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T I T L E

PROFILE OF SALMONELLA TYPHIMURIUM AT KENYATTA NATIONAL
HOSPITAL NAIROBI, KENYA

BY

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Nazir Mirza-----

DATE 24.10.91

DR. NAZIR BEGUM MIRZA

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DEDICATION

To my late father - Major A. S. Din. OBE.

And

To my husband - Dr. M. B. Mirza

For their continuous support and encouragement.

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LIST OF SYMBOLS AND ABBREVIATIONS.

AIDS	=	Acquired Immunodeficiency syndrome
AMP	=	Adenocine monophosphate
AOW	=	Adult Observation ward
BA	=	Blood Agar
CBA	=	Chocolate blood agar
°C	=	Degree Centigrade
CAMP	=	Cyclic adenocine monophosphate
CCF	=	Congestive cardiac failure
C.I.	=	Confidence Interval
CMI	=	Cell mediated immunity
CNS	=	Central Nervous System
Co ₂	=	Carbon diaoxide
CSF	=	Cerobrospinal fluid
DCA	=	Deoxycholate Citrate Agar
DEC	=	December
DST	=	Diagnostic Sensitivity Test Agar
=	=	Equals
HIV	=	Human Immunodefeciency Virus
HRS	=	Hours

Kb	=	Kilobase
KNH	=	Kenyatta National Hospital
LPS	=	Lipopolysaccharides
Mac	=	MacConkey
M.Med	=	Master of Medicine
Mcg	=	Microgram
MI	=	Mililitre
MMWR	=	Morbidity and Mortality Weekly Report
MTC	=	Medical Training College
NCTC	=	National Collection of Type Cultures
-	=	Negative
NLF	=	Non - lactose - fermenter
NS	=	Not Significant
N	=	Number
OR	=	Odds Ratio
PEW	=	Paediatric Emergency Ward
PHLS	=	Public Health Laboratory Services
%	=	Percentage
+	=	Positive
POW	=	Paediatric Observation Ward

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PUO	=	Pyrexia of Unknown Origin
R	=	Resistant
RHD	=	Rheumatic Heart Disease
RTF	=	Resistant Transfer Factor
SCD	=	Sickle Cell Disease
S	=	Sensitive
SEPT	=	September
SD	=	Standard Diviation
TSI	=	Triple Sugar Iron Agar

S U M M A R Y

Salmonella typhimurium infections at Kenyatta National Hospital (KNH) are being isolated more frequently than Salmonella typhi.

There has been a persistent rise in the isolation of S.typhimurium from blood, stools and cerebrospinal fluids (CSF) since 1970, with reports of minor outbreaks in 1972 and 1974. A major outbreak occurred in 1985 which resulted in closure of the paediatric observation wards (POW) the main admission area for children. Renovation measures in POW improved the situation for only a short time. S.typhimurium reappeared soon after reopening these wards. Over - crowding with resultant poor hygiene is one of the contributory factors to infections with S.typhimurium.

Antibiotic resistance pattern of recent S.typhimurium isolates when compared to those isolated in the early 70's (Table 20), showed an increase in resistance to most antibiotics : ampicillin (95%), Tetracycline (56%), cotrimoxazole (83%), kanamycin

(53%), gentamicin (32%), streptomycin (95%), sulphafurazole (100%) and chloramphenicol (88%). Only 2% strains were resistant to amikacin and all were sensitive to cefotaxime, the only third generation cephalosporin tested. This reflected the development of multiple drug resistance in S.typhimurium strains. S.typhi as compared to S.typhimurium is still sensitive to most of the antibiotics including chloramphenicol. While chloramphenicol remains the mainstay therapy for S.typhi infections, it cannot be used for the treatment of S.typhimurium infections. It is recommended that cefotaxime or other third generation cephalosporins would be the drugs of choice for the treatment of life-threatening infections with S.typhimurium. There is a need to look into cheaper and more effective drugs.

Thirty five strains of S.typhimurium studied for transfer of resistance, showed that they transferred their resistance to a sensitive strain of E.coli K - 12 in the conjugation experiments. Each strain carried multiple plasmids. Further characterization of these

plasmids was not possible due to restricted resources.

S.typhimurium isolated from clinical material from KNH belonged to several different phage-types. Type 56 (29.4%) was most common, followed by 193 (20.5%) and 208(17.6%). Other phage types isolated included 135 (2 strains), 132 (1 strain , 2 (1 strain), 3 aerogenic (1 strain), untypable (5 strains) and RDNC (1 strain). These phage types did not conform to any particular pattern.

In a 6 month prospective study for nosocomial and community acquired gastroenteritis a total of eleven hundred patients were screened. Nosocomial gastroenteritis was acquired by 330 (30%) patients. From 330 nosocomial gastroenteritis cases Salmonella species were isolated from 29 (8.8%) cases out of which 22 (75.9%) were S.typhimurium.

From 770 patients screened on admission with or without diarrhoea, the prevalence of rectal carriage of S.typhimurium in the community was 1.2%. The risk of nosocomial gastroenteritis with S.typhimurium was

significantly higher than in patients with community acquired infections. There was no age related significant difference in the incidence of nosocomial or prevalence of community acquired gastroenteritis with S.typhimurium, that is nosocomial and community acquired cases occurred equally in children under 5 years of age and those above 5 years.

Of 112 HIV positive patients, 33 developed nosocomial gastroenteritis. S.typhimurium was isolated from 7 (21.2%) of these, while 79 patients did not have Salmonella isolated from their rectal swabs on admission.

In a case control study, 50 cases with S.typhimurium infection were compared with 100 controls matched for age and sex. The results indicated that with S.typhimurium infections, fever was the most common presenting complaint (84%), followed by diarrhoea (56%), malnutrition (38%), and cough (36%). A few cases presented with bone and joint swellings (6%), jaundice (4%) and CNS involvement (2%). The

associated risk factors showing significant odds ratios for S.typhimurium included : residence outside Nairobi (3.14), drinking of untreated water (12.76), use of pit latrines (19.15), presence of domestic animals in the compound (5.35), chickens (2.84), chickens + cows (3.16) and drinking of untreated cows milk (10.63).

Other associated risk factors included illnesses like measles, malnutrition, bronchopneumonia, malignancies, septic arthritis with sickle cell disease and cardiac problems, which necessitated prolonged stay of the patient in hospital.

Therefore there is an urgent need to set up a surveillance system for early recognition of S.typhimurium infections in the hospital. That the laboratory should also remain vigilant and provide information early on the development of multiple drug resistance in the pathogens isolated from KNH.

C H A P T E R 1.**1.1 INTRODUCTION.**

Kenyatta National Hospital (KNH) is an 1100 bed acute care hospital, in Nairobi, Kenya. It is basically a referral hospital to the whole country, but it also serves as a community hospital for individuals residing in and around Nairobi. The hospital is serviced by a relatively well equipped Medical Microbiology Laboratory, run by well trained technical staff and supervised by qualified pathologists. This laboratory also serves as a training ground for medical undergraduates, and postgraduates of the University of Nairobi and technical staff from the Medical Training College (MTC), Ministry of Health. All the specimens for microbiology investigations from KNH are received by the laboratory for processing. As there is as yet no system for computer entry of the results, the original report is dispatched to the ward, while the duplicate is kept in the laboratory as a record for reference.

By monitoring the results of cultures and sensitivity of organisms isolated, it started to become clear in the early seventies that S. typhimurium was being isolated with greater frequency from blood culture, stool and CSF specimens. In a retrospective study, Wamola and Mirza (1981) analysed the results of diagnostic material processed by the bacteriology laboratory during a 10 year period (1970-1980). It became apparent that in these years the hospital had seen a 14 - fold increase in S. typhimurium isolates, unparalleled by other salmonellae including the well known endemic S. typhi. Also noted over the years was the high mortality and increased resistance to antibiotics associated with S. typhimurium infections compared to other salmonellae (Wamola et al. 1974). More serious outbreaks of S. typhimurium infection from KNH were also reported by Say and Wamola (1974) and Walijee (1976). Mirza and Wamola (1989) reported the 1985 outbreak which necessitated the temporary closure of the Paediatric Emergency Wards (PEW), the main

paediatric admitting area. The sources of these outbreaks remained unknown.

The problem of Salmonella infections at KNH reflects a world-wide increase in the multi-drug resistant, non-typhoidal Salmonella infections caused by S. typhimurium (Christie A.B. 1971, Lepage et al. 1984, Nesbitt et al. 1988).

1.2 LITERATURE REVIEW

S. typhimurium is widespread in man and animals, both wild and domestic. There is evidence that the incidence of non-typhoidal salmonellosis is increasing throughout the world. This has also been reported by Wamola et al. (1974), Wamola and Mirza (1981), Lepage et al. (1984), Bogaerts et al. (1985), Nesbitt et al. (1988) to mention but a few relevant studies to the African context.

