the manufacturer (Appendix I). To minimize evaporation, tubes were tightly capped and stored at 4 – 8^{o}C. This media was also used to prepare the final antimicrobial agent concentrations.

3.9.3 Preparation of inoculum

The Salmonella organism was subcultured from frozen glycerol stocks onto MacConkey or Cysteine Lactose Electrolyte Deficient (CLED) agar plates and incubated for 18-24 hours. The following day, colonies from the agar plate were directly re-suspended in sterile 0.9% saline. The cell suspension was adjusted to exactly match the 0.5 McFarland turbidity standard. This was critical because a higher inoculum would lead to inaccurate MIC values. The process to determine the MIC of the antibiotics is shown in Fig 3.1

3.9.4 Addition of inoculum

Inoculation was done with a 1mm loop. This way 0.001ml of the prepared inoculum was transferred to all the tubes except the negative control as shown in Fig. 3.1. The resulting concentration of 10^4 organisms/ml was obtained.

3.9.5 Incubation, interpretation and reporting of results

Tubes were incubated at 37^{o}C for 16 to 20 hours before being read. Growth or the lack of it in the antibiotic containing tubes was best determined by comparing them with the negative control and is generally indicated by turbidity or the lack of it. Therefore, the lowest concentration that completely inhibits visible growth as detected by the unaided eye is

recorded as the MIC (Balows et al, 1991). The MBC is defined as the lowest concentration of the antibiotic that reduced viable growth by 99.9% within 24 hours (Balows et al, 1991).

3.9.6 MBC Determination

The MBC is determined by subculturing on agar, tubes of the end point dilution for the MIC and tubes of two subsequent dilutions before and after the MIC tube (Atlas, 1995). This is done by taking 0.01ml from each of the said tubes, streaking on a Mueller-Hinton agar plate and incubating for 18-20 hours at37^{o}C. Plates with colonies less than 5 are deemed as having no growth. The MBC is defined as the lowest concentration of the antibiotic that reduced the viable growth by 99.9% within 24 hours (Balows et al, 1991).

The MBC is particularly useful in determining the appropriate concentration of an antibiotic for use in treating patients (especially those with lowered immune response).

CHAPTER FOUR

RESULTS

4.1 Age, sex and species isolated

For the year 2005, 11,809 blood cultures were done yielding 3,908 (33.1%) positive blood cultures. Out of this number 372 (9.5%) were bacteriologically confirmed Salmonella bacteraemia cases – 296 (79.6%) being children up to 12 years of age (males 159 (53.7%) and females 137 (46.3%)).

The distribution of isolates according to age and sex is in Table 4.1.

Out of the 296 isolates used in this study 258(87.2%) had *Salmonella spp* and 38(12.8%) *Salmonella typhi* isolated from their blood cultures. The distribution of isolates according to species and sex is seen in Table 4.2

Table 4.1 Distribution of isolates according to age and sex of patients

Age	Number	Sex	
		Male	Female
0-3 yrs	262 (88.5%)	140 (47.3%)	122 (41.2%)
4-7 yrs	27 (9.1%)	16 (5.4%)	11 (3.7%)
8-12yrs	7 (2.4%)	3 (1.0%)	4 (1.4%)
Total	296 (100%)	159 (53.7%)	137 (46.3%)

Table 4.2 Distribution of 296 isolates according to species using Standard Manual Method

Species	Number	Sex	
		Male	Female
Salmonella spp	258 (87.2%)	137 (46.3%)	121 (40.9%)
Salmonella typhi	38 (12.8%)	22 (7.4%)	16 (5.4%)
Total	296 (100%)	159 (53.7%)	137 (46.3%)

4.2 Comparison of isolates obtained by SMM to API

An automated processor, the mini API was used to confirm 58 of the Salmonella isolates already identified using the SMM. This was done to find out if the standard manual method would compare favourably with the API diagnosis.

Table 4.3 Comparison of isolates obtained by SMM to API

Organism	SMM	API	Variance
Salmonella spp	53	51	2
Salmonella typhi	5	5	0
Hafnia alvei	0	2	-2
Total	58	58	

Legend
SMM – Standard Manual Method
API – Analytical Profile Index

4.3 Antimicrobial susceptibility test results

(See Appendix for tables 4.4 and 4.5)

Table 4.6 Antibiogram (showing sensitivities) using standard manual method and API for *Salmonella spp* isolates-Percentages in parenthesis

Method	No of isolates tested	Antibiotics / No. sensitive						
		cot (%)	amp (%)	chl (%)	cip (%)	gen (%)	amk (%)	cxm (%)
SMM	258	41 (15.9)	29 (11.2)	52 (20.2)	255 (98.8)	242 (93.8)	253 (98.1)	221 (85.7)
API	51	20 (39.2)	8 (15.6)	13 (25.5)	49 (96.1)	46 (90.2)	45 (88.2)	5 (9.8)

Legend

ampicillin	(amp) – 10ug	cotrimoxazole	(cot) – 25ug
chloramphenicol	(chl) – 30ug	ciprofloxacin	(cip) – 5ug
gentamicin	(gen) – 10ug	amikacin	(amk) – 10ug
cefuroxime	(cxm) – 30ug		

Table 4.7 Antibiogram (showing sensitivities) using SMM and API for *Salmonella typhi* isolates – Percentages in parenthesis