The problem of Salmonella infections at (KNH) has been recognized since early 1970, and was brought to the attention at the Annual Scientific Conference of

the East African Medical Research Council, for the first time, by Say and Wamola (1974). Later, more reports were to follow showing a definite rise in the isolation of salmonellae especially S. typhimurium from various clinical specimens including blood cultures, stools, pus and cerebrospinal fluids (CSF), (Slack and Badia 1974, Mirza and Nsanzemuhire 1979, Wamola and Mirza 1981, Wamola et al. 1981, Mirza and Wamola 1983). This rise was also shown to be associated with severe malnutrition in infants and children who were also suffering from measles or whooping cough and developed complications such as bronchopneumonia, laryngotracheobronchitis or septic arthritis (Slack and Badia 1974, Shiroya 1987, Nduati 1987). Among adults with positive blood cultures there were a significant number with underlying malignant disease especially of the lymphoreticular system (Kasili 1979, Slack and Badia 1974).

Attention was drawn to the fact that infections with S.typhimurium carried a greater risk, with higher

mortality, in infants and children, and that it had developed multiple antibiotic resistance in comparison to the endemic S.typhi which still remains highly sensitive to most of the antibiotics (Wamola et al. 1974, Say and Wamola 1974, Slack and Wamola 1977, Nesbitt et al. 1988, Nesbitt and Mirza 1989).

There seems to be a world-wide rise in the isolation of multiresistant strains of S. typhimurium (Christie 1971, Editorial Lancet 1982). Within Africa there have been reports of the emergence of multiresistant strains from Kenya (Wamola et al. 1974, Say and Wamola 1974, Mirza et al. 1981, Mirza and Wamola 1983, Nesbitt et al. 1988, Nesbitt et al. 1989), Nigeria (Okubadego et al. 1971), Uganda (Phillips and Tindimwebwa 1972), Durban in South Africa (Robins - Browne et al. 1983), Rwanda (Lepage et al. 1984, Bogaerts et al. 1985) and from Tunisia (Ahmed et al. 1988).

Non-typhoidal Salmonella infections are frequently associated with chronic diseases (Fraya et al. 1985),

and in patients with known underlying defects in host defenses (Patton et al. 1985). Association of *Salmonella* infections with sickle cell anaemia, malaria and bartonellosis is well documented, and this may be in part due to haemolysis (Black 1960; Hook 1961; Kaye et al. 1967; Foy and Kendal, 1974). Haemolysis has been shown to decrease the lethal dose and increase mortality in *S.typhimurium* infections in mice. Erythrophagocytosis by fixed macrophages in the reticuloendothelial system is thought to interfere with the ability of these cells to kill *Salmonella* effectively.

Infections with non-typhoidal *Salmonella* are being described with Acquired Immunodeficiency syndrome (AIDS) with increasing frequency (Nadelman et al. 1985; Glaser et al. 1985; Fischl et al. 1986; Cellum et al. 1987; Mirza et al. 1989). Patients with AIDS have a high incidence of various haematological abnormalities (Spivak et al. 1984). The frequency of this finding raises the possibility that haemolysis contributes to

anaemia and possibly to impaired reticuloendothelial cell function. It is also known that patients with AIDS have defective cell-mediated immunity (CMI), Masur et al. 1981. It may be possible that impaired reticuloendothelial function and defective CMI are both contributing factors in some patients with AIDS who develop severe Salmonella syndromes and bacteraemia.

It has been shown that schistosomiasis is associated with invasive salmonellosis (Hathout et al. 1967; Rocha et al. 1971). All three schistosomes, S. haematobium, S. japonicum and S. mansoni have been involved (Rocha et al. 1971). It has also been shown that salmonellae as well as other gram-negative bacteria, are capable of penetrating and multiplying within these parasites, which then serve as the source for recurrent bacteraemia or bacteriuria (Ohens and Dickerson 1969). Patients with bladder involvement due to S. haematobium develop Salmonella urinary tract infection as well as bacteraemia (Farid et al. 1970, Matharu 1978).

More recently infections with non - typhoidal strains of salmonellae are being described in patients with the acquired immunodeficiency syndrome (AIDS) with increasing frequency from several countries including Kenya (Nadelman et al. 1985, Fischl et al. 1986, Cellum et al. 1987, Mirza et al. 1989). The infection with non-typhoidal salmonellae may occur in patients with an established diagnosis of AIDS or it may be the first manifestation of this disorder (Sperber and Schleupner 1987).

Salmonellosis as a sexually transmitted disease among homosexual men and AIDS patients is being reported frequently from tropical areas such as Haiti and Central Africa (Drusin et al. 1976; Smith et al. 1985).

1.3. LITERATURE REVIEW FROM EAST AFRICA

Huckstep in 1962 wrote his book "Typhoid fever and other Salmonella infections". He saw and treated over 1000 cases between 1951 and 1961 in Kenya and

later in Uganda. In his book Huckstep gives an account of pathological, medical and surgical aspects of typhoid and paratyphoid fevers based on his observations of these cases. He also included a small chapter on non-typhoidal Salmonella infections (Chapter 29 pages 252-263). Most of the food poisoning epidemics mentioned by Huckstep are those which took place in U.K., Europe and U.S.A. There is no mention of any local epidemics with non-typhoidal salmonellae. However, Huckstep has highlighted the association of sickle cell anaemia and bone involvement with Salmonella species.

MacDougal (1954) reported a case of epitrochlear abscess with S. enteritidis. This was in a one year old child with prolonged fever for 6 months, diarrhoea, vomiting, malnutrition, scabies and malaria. He thought that the infection was due to blood stream spread, similar to that of typhoid, probably precipitated by trauma resulting in an infected haematoma.

Musoke (1952) reported his clinical findings on 200 cases of typhoid fever, from Mulago Hospital, Kampala in Uganda. He described an increase in the endemicity of typhoid that occurred in and around Kampala in 1948-1949. To show an increase in the endemicity he quoted the following figures. From 1942 to 1946, the average number of cases of typhoid fever admitted to Mulago Hospital was 42 per year, while the number of cases admitted in 1948 and 1949 were 171 and 200 respectively.

Mpairwe (1968) reported 3 cases of Salmonella meningitis from Mulago Hospital, Kampala, Uganda. Two of the cases were due to S. enteritidis and one due to S. typhimurium. All were children under one year of age.

Foster and Hawgood (1966) reported one case of S. enteritidis in their 97 clinical cases of meningitis in a paper on the "Aetiology and laboratory diagnosis of meningitis in Kampala, Uganda". No particular importance was given to this one case of Salmonella

meningitis until Mpairwe (1968) reported his 3 cases of Salmonella meningitis and cited this case as one of the three.

Kagwa Nyanzi from Uganda in 1971 discussed clinical presentation and laboratory diagnosis of typhoid fever. From her work she concluded that typhoid fever in children constituted an important problem in Uganda. She thought that it was not difficult to recognize the disease if the condition was borne in mind when dealing with a child suffering from fever of some days duration, headache, abdominal pain, dry cough and furred tongue. But the ultimate diagnosis rested on laboratory investigations. The classical picture seen in adults is usually absent in children e.g. step ladder pyrexia, pulse dissociation, rose spots, leucopenia and constipation. The common complications in children were mostly of central nervous system, respiratory system and gastrointestinal tract. The mortality in her cases was low (6%). This was probably due to treatment with

chloramphenicol.

Kalya and Oduori (1972) conducted a study at KNH in 1971. They cultured rectal swabs from 180 children with diarrhoea admitted to paediatric observation wards. Twenty nine (16.1%) children had pathogenic bacteria isolated from their rectal swabs. Seventeen were Escherichia coli, 8 were Shigella species and 4 had Salmonella isolated. Three of the Salmonella species isolated were S.typhimurium and one was S.dublin. Majority of the children were under one year of age. One S.typhimurium out of the three was resistant to chloramphenicol and two were resistant to ampicillin.

Mackey (1955) from Dar es Salaam wrote a paper on Salmonella isolates from human and animal sources during the period 1948 - 1953. Although all age groups were equally affected, he found that children had more severe disease than adults. A small proportion, about 3%, were thought to be carriers. The large house lizard population in Dar es Salaam was found to be

heavily infected, but there was no host reaction or disease among these lizards, who live in close association with man. There was hardly any house in Dar es Salaam in which infected lizard droppings could not be found. Of the thirty-three different Salmonella types found in lizard droppings, twenty one were also isolated from human cases. The other sources included cockroaches, dogs, fowls, snakes and a centipede.

Vaizy (1959) from Mulago Hospital presented a paper to the Association of Physicians Annual Conference on "typhoid at Mulago Hospital - Analysis of cases between 1949 and 1957". Vaizy made a comparison of cases of typhoid fever in Mulago Hospital, Kampala in 1957 when chloramphenicol treatment was available, with those in 1949 published by Musoke (Musoke 1949) at a time when chloramphenicol was not available. The mortality was reduced following treatment with chloramphenicol from 24.5 to 7.5% and the duration of fever, and of stay in hospital, by about half. Late deaths from the 7th to the 12th day following treatment

were thought to be due to treatment failure with chloramphenicol. When sensitivity tests were performed he found that one in every twelve (8.3%) isolates was resistant to chloramphenicol.

Walijee (1976) wrote her M. Med. thesis on "Salmonella infections in children". In her series of 178 patients, a wide variety of salmonellae were isolated, but S. typhimurium was isolated in highest numbers - 78% of total isolates. It was also resistant to most antibiotics, while S. typhi was 100% sensitive. She found four cases of Salmonella with Schistosoma mansoni infection. Three of these patients had Salmonella septicaemic illness, while one had Salmonella isolated from stool only. All 4 gave a history of fever over a prolonged period.