Method	No of isolates tested	Antibiotics / No. sensitive						
		cot (%)	amp (%)	chl (%)	cip (%)	gen (%)	amk (%)	cxm (%)
SMM	38	3 (7.9)	6 (15.8)	9 (23.7)	29 (76.3)	37 (97.4)	26 (68.4)	34 (89.4)
API	5	0 (0)	0 (0)	1 (20)	5 (100)	5 (100)	5 (100)	5 (100)

Legend

ampicillin	(amp) – 10ug	cotrimoxazole	(cot) – 25ug
chloramphenicol	(chl) – 30ug	ciprofloxacin	(cip) – 5ug
gentamicin	(gen) – 10ug	amikacin	(amk) – 10ug
cefuroxime	(cxm) – 30ug		

4.4 MIC and MBC results obtained using the broth dilution technique

Ampicillin, chloramphenicol and ciprofloxacin were the three antibiotics tested. The results obtained are shown in the tables below.

4.4.1 *Salmonella spp* isolates

For ampicillin, 11 isolates were sensitive within the range 0.039-1.25ug/ml, 0 between 2.5-80ug/ml and 89 between 160-5000ug/ml. For the MBC, 2 isolates were sensitive within the range 0.039-1.25ug/ml, 17 between 2.5-80ug/ml and 81 between 160-5000ug/ml (see Tables 4.10 and 4.11).

For chloramphenicol, 0 isolates were sensitive within the range 0.039-1.25ug/ml, 22 between 2.5-80ug/ml and 78 between 160-5000ug/ml. The MBC followed the same pattern (see Tables 4.10 and 4.11).

For ciprofloxacin, 99 isolates were sensitive within the range 0.039-1.25ug/ml, 1 between 2.5-80ug/ml and 0 between 160-5000ug/ml. The MBC followed the same pattern (see Tables 4.10 and 4.1)

Table 4.8 MIC of *Salmonella spp* to ampicillin, chloramphenicol and ciprofloxacin

Drugs	MIC ug/ml					
	S. spp			*S. typhi*		
	Range	No. tested	MIC_{90}	Range	No. tested	MIC_{90}
amp	0.156 - 2560	100	>2560	0.063 - 2560	30	>2560
chl	2.5 – 2560	100	>2560	0.063 - 2560	30	>2560
cip	0.0195 - 20	100	0.078	0.039 - 40	30	0.156

	No	Concentration (ug/ml)																
		0.039	0.078	0.156	0.313	0.63	1.25	2.5	5	10	20	40	80	160	320	640	1280	2560	5000
amp	100						11							9					80
chl	100											1	20	1					78
cip	100	3	97																

Legend
amp – ampicillin, chl – chloramphenicol, cip - ciprofloxacin

Table 4.9 MBC of *Salmonella spp* to ampicillin, chloramphenicol and ciprofloxacin

	No	Concentration (ug/ml)																
		0.039	0.078	0.156	0.313	0.63	1.25	2.5	5	10	20	40	80	160	320	640	1280	2560	5000
amp	100						2	9					8	1				2	78
chl	100											1	21						78
cip	100	1		98					1										

Legend
amp – ampicillin, chl – chloramphenicol, cip - ciprofloxacin

4.4.2 *Salmonella typhi* isolates

For ampicillin, 2 isolates were sensitive within the range 0.039-1.25ug/ml, 0 between 2.5-80ug/ml and 28 between 160-5000ug/ml. For the MBC, 0 isolates were sensitive within the range 0.039-1.25ug/ml, 2 between 2.5-80ug/ml and 28 between 160-5000ug/ml (see Tables 4.12 and 4.13).

For chloramphenicol, 0 isolates were sensitive within the range 0.039-1.25ug/ml, 5 between 2.5-80ug/ml and 25 between 160-5000ug/ml. The MBC followed the same pattern as that for ampicillin (see Tables 4.12 and 4.13).

For ciprofloxacin, 29 isolates were sensitive within the range 0.039-1.25ug/ml, 1 between 2.5-80ug/ml and 0 between 160-5000ug/ml. The MBC followed the same pattern (see Tables 4.10 and 4.11).

Table 4.10 MIC of *Salmonella typhi* to ampicillin, chloramphenicol and ciprofloxacin

		Concentration (ug/ml)																
	No	0.039	0.078	0.156	0.313	0.63	1.25	2.5	5	10	20	40	80	160	320	640	1280	2560	5000
amp	30						2												28
chl	30												5						25
cip	30	1	28								1								

Legend
amp – ampicillin, chl – chloramphenicol, cip - ciprofloxacin

Table 4.11 MBC of *Salmonella typhi* to ampicillin, chloramphenicol and ciprofloxacin

		Concentration (ug/ml)																
	No	0.039	0.078	0.156	0.313	0.63	1.25	2.5	5	10	20	40	80	160	320	640	1280	2560	5000
amp	30							2											28
chl	30												2	3					25
cip	30		25	4								1							

Legend
amp – ampicillin, chl – chloramphenicol, cip - ciprofloxacin

CHAPTER FIVE

DISCUSSION, CONCLUSION, SUMMARY AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Incidence of Salmonella bacteraemia

For the year 2005, 11,809 blood cultures were done yielding 33.1% (3,908) positive blood cultures. Out of this number 3.1% (372) were bacteriologically confirmed Salmonella bacteraemia cases – 79.6% (296) being children up to 12 years of age. Though the value obtained in my study is lower than the 93.3% obtained by Udgaonkar et al in 1995 in India, it is higher than the 50.4% obtained in South Australia in the same year (Infectious and Notifiable Diseases in South Australia, 1995).