Matharu's (1978) M. Med thesis on "Chronic salmonellosis complicating S.mansoni infection", was mainly a clinical and pathological study carried out at KNH over a two year period. His observation was that chronic salmonellosis complicated an underlying

S.mansoni infection. The duration of symptoms characterized by night sweats, abdominal tenderness, with hepatomegaly was between 2 - 16 months with an average of 7 months. Blood cultures were the most reliable method for isolation of the Salmonella species. He found the response to treatment for both the infections with a combination of cotrimoxazole or chloramphenicol, and hycanthonone or oxamniquine was excellent and there was no relapse of either infection, in a two year follow up.

Patel (1984) in his M. Med. thesis on " The Pattern of Infections in children with haematological malignancies undergoing treatment at KNH ", found only 3 cases positive with Salmonella out of 35 blood cultures. Two were infected with S.typhi and one had S.typhimurium infection.

Shiroya (1987) reported from Kisumu on the oral treatment of Salmonella septic arthritis with chloramphenicol. His study was prompted by the fact that septic arthritis is a common problem in Kisumu

(Kenya) and a pilot project established that Salmonella was the most common aetiological agent. In the 19 patients under 2 years of age treated with oral chloramphenicol, he found the response to oral treatment quite satisfactory.

Nduati's (M.Med. thesis 1987) work on "Acute septic arthritis as it is seen in children at KNH" was to elucidate the clinical characteristics and aetiological agents of septic arthritis. She found that non-typhoidal Salmonella species especially S.typhimurium were the most frequently isolated pathogens in infants under one year of age.

From clinically diagnosed 60 septicaemic patients at KNH, Odhiambo (M.Med thesis 1988) found S.typhimurium (63%) to be the most frequent pathogen isolated from blood cultures of children under 4 years. The highest (46%) were in children under one year of age.

It is noticeable from the above review of literature from East Africa, that there has not been

much written about non-typhoidal Salmonella infections before the seventies. Most of the reviews indicate the isolation of Salmonella as an incidental finding. After 1970, however, the Salmonella isolation rate appeared to be increasing and it started to become noticeable that along with other gram negative organisms, Salmonella species were also becoming resistant to most antibiotics. This was reported from Dar es Salaam (Nsanzemuhire et al. 1974), from Uganda (Kagwa Nyanzi 1971, Phillips and Tindimwebwa 1972) and on several occasions from Kenya (Say 1974, Wamola et al. 1974, Say and Wamola 1974; Slack and Badia 1974, Walijee 1976, Slack and Wamola 1977, Wamola and Mirza 1981, Wamola et al. 1981, Mirza and Wamola 1983, Nesbitt et al. 1988, Mirza and Wamola 1989).

1.4. STUDIES IN ANIMALS

In a study designed to survey the occurrence of salmonellosis on 4 farms and a slaughterhouse in the neighbourhood of Nairobi, Bebora (1979) reported, that

there was an apparent low occurrence of Salmonella infection in birds from farms and the slaughterhouse, but serologically there was evidence that the occurrence of salmonellosis was quite high among the birds. The failure to isolate Salmonella on cloacal swabs from chicken was explained by the fact that the excretion of Salmonella from infected birds is intermittent (Magwood et al. 1962, Brownel et al. 1969, Smith et al. 1972, Brown et al. 1975), and thus, if one does not detect Salmonella bacteria with the cloacal swab method, it does not mean that the animal is free from infection. The shedding of Salmonella from the birds is influenced by muscular fatigue, cold, heat, wetness, limitation of food and water and concurrent infection (Brown et al 1973). It has been found by Brown and others (Brown et al 1975) that cloacal excretion of S. typhimurium occurred during the first 5 days of infection, after which the excretion rate dropped considerably.

The ability of Salmonella bacteria to reside within macrophages (Campbell, 1976) offers another possible explanation for the failure to isolate salmonellae from faeces of animals or birds.

In his work on "Salmonella reservoirs in animals as sources of human infections", Kayihura (MSc thesis 1982) concluded that a wide range of Salmonella serotypes existed in the animal population in the Nairobi area and its vicinity, but there was little overlap between serotypes found in animals and humans. He was unable to draw definite conclusions with regard to epidemiological pattern or zoonotic aspect of human salmonellosis, except, perhaps in the case of S. enteritidis. During his study period, S. enteritidis was the most common serotype after S. typhimurium, to be isolated from stools of patients, and was also the most frequent isolate from wild rodents. The high carrier rate (7.3%) in rodents and their tendency to invade houses, with subsequent contamination of foods or feeds, supports the idea that wild rodents may play

a major role in the epidemiology of both human and animal salmonellosis. Out of his 1500 specimens investigated, Kayihura identified 32 different Salmonella serotypes. Only two of these were S.typhimurium, both being isolated from rodents.

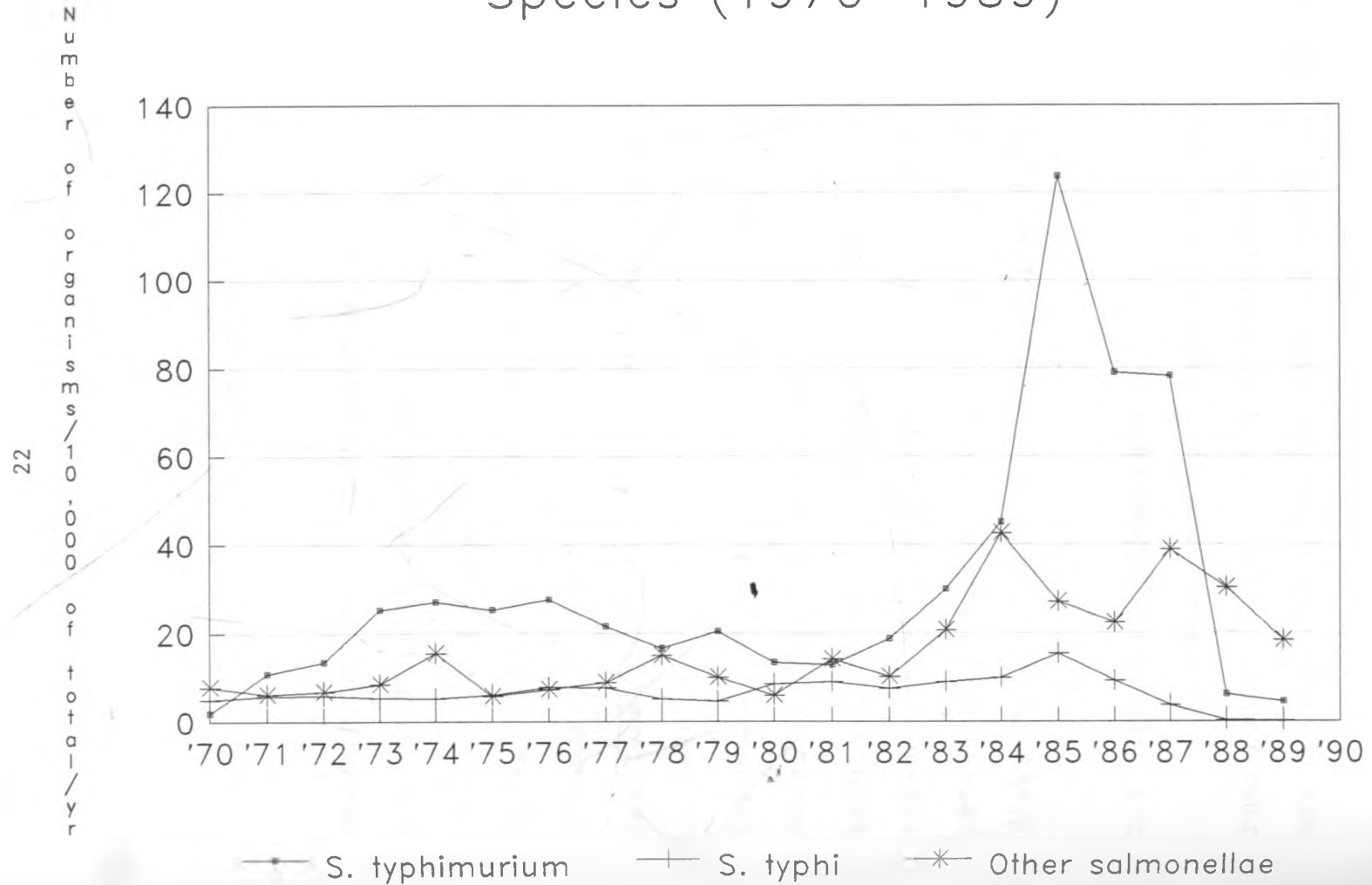
From 1970 to date, S. typhimurium is one of the most common Salmonella species being isolated from clinical specimens at KNH compared to S. typhi, and other species (Fig. 1). Since 1983 Salmonella havana was endemic in the nursery until recently which added to the numbers of other salmonellae, as seen in Fig 1.

S.typhimurium contributes to a large extent to morbidity and mortality in neonates, infants, children and the immunocompromised patients. From Fig.1 which gives rate of organisms per 10,000 of total specimens received in the laboratory per year, it is obvious that in 1985 there was a steep rise in the isolation of this pathogen which coincided with the epidemic of 1985 (Mirza and Wamola 1989).

Given that there has been no change in the methodology of processing of specimens and identification of microorganisms in the laboratory in the last 10 years, I noticed a persistent rise in the isolation of S.typhimurium from various clinical specimens.

Therefore it became necessary to examine this problem in more detail and to identify effective intervention measures to limit the number of S.typhimurium infections at KNH.

Figure 1: Yearly Isolates of Salmonella Species (1970–1989)



CHAPTER 2

DESCRIPTION OF THE GENUS SALMONELLA

2.1 HISTORICAL BACKGROUND

Salmonella typhimurium was first isolated in 1892 by Loeffler from rodents suffering from a typhoid-like illness. Other specific names given to it included Psittacosis, by Nocard in 1893, aertrycke by de Noble in 1898 and Pestis cavia by Wherry in 1908. It was frequently referred to as the "Breslau bacillus" in the German literature as quoted by Parker (1983). S. typhimurium has a very definite invasive power for tissues of mice, and other laboratory rodents. In fact it was given the name "typhimurium" because of the first isolates were from mice (Parker 1983)

2.2 THE GENUS SALMONELLA

Salmonellae are gram-negative non spore-forming rods belonging to the family Enterobacteriaceae. There are approximately 2000 or more serotypes or variants

that are potentially pathogenic for man and animals. Salmonellae have been classified into three primary species: Salmonella typhi, Salmonella cholerasuis and Salmonella enteritidis (Ewing 1972). S. typhi and S. cholerasuis have only one serotype each, while the remaining 2,000 or more serotypes belong to Salmonella enteritidis species (Ewing 1972, Buchanan et al. 1974). Most serotypes are motile with peritrichous flagellae, except S. gallinarium and S. pullorum, which have no flagellae and are non-motile.

Differentiation of salmonellae from other members of Enterobacteriaceae is on the basis of biochemical tests and fermentation reactions with specific sugars. Most salmonellae

ferment glucose and mannose with the production of acid and gas but do not ferment lactose, sucrose or salicin (Edwards and Ewing 1972). S. typhi does not produce gas. Salmonella species can also be identified by their antigenic analysis.

2.3. ANTIGENIC STRUCTURE

Salmonellae like other Enterobacteria have surface antigens (Figure 2). These are somatic or "O" antigens [1,4 (5) 12] which are the lipopolysaccharide component of the cell wall of both motile and non-motile forms. These antigens are resistant to prolonged heating at 100⁰C, to alcohol and to dilute acids. "O" antigens are prepared from non-motile forms or by treatment with heat and alcohol. Antibodies to "O" antigens are predominantly IgM. With sera containing anti "O" antibodies, such antigens agglutinate slowly in granular masses.

Flagellar or "H" antigens [i,1,2] are proteins and are inactivated by heating over 60⁰C, and also by acids and alcohol. They are best prepared for serologic testing by adding formalin to young motile broth cultures. With sera containing anti "H" antibodies, such antigens agglutinate rapidly in large fluffy clumps. These "H" antigens contain several immunological components. Within a single Salmonella

species, flagellar antigens may occur in either or both of 2 forms called phase I and phase II. The organisms tend to mutate from one phase to the other. This is called phase variation. Antibodies to "H" antigens are predominantly IgG.

The "Vi" antigen present at the extreme periphery of the body of S. typhi often interferes with agglutination of freshly isolated strains by antisera containing mainly anti-"O" agglutinins. It is destroyed by heating for one hour at 60°C and by acids and phenol. Certain serotypes e.g S. typhi that possess "Vi" antigens tend to be more virulent than those lacking it.

According to the Kauffman-White schema (Edwards and Ewing 1972, Buchanan et al. 1974) salmonellae are divided into groups on the basis of their somatic antigens (Table 1). Each group has a major determinant which is a strongly reacting somatic antigen and one or more minor somatic antigens. For identification of specific serotypes, flagellar antigens of phase 1 and 2

are defined in addition to the somatic antigens.

Identification of Salmonella variants with identical antigenic composition but different sugar fermentation reactions makes initial screening for Salmonella by lactose fermentation somewhat unreliable in endemic areas, e.g. a lactose fermenting S. typhimurium became endemic in Sao Paulo, Brazil in early 1970 and was easily confused with Escherichia coli (Falcao et al. 1975). In 1977 a case of endarteritis and septicaemia caused by a lactose fermenting S. typhimurium was initially identified as Enterobacter aerogenes and was reported in the United States (Porschen et al. 1977). Lactose fermenting strains of S. typhi were reported as early as 1959. This property was thought to be plasmid mediated, while some other strains of Salmonella were reported to be lactose fermenters and this property was transmissible in some and not in others (Falcow and Baron 1962, Hall et al. 1978). Anand et al. (1980) also reported an institutional outbreak by lactose fermenting S.

newport. Rarely there are other atypical Salmonella such as the anaerobic S. typhi reported by Huber et al. (1975).

2.4. EFFECT OF HEAT

Salmonella are readily killed by heat - in one hour at 55°C, and in 15 to 20 minutes at 60°C (Bowmer 1964). They are destroyed by 60°C pasteurization temperatures in milk (Taylor Joan 1962), and in liquid whole eggs (Murdock et al. 1960). Baking can be expected to kill all salmonellae even when the liquid egg used is highly contaminated.

2.5 EFFECT OF COLD

At low temperatures Salmonella are not killed, but they do not multiply. The critical temperature appears to be about 5°C, Below this temperature, salmonellae do not multiply, above it they begin to do so, at temperatures and speeds which vary with the nature of the infected food. Clearly therefore, temperature is of

very great importance in the storage of cooked or prepared foods.

2.6 EFFECT OF CHEMICALS

Salmonella can be destroyed by a wide range of chemicals including phenols, mercuric chloride, formaldehyde and quaternary ammonium compounds. Any of these can be used for disinfecting surfaces and utensils, (Bowmer 1964). Chlorine and potassium permanganate can be used for treating food. Thus lettuce can be freed from Salmonella in 30 seconds by washing in water containing 80 parts/million chlorine (Hobbs et al. 1962). Chicken carcasses can be rendered free of Salmonella by treating them for 10 minutes in water containing 200 parts per million of chlorine (Dixon and Pooley, 1961).

2.7 HABITAT

The normal habitat of Salmonella is the intestinal canal of the very large number of hosts including man

and animals. They have been isolated from the intestine of pythons (Rewell et al. 1948), camels (Cheyne et al. 1977), faeces of pet tortoises (Douglas et al. 1954., Thomas 1957, Ang"O" et al. 1973), dogs (Wolff et al.1948.,Mackel et al.1952),and cats (Mackel et al.1952). London pigeons have been shown to be carriers of salmonellae (Farrant 1964). Rats are often heavily infected especially if they have access to offals and animal by-products (Ludlam, 1954). Several types of Salmonella have been also isolated from mussels and other shellfish (McDonald et al. 1948) and from reptiles in zoos (Koopman et al. 1973), fresh water snails (Andrews et al. 1975, Bartlett and Trust 1976), mink (William and Bellhouse 1974), frogs (Ang'O' et al. 1973), and toads (Cruickshank and Williams 1978). Salmonella can survive in soil for greater part of a year (Mair and Ross 1960) and have been found in ward dust (Black et al. 1960) in the water pipes from wash basins, water closets (Black et al. 1960, Harvey and Philip 1961) and in sludge and effluent from sewage

works (Black et al. 1960, Kampelmacher et al. 1976).

2.8 BIOLOGICAL CHARACTERISTICS OF SALMONELLA

There are several biological characteristics that make Salmonella virulent for man and animals. Included among these properties are:

- invasiveness
- the ability to resist phagocytosis and the action of bactericidal antibody
- the production of enterotoxins
- and the presence of antibiotic resistance.

The "O" (somatic) antigen contained within the lipopolysaccharide (LPS) somatic capsule appears to be an important determinant of virulence for non-typhoidal Salmonella. Organisms that lack the O-specific side chains of the LPS are called "rough" mutants and are unable to cause disease in experimental animals (Makela et al. 1973; Fields et al. 1986). Strains that have some component of the LPS, but not all of it, have been shown to be moderately invasive, whereas "smooth"

strains, which have an intact LPS, are fully virulent (Nakano and Saito 1969). In addition to the O antigen in "smooth" organisms, the presence of LPS is important in the pathogenesis of salmonellosis, especially during blood stream invasion. The outer portion of the cell wall of Salmonella contains endotoxin, like that of other Enterobacteriaceae. Lipopolysaccharide of all Enterobacteriaceae and of Salmonella in particular, can produce fever, activate complement, activate clotting cascades, alter host defense and cause the syndrome of "septic shock" (Elin and Wolff 1976). In studies in experimental animals it has been shown that Salmonella may initiate the synthesis of prostaglandins which in turn stimulate adenylate cyclase-cyclic AMP (CAMP) synthesis with resultant fluid accumulation (Giannella et al. 1975). Whether prostaglandin synthesis is stimulated by enterotoxin or by local inflammation is unclear (Giannella et al. 1973). Skin permeability factors and a long-acting, heat-labile factor from culture filtrates of S. typhimurium have been described

(Sandefur and Peterson 1976). In addition, Kuopal and Deibel (1975) have detected a Salmonella "enterotoxin" using the suckling-mouse assay. Cell-free filtrates of pathogenic salmonellae were able to cause fluid accumulation in the rabbit ileal loop when the intestinal lumen was pre-washed with a mucolytic agent (Sedlock and Deibel 1978). The relationship among invasiveness, skin permeability factors and the presumptive enterotoxin in the pathogenesis of diarrhoeal disease needs further study (Takeuchi 1966, Takeuchi and Sprinz 1967, Giannella et al. 1976, Kinsey et al. 1976, Turnbull and Richmond 1978, Eisenstein 1983). It has been shown in animal studies that salmonellae can be differentiated into three groups.