An overall incidence of 3.1 per 100 people screened for sepsis at KATH was seen for the year 2005. The incidence among children was seen to be 7.6 per 100. This is higher than values obtained by Yagupsky (2002) and Broide (2005) which were 9.3 per 100,000 to 26.8 per 100,000 and 3.9 per 1,000 people respectively.

The incidence of *Salmonella typhi* 12.8% (38) was lower than *Salmonella spp* 87.2% (258). This conforms to the incidence rate reported by Wilkens et al in 1997 in Accra. Out of the 21.6% children with Salmonella bacteraemia, 59% (14) was due to *Salmonella spp* and 25% (6) was due to *Salmonella typhi.* The incidence of Salmonella typhi 12.8% (38) however is still higher than what was earlier recorded (1%) in children in Nigeria by Christen (1980).

Of the 296 isolates obtained, males were 53.7% (159) and females 46.3% (137) showing a slight male preponderance. Children between 0-3 years of age accounted for 88.5% of the total. This single peak is similar to that obtained (5 – 11 years) by Ogunyele et al (2005) but does not conform to the peaks noted by Broide et al (2005) - two peaks: 1-5years and 15-65years. It rather conformed to studies done by Chui et al (1994), Diez et al (2004) and Yagupsky et al (2002).

Chui et al saw in their study in Taiwan that children under 3 years had a high probability of extra-intestinal infection with NTS. The mean age of the 27 patients used by Diez et al (2004), their study in Spain was 11.1 months. Yagupsky et al noted a significant rise in the incidence of NTS bacteraemia in children less than 4 years.

Marbell et al (1974) stated that there is seasonality in the occurrence and frequency of Salmonella infections in Ghana, with *Salmonella typhi* infections following the rainfall pattern with a peak in July. During the period of sample collection, August had the highest number of *Salmonella spp* isolated followed by November. The highest number of *Salmonella typhi* was also seen in November.

5.1.2 Characterization of isolates

Out of the 296 isolates from blood culture, 87.2% (258) were *Salmonella spp* and 12.8% (38) were *Salmonella typhi* using the Standard Manual Method (SMM). Fifty-eight (58) isolates obtained by SMM (53 *Salmonella spp* and 5 *Salmonella typhi*) were re-characterized using the automated API. The API identified 51 out of

the 53 *Salmonella spp* as true *Salmonella spp*. The remaining 2 were identified as *Hafnia alvei*. The 5 *Salmonella typhi* were identified as true *Salmonella typhi*. This could mean that if all the 258 Salmonella spp isolates had been tested by API there could have been more *Hafnia alvei* isolated. Even though it is evident that the API is more accurate in identification because it has 32 biochemical tests, it is also more expensive and requires more expertise. The SMM was 98.3% accurate compared to the API, far less expensive and easier to use. The standard manual method in my opinion should still be used.

5.1.3 Disk diffusion susceptibility testing

For *in vitro* antibiotic sensitivity patterns, a range of 3.7 – 99.3% sensitivity was demonstrated by the antibiotics.

5.1.3.1 *Salmonella spp* isolates

Sensitivity was highest to amikacin (98.1%) and ceftazidime (98.0%). The sensitivities of the other antibiotics were as follows: ciprofloxacin (97.9%), ceftriaxone (95.8%), gentamicin (95.7%) and cefuroxime (82.4%).

Guerra et al (2000) in Spain analyzed the resistance profile for 15 antimicrobial agents of 333 Salmonella strains and reported that all the strains were susceptible to amikacin, ceftazidime and ciprofloxacin. In a study in South Africa by the Water Research Commission (www.wrc.org.za) enteric pathogens such as Salmonella showed marked sensitivity to ciprofloxacin (96.6%), amikacin (100%) and ceftriaxone (93%). Similar results were obtained by Yurdakok et al, 1997 and Wasfy et al, 2000 in South Africa.

The highest percentage resistance was towards ampicillin (87.9%), cotrimoxazole (81.6%) and chloramphenicol (78.7%). Guerra et al (2000) in Spain analyzed the resistance profile for 15 antimicrobial agents of 333 Salmonella strains and reported that resistance to ampicillin and chloramphenicol ranged between 22-46%. Likewise Dobardzic (1996) in Kuwait found the resistance of chloramphenicol, ampicillin and cotrimoxazole to vary between 18% and 50%. Kariuki et al (2005) in their study in Kenya reported that the prevalence of NTS which are multiple resistant to commonly used drugs including ampicillin and chloramphenicol rose from 31% in 1994 to 42% in 2003. Parry (2003) believes that antimicrobial resistance in Salmonella to chloramphenicol, ampicillin and cotrimoxazole is common in Africa. I share the view of Coovadia et al (1992) and Su et al (2004) in feeling that the emergence of multidrug resistant Salmonellae to ampicillin, chloramphenicol and cotrimoxazole has become a global challenge.

5.1.3.2 *Salmonella typhi* isolates

This study revealed that *Salmonella typhi* showed greater resistance against drugs, which is in agreement with other findings of other workers (Farooqi et al, 1990), who reported that high resistance was shown against ampicillin and chloramphenicol.

The least sensitivity was towards cotrimoxazole (3.7%), ampicillin (8.3%), and chloramphenicol (17.9%) thereby giving resistances of 96.3%, 91.7% and 82.1% respectively.

Since its introduction in 1948, chloramphenicol has been the treatment of choice for typhoid fever and remained the standard against which newer antimicrobials were compared (Mandal et al, 2004). Treatment with chloramphenicol reduced mortality from 20% to 1%. Despite this chloramphenicol treatment is associated with higher rate of continued and chronic carriage and high mortality rate in some recent series reported from developing countries (Miller et al, 2000; Mandal et al, 2004). Ampicillin and cotrimoxazole were found to be effective alternate drugs (Parry, 2004).

In the present study, ampicillin and cotrimoxazole were not as effective against *S. typhi* as they used to be. Probably these drugs could once again be used in the future for enteric fever (Rodrigues et al, 2003).

Cephalosporins, in the recent past, have gained importance for the treatment of enteric infections (Gautam et al, 2002). Parenterally administered 3rd generation cephalosporins are effective in the treatment of typhoid fever. In this study, *S. typhi* was quite sensitive to ceftriaxone (73.2%). Results of 92–100% sensitivity to 3rd and 4th generation cephalosporins were observed by Gautam et al, 2002 and Nath et al, 2003. Notwithstanding this, some authors have reported treatment failure with these cephalosporins in recent years (Mandal et al, 2004). In this study cefuroxime (69.4%) and ceftazidime (62.5%) were not too sensitive as compared to ceftriaxone .

Quinolones are highly effective against salmonellae *in vitro*. Ciprofloxacin is considered the drug of choice for the treatment of multidrug resistant typhoid,

replacing chloramphenicol (Mandal, 2004). Hemalatha et al in India reported a sensitivity of 95% to ciprofloxacin in 1999.

However, due to the widespread use of ciprofloxacin since its introduction in the early1900s, resistance and treatment failures were being increasingly observed and reported. Gautam et al reported a decrease in sensitivity of *S. typhi* to ciprofloxacin from 89% to 81% (1997-2001). Threlfall et al, 2001, noted that 23% of *S. typhi* in 1999 in the UK showed decreased susceptibility to ciprofloxacin. The present study also documents lower findings of 61.5%.

Gentamicin had a sensitivity of 72.9% followed amikacin (53.5%).

Tables 4.6 - 4.9 show the disc diffusion test results for 58 isolates using both SMM and API methods. For *Salmonella spp* isolates, whilst most isolates were found to be sensitive (90.2 – 100%) to the antibiotics used, only 20.2% was sensitive to cotrimoxazole (SMM being considered). Results using the API were quite peculiar. Cefuroxime, a 3rd generation cephalosporin, had 9.8% sensitivity compared to 96.1% using SMM. Again, ceftazidime had 68.6% respectively as compared to 98.5% using the standard manual method. However, the results of API are doubtful. It could be attributed to some non-functioning reagents. This is because clinical results show many patients still respond to treatment by these drugs.

5.1.4 MIC Results

Generally, a margin of safety 10 times the MIC is desirable to ensure successful treatment of the disease (Atlas, 1995)

5.1.4.1 MIC of *Salmonella spp* isolates to ampicillin

For ampicillin, 11 out of 100 were sensitive. The MIC of the sensitive isolates was 1.25ug/ml. MIC interpretive standards for dilution susceptibility testing states a dilution value of less than 8ug/ml as susceptible and 16ug/ml as moderately susceptible (Balows et al, 1991).

The value obtained in this study is well below the susceptibility breakpoint value (10 ug) and agrees with Dobardzic (1996) who reported that all ampicillin-susceptible isolates had extremely low MIC values.

Nine (9) of the 100 isolates clustered around 160ug/ml dilution giving a MIC of 160ug/ml (Table 4.10). The answer to whether these should not be considered as resistant is that they should rather be taken as those of intermediate susceptibility.

The remaining isolates (80%) which were resistant had an MIC greater than 320ug/ml (Table 4.10). This value is ten times higher than the 32ug/ml stated as the interpretive standard for resistance to ampicillin (Balows et al, 1991).

5.1.4.2 MIC of *Salmonella spp* isolates to chloramphenicol

For chloramphenicol, there were 21 sensitive, 1 intermediate and 78 resistant isolates. The MIC of the sensitive isolates was 80ug/ml, that for intermediate was 160ug/ml and that for the resistant ones was 5000ug/ml (see Tables 4.10). Kariuki et al (2005) in Kenya noted the large increase in MICs of all commonly used drugs including chloramphenicol and ampicillin over the last decade.

Rampant use of chloramphenicol and ampicillin, not only for Salmonella but for other infections too, has gradually led to increased MICs of the drugs, threatening their therapeutic efficacy. The question then is whether the high level of MICs, in resistant isolates, is determined by the acquisition of R-plasmid under selective pressure and that in contrast, loss of R-plasmid causes emergence of sensitive strains showing very low MICs. I believe more research is needed here.

5.1.4.3 MIC of *Salmonella spp* isolates to ciprofloxacin

In my study, all the isolates were sensitive to ciprofloxacin using the standard manual but 55 (94.8%) out of the 58 were sensitive by API method. All the isolates (100%) were sensitive using the broth dilution method. Three (3) gave a MIC of 0.039ug/ml whilst ninety seven (97) gave a MIC of 0.078ug/ml (Table 4.10). This is well below the breakpoint value of 5ug.