1. those that are invasive and cause enteritis and the secretion of fluid.
2. those that have none of these properties and
3. those that invade and cause enteritis, but do not cause secretion of fluid.

Another fundamental biological characteristic of

Salmonella is their resistance to phagocytosis. These organisms are able to multiply within the macrophages of the reticuloendothelial system. The incidence of systemic salmonellosis in patients with sickle-cell anaemia and in females who are heterozygous for the chronic granulomatous diseases gene demonstrates the need for phagocytes to contain the infection (Hook 1961, Moellering and Weinberg 1970). Furthermore, the ability to develop delayed hypersensitivity to an extract of S. typhimurium has been shown to play an important role in the susceptibility of mice to this organism. This cell mediated immunity seems to be an important biological characteristic of Salmonella infections (Plant and Glynn 1974, Waiyaki 1974).

2.9 TRANSMISSION OF SALMONELLA IN MAN

Salmonellosis can be transmitted from an infected common source, such as contaminated food or water and from person to person. The former mechanism is thought to be the predominant means of transmission in

community acquired cases (Rice et al. 1976), while the latter mechanism is well recognized in an institutional set up (Schroeder et al. 1968, Baine et al. 1973; Steere et al. 1975). The marked similarity in the frequency of serotypes isolated from human and animal sources suggests that animal reservoirs play a crucial role in the transmission of the infection (Madwell and McChesney 1975; Morse and Duncan 1975). When known common source outbreaks are examined, the importance of animal reservoirs becomes more than obvious. The role of animals particularly food animals in the Salmonella cycle (animal feed-animals-food-man-animal feed) has been well recognized for a long time (Buxton 1957, Van Oye 1964, Rowe 1973). It is also well established that cattle, pigs and poultry are the major source of S.typhimurium infection. Most Salmonella infections in animals and birds are symptomless and may remain unsuspected until an outbreak of human infection follows the consumption of infected food (Bryan 1979, Roberts 1982a). Sources of infection in animals are

fertilizers and animal feeds contaminated with Salmonella from man or other animals as shown in several investigations (Walker 1957, Report 1959, Dixon 1960, Galbraith et al. 1962). This reservoir among domestic animals is of fundamental importance in the epidemiology of salmonellosis. Animal-to-animal transmission may then occur either directly or indirectly. Prevalence of infection among groups of animals may be low, but stress and crowding in animal feeding and holding pens before slaughter increase the spread of infection. Processing equipment and the environments of abattoirs and packing plants become contaminated from carcasses of infected animals and contaminate previously uninfected meats passing through the processing line. Thus contamination of poultry, meat and other food products probably accounts for the fact that food handlers are more likely to be asymptomatic carriers of Salmonella than are other members of the general population.

Besides transmission of salmonellae by

contaminated food, direct or indirect (secondary) transmission from man-to-man may also take place. Spread by direct contact is likely when individuals - patients or hospital personnel-become infected, (Steere et al. 1973) and they in turn, unknowingly expose to infection other patients or personnel as a consequence of poor personal hygiene or faulty patient-care technique (Pether and Scott 1982; Fekety 1983).

Salmonellae have been isolated from a variety of biting and non-biting arthropods including fleas (Jadin 1951), ticks (Reitler and Mentzel 1946, Jadin 1951), human lice (Liu et al. 1938), and animal lice (Messerlin and Courzi 1942, Milner et al. 1957), cockroaches (Oothuman et al. 1989), and flies (Hobbs and Gilbert 1978).

2.10 PATHOGENESIS

The term 'Salmonellosis' refers to infections caused by Salmonella species. This term however does not differentiate between asymptomatic infections and

symptomatic infections including acute enterocolitis, bacteraemia, localized infection, typhoid fever or paratyphoid fevers.

However the most common cause of non-typhoidal salmonellosis is S. enteritidis serotype S. typhimurium (Youmans et al. 1975). Salmonellosis caused by S. typhimurium, may result in enterocolitis in which case the symptoms may be brief, mild and self-limiting with diarrhoea and vomiting; or severe and invasive with resultant septicaemia especially in the very young, very old or immunocompromised patients (Saphra 1950, Womack 1957, Han et al. 1967, Wolfe et al. 1971, Grossman et al. 1972, Kasili 1979, Steven et al. 1987).

After the organisms have been swallowed in contaminated food or drink they must reach the intestine before they can cause disease. This means getting through the stomach acid barrier where the salmonellae can be destroyed. If the number swallowed is small, all may perish and none may get through to cause disease. If they are swallowed in large numbers

($10^8 - 10^9$) then enough may reach the intestines alive. This probably depends on the speed with which the stomach empties. If the salmonellae are swallowed with water, their passage through the stomach may be rapid, whereas if swallowed with food, they may be held up for long enough and get destroyed by the acidity of the stomach. Patients who take antacids, those with achlorhydria, and those who have undergone partial or total gastrectomy, are more liable than normal persons to suffer from Salmonella infection (Waddell and Kunz 1956, Giannella et al. 1970, Gray and Trieman 1971, Giannella et al. 1973). Having survived the passage through the stomach, the salmonellae must then establish themselves in the small intestine and the colon if they are not to get swept away by motility and peristaltic action of the intestines. This the salmonellae do by adsorbing themselves to the intestinal epithelial cells. They then penetrate the epithelial cells, migrate through them and are extruded into the lamina propria as has been demonstrated in

electron microscopic studies in animal experiments (Takeuchi 1966). Here they cause an inflammatory response, with polymorphonuclear leucocytes in most cases, but in the case of S.typhi this inflammatory response involves macrophages (Takeuchi and Sprinz 1967). Carried by these macrophages, S.typhi invades the body and settles down to multiply in the reticuloendothelial system. The other salmonellae (non-typhoidal) which cause gastroenteritis remain in the intestinal wall causing varying degrees of inflammatory reaction. Once established in the intestine, they cause clinical disease. The incubation period is normally short varying from 6-48 hours. Diarrhoea and vomiting may develop abruptly and be profuse and frequent, leading to dehydration and collapse; or the symptoms may be mild and self-limiting lasting 1 to 5 days. Abdominal pain and cramps are common. Stools may contain blood. Fever and toxicity are present at the onset of the illness, but may subside as diarrhoea and vomiting respond to treatment.

Persistence of fever and systemic disturbance suggests that blood stream invasion has occurred, and that the patient has developed Salmonella septicaemia. The disease in children may be more severe (Kazemi et al. 1974) than adults. Once the organism invades the blood stream, almost any organ can become involved. Meningitis, arteritis, endocarditis, osteomyelitis, wound infections, septic arthritis, and focal abscesses in virtually all anatomical sites have been recorded. (Saphra and Winters 1957, Black et al. 1960, Mirza and Nsanzumuhire 1979)

2.11 CARRIERS

Patients may become symptomless carriers as a result of symptomatic or asymptomatic infection (McCoy 1974). There are three categories of symptomless carriers;-

(a) Those who excrete salmonellae during a certain time after being exposed to infection, but never show any morbid symptoms. These carriers are of little

epidemiological significance because as a rule the excretion is sporadic and of rather short duration.

(b) Those who temporarily excrete salmonellae after recovering from clinical salmonellosis. This category is of more importance. It may contain children who can spread infection both within and outside a hospital.

(c) The third category consists of permanent Salmonella excreters. These are rare, but dangerous from an epidemiological point of view.

In the permanent Salmonella excreters there must be a reservoir in the body where the bacteria reproduce themselves continuously. In most cases it is the gall bladder, but in some cases there is an intestinal diverticulum or an anatomical anomaly of the urinary tract which may serve as the reservoir. Many chronic carriers of Salmonella species have diseased gall bladders with cholelithiasis, or renal lithiasis (Perkins et al. 1966). In addition, persons with Schistosoma haematobium infections of the urinary tract

or gut infections due to Schistosoma mansoni or Schistosoma japonicum may also pass salmonellae asymptotically or have prolonged Salmonella bacteraemia with intestinal carriage. Treatment of Salmonella infection may be facilitated by treatment of the schistosomiasis, for Salmonella infections have been known to clear spontaneously following schistosomal therapy (Neves et al. 1969, Rocha et al. 1971). Patients with hepatic infection due to Opisthorchis sinensis may also become chronic carriers of Salmonella (McFadzean and Ong 1966).