The MIC interpretive standard for susceptibility to ciprofloxacin is a dilution of less than 1ug/ml. The value of 0.078ug/ml obtained still agrees with Dobardzic (1996) who reported that the MIC of ciprofloxacin of all Salmonella isolates tested was well below its susceptibility breakpoint but not with Parry (2003) who believes there is growing resistance to ciprofloxacin.

5.1.4.4 MIC of *Salmonella typhi* isolates to ampicillin

For ampicillin, there were 2 sensitive and 28 resistant isolates. The MIC of the sensitive isolates was 1.25ug/ml and that for the resistant ones was 5000ug/ml (see Table 4.12). The MIC interpretive standard for dilution susceptibility testing states a dilution value of less than 8ug/ml as susceptible and 16ug/ml as moderately susceptible (Balows et al, 1991).

The value obtained in this study is far below the susceptibility breakpoint value (10 ug) and agrees with Dobardzic (1996) who reported that all ampicillin-susceptible isolates had extremely low MIC values.

5.1.4.5 MIC of *Salmonella typhi* isolates to chloramphenicol

For chloramphenicol, there were 5 sensitive and 25 resistant isolates. The MIC of the sensitive isolates was 80ug/ml and that for the resistant ones was 5000ug/ml (see Tables 4.12).

5.1.4.6 MIC of *Salmonella typhi* isolates to ciprofloxacin

For ciprofloxacin, there were 29 sensitive and 1 resistant isolates. The MIC of the sensitive isolates was 0.078ug/ml and that for the resistant ones was 20ug/ml (see Tables 4.12).

The MIC interpretive standard for ciprofloxacin is given as < 1ug/ml for susceptible and > 8ug/ml for resistant strains. This study obtained a value of 0.078ug/ml and it is higher than that obtained by Madhuilka et al (2004) in India. They found the MICs of ciprofloxacin to be less than 0.5mg/l.

The resistant value conforms to the study by Parry (2003) who believes there is growing resistance to ciprofloxacin.

5.2 Summary

In summary, for the year under review, 3,908(33.1%) positive blood cultures were obtained. Out of this number 372(3.1%) were bacteriologically confirmed Salmonella cases – 296(79.6%) being children. There was no gender bias.

Out of the 296 isolates, 258(87.2%) had NTS isolated. Though this value is not as high as that obtained by Udgaonkar et al (1995) of 93.3% in India, it is higher than 21.6% by Wilkens et al (1997) in Accra and 50.4% by the Public Health Sector of South Australia (1995).

A higher percentage of the children in this study were between the ages of 0-3 years (88.5%). Similar results were obtained by Chui et al (1994), Udgaonkar et al (1995), Yagupsky et al (2002) and Diez et al (2004) in Taiwan, India, Israel and Spain respectively.

Ampicillin and chloramphenicol seem not to be effective against Salmonella bacteraemia due to the very high resistance seen in this study. Ampicillin and chloramphenicol had sensitivities between 6 – 17.2% and 8.6 – 15% respectively. This implies a resistance of 82.8 – 94% and 85 – 91.4% respectively for ampicillin and chloramphenicol. These values are far higher than resistances of 22 – 46% by Guerra et al (2000) in Spain, 18 – 50% by Dobardzic (1996) in Kuwait and 31 – 42 %(1994-2003) by Kariuki et al (2005) in Kenya.

Ciprofloxacin however seems to work effectively against Salmonella infections in children.

5.3 Conclusion

Salmonella bacteraemia is a health problem for children in Kumasi with children between 0 and 3 years being the most affected. It may be complicated by resistance to commonly available antibiotics. Regular screening of all febrile children within this age group by means of blood cultures might go a long way in early detection and prompt treatment.

More resistance was seen in *Salmonella typhi* than in *S. spp* with an observed resistance of 82.8 – 94% to commonly available drugs.
Enteric fever continues to be a major health problem despite the use of antibiotics and the development of newer antibacterial drugs.

5.4 Recommendations

The Standard Manual Method should be maintained as the primary means of identification of organisms since it compares favourably with the API method.

I recommend that more education on the rational use of antibiotics is needed to prevent selective pressure mounting against them.

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APPENDIX I

Media used, composition and mode of preparation

Brain Heart Infusion broth (Oxoid, England)

Composition (typical g/L)	Brain-Heart Infusion Solids (Porcine)	17.5
	Tryptose	10.0
	Glucose	2.0
	Sodium Chloride	5.0
	Disodium Hydrogen Phosphate	2.5
	pH	7.4+/- 0.2

Mode of preparation – Disperse 37g in 1L of deionised water and soak for 10minutes. Swirl to mix and warm gently to dissolve. Dispense into final containers and sterilize by autoclaving for 15minutes at 121^0C.

Blood Agar Base (Oxoid, England)

Formula in g/l	Nutrient substrate (peptones, extracts)	22
	Sodium chloride	5
	Agar	13
	pH	7.3(approx.)

Mode of preparation – Suspend 40g in 950ml of distilled water and boil to dissolve the medium completely. Sterilize by autoclaving at 121^0C for 15minutes. Cool to 50^0C and aseptically add 5-7% sterile defibrinated blood. Mix well before pouring.

MacConkey Agar (Oxoid, England)

Standard formula in g/l	Peptic digest of animal tissue	20.00
	Lactose	10.00

Bile salts	5.00
Sodium chloride	5.00
Neutral red	0.07
Agar	15.00
Final pH (at 250C)	7.5+/- 0.2

Mode of preparation – Suspend 55.07g in 1000ml distilled water and heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121^0C) for 15 minutes. Avoid overheating. Cool to 45-50^0C and pour into sterile Petri plates.

Cysteine Lactose Electrolyte Deficient Agar (Oxoid, England)

(Bevis modification)

Composition (typical g/L)	Balanced Peptone No.1	4.0
	Beef Extract	3.0
	Tryptone	4.0
	Lactose	10.0
	L-Cystine	0.128
	Bromothymol Blue Indicator	0.02
	Andrade's Indicator	0.08
	Agar No. 1	15.0
	pH	7.5+/- 0.2

Mode of preparation – Disperse 36g in 1L of deionised water and soak for 10 minutes. Swirl to mix and sterilize by autoclaving for 15 minutes at 121^0C. Cool to 47^0C and mix before pouring into Petri dishes and then dry the agar surface.

Nutrient Agar (Oxoid, England)

Composition (typical g/L)	Peptone	5.0

Beef Extract	3.0
Sodium chloride	8.0
Agar No. 2	12.0
pH	7.3 +/- 0.2

Mode of preparation – Weigh 28g of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then sterilize by autoclaving for 15 minutes at 121^0C. cool to 47^0C, mix well and pour plates.

TSI Agar (Oxoid, England)

This is a medium for the differentiation of gram negative enteric bacteria on the basis of carbohydrate fermentation and the production of hydrogen sulphide.

Formula in g/l Peptones	27.4
Glucose	1.0
Lactose	10.0
Sucrose	10.0
Sodium chloride	5.0
Sodium thiosulphate	0.3
Ferric citrate	0.3
Phenol red	0.03
Agar	12.0
pH	7.4 (approx.)

Mode of preparation – Suspend 66g in one litre of distilled water and boil to dissolve the medium completely. Dispense into test tubes and sterilize by autoclaving at121^0C for 15 minutes. Allow to cool in a slanted position such that deep butts are formed.

Simmons Citrate Agar (Biotec, UK)

This is a medium used in the differentiation of Enterobacteriaceae.

Formula in g/l	Magnesium sulphate	0.2
	Ammonium dihydrogen phosphate	1.0
	Dipotassium phosphate	1.0
	Sodium citrate	2.0
	Sodium chloride	5.0
	Bromothymol blue	0.08
	Agar Agar	15.0
	pH	6.9 +/- 0.

Mode of preparation – Weigh 24g of powder and add to 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and then heat to dissolve. Dispense into tubes or bottles then sterilize by autoclaving at 121^0C for 15 minutes. Allow to set as slopes.

Urea Agar Base (Oxoid, England)

Composition (typical g/L) –	Peptone	1.0
	Glucose	1.0
	Sodium chloride	5.0
	Disodium phosphate	1.2
	Potassium dihydrogen phosphate	0.8
	Phenol red	0.012
	Agar No. 1	12.0
	pH	6.8 +/- 0.2

Mode of preparation – Disperse 2.1g in 95ml of deionised water and soak for 10 minutes. Swirl to mix and sterilize by autoclaving for 15 minutes at 121^0C. cool to 47^0C and add 5ml of sterile urea solution, supplement X130 or X135. Dispense into final containers and set in a sloped position.

Tryptone Soya Broth (Oxoid, England)

Typical formula in g/l –	Pancreatic digest of casein	17.0
	Papaic digest of soybean meal	3.0
	Sodium chloride	5.0
	Di-basic potassium phosphate	2.5
	Glucose	2.5
	pH	7.3 +/- 0.2

Mode of preparation – Dissolve 30g in 1 litre of distilled water and distribute into final containers. Sterilize by autoclaving at 121^0C for 15 minutes.

Table 4.4 Antibiogram of *Salmonella spp* showing sensitivities for the year 2005 - Percentages in parenthesis

	Isolates n	cot (%)	amp (%)	chl (%)	gen (%)	amk (%)	cro (%)	cxm (%)	cip (%)	caz (%)
JAN.	28	14 (50)	1 (3.6)	9 (32.1)	27 (96.4)	28 (100)	28 (100)	19 (67.9)	28 (100)	28 (100)
FEB.	16	4 (25)	0	4 (25)	16 (100)	16 (100)	16 (100)	12 (75)	16 (100)	15 (93.8)
MAR.	16	2 (12.5)	0	2 (12.5)	16 (100)	16 (100)	16 (100)	2 (12.5)	16 (100)	16 (100)
APR.	10	5 (50)	1 (10)	3 (30)	9 (90)	10 (100)	10 (100)	9 (90)	9 (90)	10 (100)
MAY	13	2 (15.4)	2 (15.4)	2 (15.4)	12 (92.3)	13 (100)	13 (100)	13 (100)	13 (100)	11 (91.7)
JUN.	12	3 (25)	4 (33.3)	4 (33.3)	11 (91.7)	12 (100)	12 (100)	8 (66.7)	11 (91.7)	12 (100)
JUL.	33	0	0	6 (18.2)	33 (100)	33 (100)	33 (100)	30 (90.9)	33 (100)	32 (97)
AUG.	37	4 (10.8)	6 (16.2)	6 (16.2)	33 (89.2)	37 (100)	37 (100)	37 (100)	37 (100)	37 (100)
SEPT.	14	1 (7.1)	3 (21.4)	3 (21.4)	14 (100)	14 (100)	14 (100)	12 (85.7)	13 (92.9)	14 (100)
OCT.	27	2 (7.4)	3 (11.1)	4 (14.8)	27 (100)	27 (100)	27 (100)	27 (100)	27 (100)	27 (100)
NOV.	34	2 (5.9)	6 (17.7)	5 (14.7)	32 (94.1)	32 (94.1)	17 (50)	34 (100)	34 (100)	34 (100)
DEC.	18	2 (11.1)	3 (16.7)	4 (22.2)	17 (94.4)	15 (83.3)	18 (100)	18 (100)	18 (100)	18 (100)
Total	258	18.4	12.1	21.3	95.7	98.1	95.8	82.4	97.9	98.0