2.12 ANTIBIOTIC RESISTANCE

Resistance to antimicrobials has become a significant issue in the epidemiology of Salmonella infections. Of special significance is the fact that strains isolated in animals and humans have been resistant to several antimicrobial agents, that resistance is plasmid-mediated (Anderson 1968b; Threfall et al. 1980) and that Salmonella are capable

of transferring resistance for multiple antibiotics (Smith 1966; Bhatia et al. 1981). Resistance to streptomycin, tetracycline, sulphathiazole and ampicillin was reported to be most common (Schroeder 1967), and S.typhimurium was the serotype most frequently found resistant to one or more antibiotics. More than 80% of multiple resistant strains could transfer their resistance to E.coli (Schroeder 1967).

Neu et al. (1975) in studies from New York demonstrated an increase in the prevalence of salmonellae resistant to multiple antibiotics. Over 50% of isolates of S.typhimurium and S. newport were resistant to four drugs. Interestingly resistance in salmonellae isolated from animals tended to parallel the resistance of human isolates. Similar results have been reported from Great Britain, Canada and the Netherlands (Anderson 1968; Manten et al. 1971; Finland 1975; Gran and Di Mambro 1977).

There are no similar reports on the antibiotic sensitivity of Salmonella isolates from animal sources

from Kenya.

It has been suggested that the use of animal feeds supplemented with antibiotics has been responsible for the selection of salmonellae in animals that are resistant to these antibiotics (Finlayson and Barnum 1973, Gardener 1978). Supporting evidence came from prospective epidemiological studies on farms in which both the animals and humans had similar patterns of antibiotic resistance in their E. coli and salmonellae (Marsik et al. 1975). Although the strains were not identical, the presence of transferable multiple drug resistance suggested plasmid transmission within strains and cross-infection from animals to man or vice-versa (Marsik et al. 1975)..

In general, chloramphenicol resistance among non-typhoidal salmonellae has been infrequent and is usually associated with the therapeutic use of this drug in humans (Cherubin et al. 1977). There have been some interesting exceptions. One involved the emergence of plasmid-containing strains of

S.typhimurium in a burns unit. These strains were resistant to silver nitrate, mercuric chloride and multiple antibiotics including chloramphenicol (McHugh et al 1975). Another was the appearance of chloramphenicol resistant, non-typhoidal Salmonella following the outbreak of chloramphenicol resistant S.typhi in Mexico (Olarate and Galindo 1973, Gonzales-Corte et al. 1973, MMWR 1973, Bissett et al. 1974, Baine et al. 1977)

Takeuchi (1966) also during his studies in animal populations also noted a high prevalence of chloramphenicol resistance among salmonellae. It has been fortunate that the plasmid associated with the Mexican outbreak seems to have disappeared (Gangarosa et al.1972).

A study from Great Britain indicated the role that plasmids can play in the epidemiology of Salmonella infections. S.wien, an unusual isolate caused outbreaks in paediatric wards in England, Algeria, France, Austria and Southern Europe (McConnell et al. 1979).

Several plasmids had been found, some being transferrable and conferring resistance to ampicillin, chloramphenicol, tetracycline, kanamycin, streptomycin, spectinomycin, and sulphonamides. Plasmid-compatibility studies indicated a clonal origin for this plasmid and suggested that S.wien had spread throughout these areas by some as yet undetected sources. Furthermore, other studies in Great Britain indicated that S.typhimurium phage type 204, found frequently in both animals and human outbreaks, had acquired plasmid mediated resistance to chloramphenicol (Editorial 1979). In addition, some strains of S.typhimurium, phage type 193, had plasmids derived from the phage 204 strain of S.typhimurium and acquired resistance to trimethoprim that may have been carried on a "transposon" (Editorial 1982). Further molecular genetic studies are needed in the future to determine the molecular epidemiology of these and other isolates in both animals and humans, with a view to breaking the chain of transmission from one to the

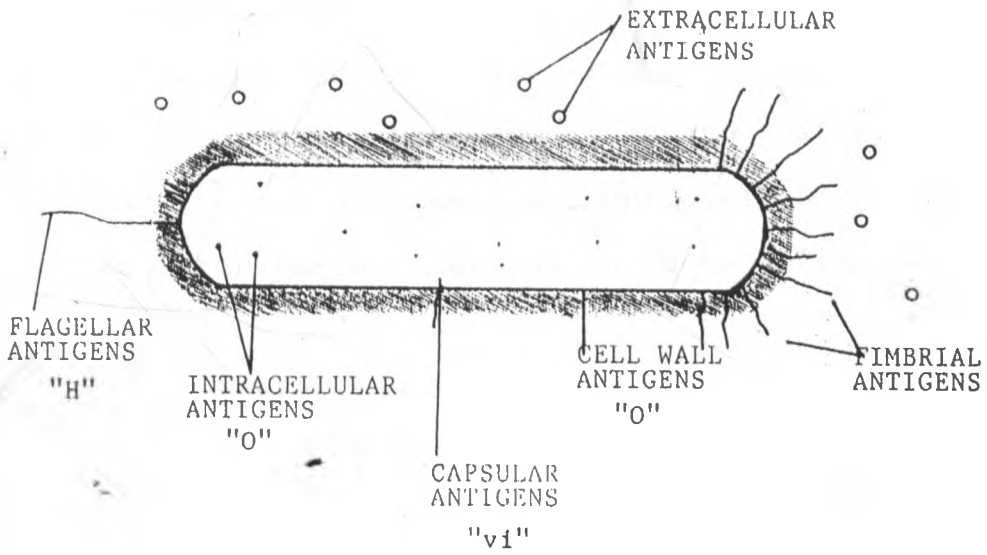
other, and so reducing the number of infections both in man and animals.

TABLE I. EXAMPLES OF ANTIGENIC SCHEMATA FOR SOME COMMON SALMONELLAE SEROTYPES

Species and serotypes	Group	O antigens	H Ag	
			Phase I	II
S.paratyphi A	A	1,2,12	a	-
S.paratyphi B	B	1,4,5,12	b	1,2
S.saint-paul	B	1,4,5,12	e,h	1,2
S.derby	B	1,4,5,12	f,g	-
S.typhimurium	B	1,4,5,12	i	1,2
S.heidelberg	B	1,4,5,12	r	1,2
S.choleraesuis	C ₁	6,7	c	1,5
S.oranienburg	C ₁	6,7	m,t	-
S.thompson	C ₁	6,7	k	1,5
S.typhi	D	9,12,vi	d -	S
S.enteritidis	D	1,9,12	g,m	-

Ag = antigens

FIG. 2 : BACTERIAL ANTIGENS



CHAPTER 3

3.1 HYPOTHESIS

S.typhimurium is a serious problem at K.N.H. and that it probably goes unnoticed and that after this study it will have been appropriately high-lighted so that intervention measures could be introduced.

3.2 JUSTIFICATION

A rise in the isolation of S.typhimurium from clinical specimens at KNH has been observed over the past few years. Lately this problem is becoming more acute with S.typhimurium being isolated from blood, CSf, stools, pus etc. The age group that appears to be affected is mainly infants with a high mortality rate. This has been reported in earlier studies by Wamola and Mirza (1981), Mirza and Wamola (1983).

The organism has also acquired very high resistance to most antibiotics, including chloramphenicol (Say and Wamola 1974, Slack and Badia 1974, Wamola and Mirza 1981, Nesbitt et al. 1988).

Outbreaks of infection due to drug resistant organisms are an increasing problem not only with Salmonella but with other gram negative organisms as well (Mirza et al. 1981). Delay in recognition of drug resistance causes unnecessary morbidity and loss of life.

The mode of nosocomial transmission is not clear. S.typhimurium has also recently been reported as a cause of gastroenteritis and bacteraemia in patients with acquired immunodeficiency syndrome (Glaser et al. 1985., Jacobs et al. 1985., Smith et al. 1985). Although these infections have appeared to be community acquired, frequent recurrences are common, sometimes while the patient is hospitalised. There is no previous data on nosocomial infections at KNH. Study of S.typhimurium nosocomial transmission will highlight this aspect and assist in preventing further infections.

Phage - typing of the isolates will make it possible to divide the species of S.typhimurium into phage-types - i.e. groups of strains having the same or

closely similar phage - susceptibilities and resistance. This may assist in identifying the source of infections. Stool cultures of the staff and all other regular visitors to the ward may reveal one or more carrier of the same phage - type as that of the patient.

Transfer of resistance is a relatively important mechanism by which resistance is spread in the hospital. Levy (1982) has found that some organisms with chromosome-mediated resistances remain a current therapeutic problem, but that most organisms currently of concern have their resistance mediated by plasmid transfer factors or transposons. Several resistance determinants can exist on a single plasmid. This finding explains the simultaneous development of resistance to unrelated antibiotics in a single bacterial strain (Casewell and Phillips 1981, Hart 1982). The impact of this is considered to be serious, since resistance is often to multiple antibiotics, and any agent may select for resistance, not only to

itself, but also to other agents, that are linked on the same resistance determinant (Murray and Moellering 1978, Schaberg et al. 1981, Levy 1982).

It is therefore important to know whether the multiple antibiotic resistance of S.typhimurium is plasmid mediated, and whether it is possible to focus efforts on interruption of transmission of resistance of this type (Plasmid mediated) by careful management of the microbial agents that currently are available for use in the hospitalized patients.

The determination of the antimicrobial susceptibility will be used in development of a drug policy on the appropriate use of antibiotics in the hospital.

3.3 AIMS AND OBJECTIVES

The major aim of this study is to determine the Profile of Salmonella typhimurium at KNH, by defining the following minor objectives:

1. An investigation of nosocomial and community

acquired S.typhimurium infections at KNH.

2. To define the major phage types of S.typhimurium isolated at KNH from various pathological conditions.
3. To study the antibiotic sensitivity pattern of S.typhimurium isolates from clinical material at KNH.
4. To study the mechanism of antibiotic resistance of S.typhimurium strains by plasmid transfer experiments.

3.4 MATERIALS AND METHODS

(i). Patients

The study will be carried out in the medical, paediatric, and surgical wards, and in the paediatric and adult observations wards at KNH. All patients with positive blood, stool, CSF, and/or urine cultures for S.typhimurium will be included in the study. Adults, and in case of children, his/her parent/guardian, will be interviewed as per questionnaire (Appendix I).

Details of age, sex, home location, sources of drinking water and milk will be obtained. Methods of sewage disposal, contact with domestic animals and birds, number of other individuals in the household sharing the room with the patient, and details of any recently ill person with diarrhoea and/or fever within the household will be obtained and included in the history. Type of diet eaten at home will be recorded and nutritional status of the patient will be assessed clinically (Wellcome classification 1970) in the hospital on admission.

(ii). Definition of nosocomial gastroenteritis

Nosocomial gastroenteritis will be the new onset of diarrhoea characterized by at least 3 or more loose stools per day, 72 hours or more after admission to hospital.

Any patient who develops fever after 72 hours or more after admission and has S.typhimurium isolated from blood, CSF, stool or rectal swab, and/or urine

will be regarded as having nosocomial infection, on the basis of criteria suggested by the US department of Health (1972). Such patients will be included in the study as cases and interviewed as above.

Cases will be identified through personal interviews with staff numbers of the wards by a questionnaire survey.

(iii). **Control group**

Controls will be patients comparable to cases for age and sex admitted to the wards of KNH during the study period, who give no history of gastro - intestinal disturbances or fever at the time of admission. The same questionnaire as for cases will be used to interview these controls.

(iv). **Follow-up**

All patients who will be identified as cases of Salmonella infection after one of the specimens (blood, stool/rectal swabs, urine, pus, CSF etc) has been

culture positive will be followed up in the relevant wards by the researcher. The questionnaire will be completed as far as possible on the first visit to the patient. The same questionnaire will be used both for controls and cases.

Any patient who is admitted in the control group will have stool/rectal swab culture done on admission. Any of the control group patient who has a positive stool/rectal swab culture for S.typhimurium will be admitted in the study group and followed up as a case. Blood for culture (5-10 mls of blood) will only be done on control group if the patient develops fever after admission to the ward.

(v). Environmental cultures in the wards

Once a week surface cultures of benches, cribs, mattresses, baby feed bottles, tap water supply, sinks and toilets etc. will be done. Staff members of wards with cases of nosocomial salmonella infection will be requested to provide stool specimen for culture.

Relatives who visit the patient will be requested to provide a stool specimen for culture. If found to be culture positive for S.typhimurium he/she will be treated and advised on personal hygiene e.g. washing of hands thoroughly after going to the toilet, or before handling food.

3.5 METHODS

Methods used in the laboratory for isolation and identification of organisms from clinical specimens of stools/rectal swabs, blood, cerebrospinal fluid (CSF) urine, pus, are the standard methods recommended in Cowan and Steel (1972), Stokes and Ridgway (1987).

Blood for culture is inoculated into a set of culture bottles, one containing triptycase soy broth and the other thioglycollate media. Liquoid (0.03 to 0.05% Sodium polyanethol sulphate) is used as an anticoagulant. After specimen collection the blood culture bottles are delivered to the laboratory where they are incubated overnight at 37⁰C, then subcultured

onto MacConkeys agar, blood agar (BA) and chocolated blood agar media (CBA). Incubation is for 48 to 72 hours. Blood culture bottles are continued to be incubated for a month, and subcultures are made at intervals to detect growth. If there is no growth after one month, the blood culture bottles are discarded and reported as no growth obtained.

Stools or rectal swabs are cultured on Deoxycholate Citrate agar (DCA), MacConkey agar (Mac) and inoculated into selenite F broth for enrichment. Urine is inoculated on CLED which is Cystine-Lactose-Electrolytes Deficient medium, while CSF and pus are cultured on Mac agar and CBA. All cultures are incubated aerobically except CBA which is incubated in a candle extinction jar to provide a carbon-dioxide (CO₂) rich atmosphere. Incubation temperature is maintained at 37⁰C. Identification of organisms is by the standard methods as described by Cowan and Steel (1972), Stokes and Ridgway (1987).

3.6 BIAS

There are limitations to this study.

Laboratory testing was limited due to limited availability of supplies. Therefore only one culture per patient was taken.

Rectal swabs instead of stool specimens were used in the nosocomial study. Previous studies (Buchwald and Blaser 1984) have shown that rectal swab do not afford maximum recovery of organisms and are of less value than stool specimens in carrier surveys and in the examination of convalescent patients, as the excretion of Salmonella may be intermittent (McCall et al. 1966).

Thus the true incidence of S.typhimurium infections is likely to be underestimated.

The incubation period of Salmonella gastroenteritis is short 6 - 48 hours (Harwitz 1977) and is dose dependent. In the nosocomial study the patients were acute cases and therefore it is hoped that they were not intermittent excreters. If there were intermittent

excreters or carriers, they would be very few.

Exclusion from the nosocomial study was history of recent treatment with antibiotics, as it is a well documented fact that between 4 to 50% of patients developed loose stools while on treatment with antibiotics. (Editorial 1975, George et al. 1978).

3.7 ETHICAL CONSIDERATIONS

There are no serious ethical issues involved in this study. Most of the investigations will be routine ones as carried out on any patient who is admitted to the wards for fever and diarrhoea (e.g. blood and stool for culture). However, before including the patient in the study, he/she will be fully informed regarding the purpose of the questionnaire and any repeat investigation that may be necessary for follow up. After the patient has understood and agreed to be included in the study, he/she will be requested to give a verbal or sign a consent form (Appendix II). The protocol will be submitted to Ethical Committee for

clearance before any work is started in the wards.

3.8 DATA ANALYSIS

The prevalence on admission and incidence of Salmonella infection will be determined using standard methods. The denominator for the prevalence will be the total number of cases screened. For the incidence of nosocomial infection, rates will be expressed both as the percent of admissions and the rate per hospital day. For the case control study, the dependent variable will be the presence of Salmonella infection. All other factors will be considered to be independent variables, and an odds ratio will be calculated to determine the risk associated with the factor. In addition, a logistic regression analysis will be undertaken to determine which factors are independently significant.

CHAPTER 4.

AN INVESTIGATION OF NOSOCOMIAL AND COMMUNITY
ACQUIRED SALMONELLA TYPHIMURIUM INFECTIONS AT KNH.

4.1 INTRODUCTION.

From the combination of Greek nosos (disease) with Komein (to take care of) as nosokomeion (hospital), and through Latin nosocomium (hospital) comes from English the word "nosocomial" (pertaining to a hospital). Nosocomial infections, then, are infections that develop and are recognized in patients and personnel in health-care institutions. These infections are not present or incubating on admission, and certain nosocomial infections may not be clinically evident until after discharge from the hospital. All other infections that fail to meet these criteria are commonly classified as "community acquired" infections.

Nosocomial transmission of Salmonella infections is well known (MacGregor and Reinhart 1977, Abbot 1980, Cruickshank 1984). Acute care hospitals, paediatric

wards, and nurseries for newborns account for about two thirds of reported institutional outbreaks; nursing homes, psychiatric hospitals, and institutions for the retarded account for the remainder (Baine et al.1973). About 85% of all outbreaks are associated with a contaminated common vehicle; usually food or drink, and about 10% are spread by cross-infection. The common vehicles of nosocomial outbreaks include a variety of foods, such as poultry, eggs, meats, dried coconut, (Galbraith et al. 1960, Semple et al. 1961), protein food supplements, or yeasts (Rowe et al. 1975). Outbreaks have also been related to pharmacologic or diagnostic preparations, especially those of animal origin. Pancreatic extract, liver extract, carmine dye, (Kunz and Ouchterlony 1955, Lang et al. 1967), bile salts, pepsin, gelatin, vitamins, thyroid extract, pituitary extract, platelets transfusion (Rhame et al. 1973), and adrenal cortical extract have all been implicated as sources of infection (Baine et al 1973). Cross-infection is especially common in medical

institutions, accounting for about 60% of the nosocomial outbreaks in which the mode of spread is known (Baine et al. 1973). Cross-infection with spread by person to person contact is responsible for virtually all outbreaks in nurseries for newborns and in paediatric wards, and is important in many outbreaks among hospitalized adults (Baine et al. 1973, Rice et al. 1976, Mhalu et al. 1984). Cross infection occurs when salmonellae are introduced into the hospital by admission of an acute case of salmonellosis or an asymptomatic carrier with another disease, or by the introduction of a common-source vehicle. Hospital personnel then carry infection on their hands or clothing from patient to patient. In some cases, fomites including dust, (Bate and James 1958), towels (Rubbo 1948), delivery room suction apparatus (Rubenstein and Fowler 1975, Ip et al. 1976), rectal thermometers (McAllister et al. 1986) and furniture may be implicated in transmission (Baine et al. 1973). Diagnostic equipment which may be difficult to

sterilize (e.g. fibre-optic endoscopes) have also been vehicles in transmission of Salmonella infections (Chmel and Armstrong 1976; Beecham et al. 1979). Hospital personnel who are excreting Salmonella in stools may serve occasionally to transmit infection to patients (Sanders et al. 1963, Musher and Rubenstein 1975, Steere et al. 1975).