Legend: cot-cotrimoxazole; amp-ampicillin; chl-chloramphenicol; gen-gentamicin; amk-amikacin; cro-ceftriaxone; cxm-cefuroxime; cip-ciprofloxacin; caz-ceftazidime

Figure 3.2 Antibiogram of *Salmonella spp*. isolates showing sensitivities for the year 2005

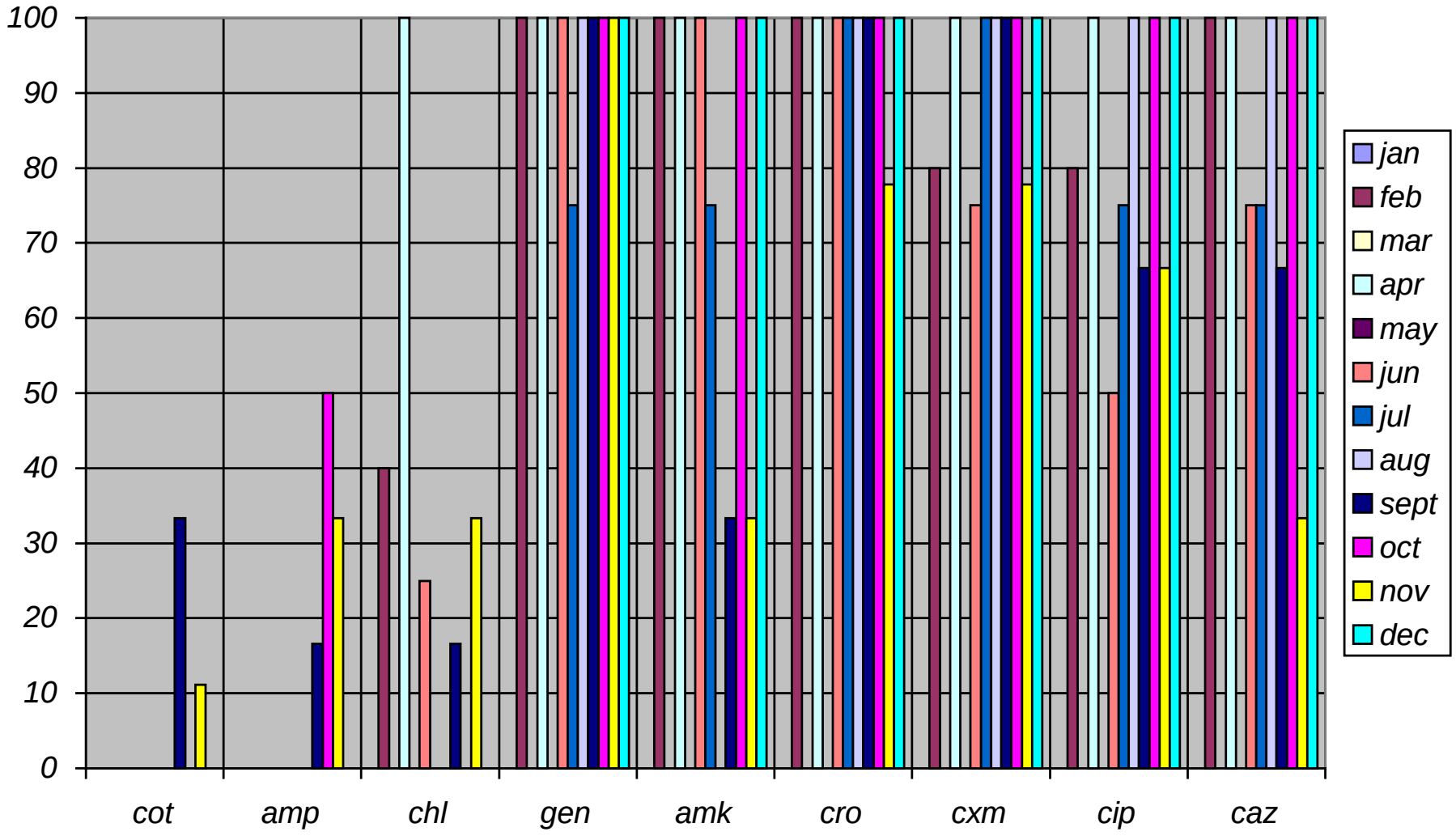
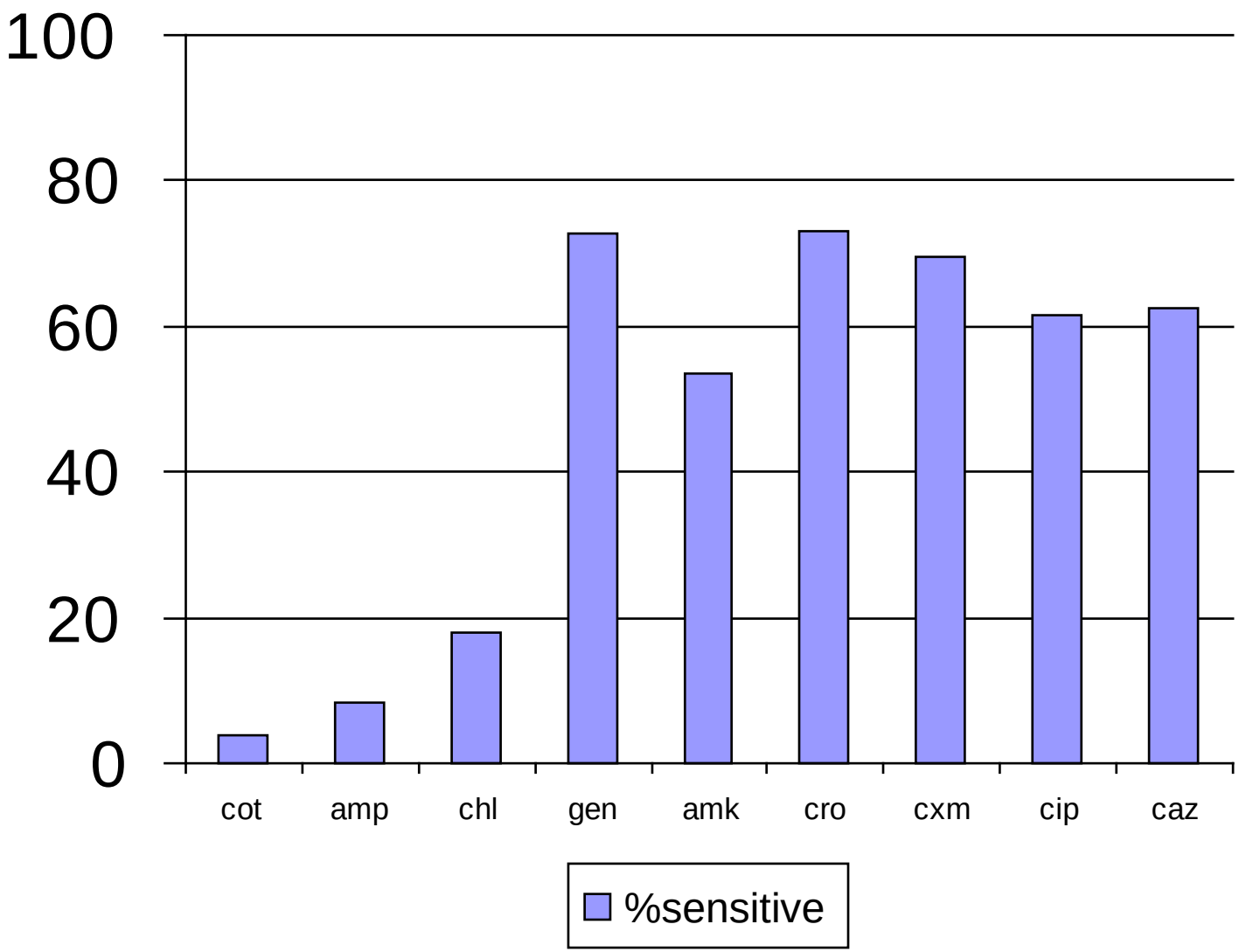


Table 4.5 Antibiogram of *Salmonella typhi* showing sensitivities for the year 2005 - Percentages in parenthesis

	Isolates n	cot (%)	amp (%)	chl (%)	gen (%)	amk (%)	cro (%)	cxm (%)	cip (%)	caz (%)
JAN.	0	0	0	0	0	0	0	0	0	0
FEB.	5	0	0	2 (40)	5 (100)	5 (100)	5 (100)	4 (80)	4 (80)	5 (100)
MAR.	0	0	0	0	0	0	0	0	0	0
APR.	2	0	0	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)
MAY	0	0	0	0	0	0	0	0	0	0
JUN.	4	0	0	1 (25)	4 (100)	4 (100)	4 (100)	3 (75)	2 (50)	3 (75)
JUL.	4	0	0	0	3 (75)	3 (75)	4 (100)	4 (100)	3 (75)	3 (75)
AUG.	1	0	0	0	1 (100)	0	1 (100)	1 (100)	1 (100)	1 (100)
SEPT.	6	2 (33.3)	1 (16.6)	1 (16.6)	6 (100)	2 (33.3)	6 (100)	6 (100)	4 (66.7)	4 (66.7)
OCT.	4	0	2 (50)	0	4 (100)	4 (100)	4 (100)	4 (100)	4 (100)	4 (100)
NOV.	9	1 (11.1)	3 (33.3)	3 (33.3)	9 (100)	3 (33.3)	7 (77.8)	7 (77.8)	6 (66.7)	3 (33.3)
DEC.	3	0	0	0	3 100	3 100	3 100	3 100	3 100	3 100
Total		3.7	8.3	17.9	72.9	53.5	73.2	69.4	61.5	62.5

Legend: cot-cotrimoxazole; amp-ampicillin; chl-chloramphenicol; gen-gentamicin; amk-amikacin; cro-ceftriaxone; cxm-cefuroxime; cip-ciprofloxacin; caz-ceftazidime

Figure 3.3 Antibiogram of *Salmonella typhi* isolates showing sensitivities for the year 2005


100
80
60
40
20
0
cot
amp
chl
gen
amk
cro
cxm
cip
caz
%sensitive