The vulnerability of hospitalized patients to Salmonella infections is related in part to the presence of major underlying diseases (Wolfe et al. 1971, Kasili 1979, Steven et al. 1987) and to chemotherapeutic agents which alter resistance to infection, (Kaye et al. 1967, Grossman et al. 1972, Lorian and Topf 1972). Antibiotic treatment contributes to the risk of nosocomial infection by several means. In addition to modifying the endogenous microflora of the host, several antibiotics interfere with immune function (chloramphenicol and rifampicin), or contribute to renal (aminoglycosides), hepatic (erythromycin and isoniazid) or cutaneous

(pencillins) side effects that increase susceptibility to infection (Kaye et al. 1967, Grossman et al. 1972, Lorian and Topf 1972).

Under pressure of increased antibiotic use, the environmental microbial flora of medical-care institutions shows increasing antibiotic resistance (Grossman et al. 1972, Lorian and Topf 1972, Riley et al. 1984). Patients acquisition of this resistant microbial flora begins shortly after admission and is accelerated by antibiotic treatment or prophylaxis, reaching in some studies as much as 50 % of the patient population by the third week. (Seldon et al. 1971). This acquisition is paralleled by an increase in the appearance of these bacteria in the causation of nosocomial infection. Those drugs with side effects that decrease normal host defenses have the most profound role in increasing risk of nosocomial infection. Corticosteroids modify inflammatory response, interfere with leucocyte function, and depress cellular and humoral immune responses (Womack

1959, MIMS 1982). Antimetabolites and a variety of other cancer chemotherapy drugs have an effect on all rapidly proliferating tissues. Haemopoietic side effects decrease immune function, and gastrointestinal side effects interfere with secretory protection and cause loss of anatomical barriers, for example through ulceration. Such patients are at high risk of nosocomial infections (Schimpff 1979).

S.typhimurium is one of the most common Salmonella species isolated from all specimens at KNH. It has become an important cause of morbidity and mortality especially in neonates, infants, children and immunocompromised patients in the hospital. It has also developed very high levels of resistance to most of the common antibiotics used at KNH. including chloramphenicol (Slack and Badia 1974, Say and Wamola 1974, Wamola et al. 1981, Nesbitt et al. 1988, Mirza and Wamola 1989).

Keeping in mind the above points, it was decided to investigate nosocomial transmission of S.typhimurium

infections at KNH. This was done in separate studies as follows :

- (a) A study of nosocomial and community acquired gastroenteritis
- (b) A study of nosocomial S.typhimurium septicaemia.
- (c) A study of contamination of the hospital environment with S.typhimurium.
- (d) A study of clinical and epidemiological features of S.typhimurium infections.

4.2 a. A STUDY OF NOSOCOMIAL AND COMMUNITY ACQUIRED GASTROENTERITIS

Materials and Methods

For identification of nosocomial transmission the study was conducted over a six month period (April - October, 1989) in the pediatric observation wards (POW) and adult observation wards (AOW), and general medical and paediatric wards of KNH. Hospitalized patients who developed diarrhoea characterized by at least 3 or more loose stools in a day, 72 hours or more after admission

to the ward were identified through daily surveillance of the study wards by the hospital infection control nurse, and were enrolled if they consented to join the study. All human immunodeficiency virus (HIV) positive patients with or without diarrhoea in the wards, whose diagnosis had been confirmed by Western blot were enrolled in the study if consent was obtained from them. At the same time patients admitted to POW or AOW within the previous 24 hours, with or without diarrhoea, were enrolled if they consented to join the study. Any HIV confirmed positive patients who came for admission to KNH were also enrolled if they consented to be included in the study. All these patients were selected randomly to bring up the total number enrolled to 10 patients daily from Monday to Friday. To avoid an excessive work load in the laboratory this was considered to be the maximum number to enrol per day. Having obtained verbal consent from the patient or parent/guardian in case of a child, a questionnaire (Appendix I) was completed on each patient.

Rectal swabs were taken from each patient. The procedure was to moisten the swab in sterile water, then insert it in the anal canal of the patient and rotate it while the patient was asked to "bear down". The swab was then inoculated on Deoxycholate Citrate Agar (DCA), and selenite F broth for enrichment at the patients bedside, and delivered to the laboratory to be incubated at 37⁰C aerobically for 18 - 24 hours. After overnight incubation non-lactose-fermenting (NLF) colonies on DCA, if present, were further processed for identification of Salmonella species. Using a sterile straight wire the surface of an individual colony on DCA was touched and inoculated into urea medium. This was incubated at 37⁰C for about 4 hours and examined at hourly intervals for a pink colour development throughout the medium, indicating urease production. A positive urease test indicated that the organism was not a Salmonella and no further tests were required on the culture. If the urease test was negative, then using a sterile straight wire, a

Triple Sugar Iron (TSI) agar slope was inoculated and incubated at 37°C overnight.

After overnight incubation of TSI, salmonellae produce pink-red slope and yellow butt indicating the fermentation of glucose but not lactose. S.typhimurium and many other salmonellae (except S.typhi) produce blackening due to hydrogen sulphide production and cracks in the medium due to gas production from glucose fermentation. Any isolates that gave a reaction suggestive of Salmonella species were confirmed serologically by slide agglutination tests using known commercially prepared Wellcome antisera (Edwards and Ewing 1972).

TSI reactions are shown in colour plate I. Colour plate II shows NLF colonies on DCA with black centers indicating hydrogen sulphide production by S.typhimurium.

The reasons for taking rectal swabs instead of stool specimens were as follows:-

a. Either the patient was too ill to provide a stool

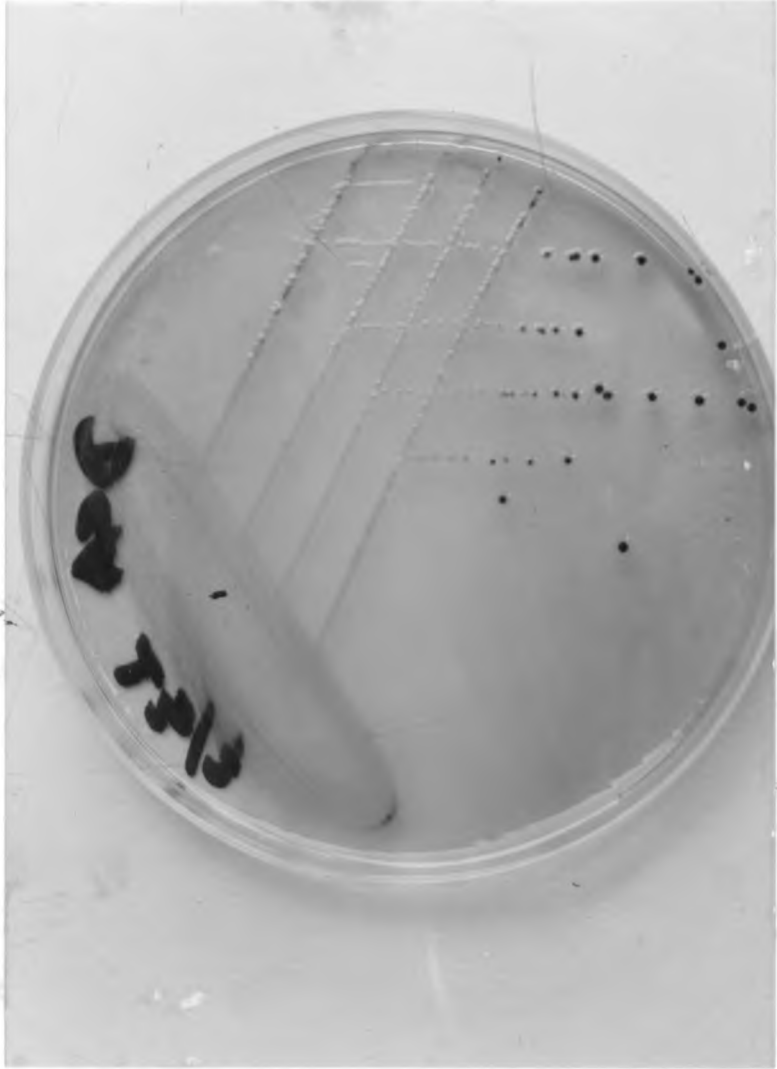
specimen, or he forgot to take his container to the toilet and therefore missed his collection of the specimen.

- b. Or sometimes the staff were too busy and overworked to remind each patient about stool specimen collection, and make sure the patient took the stool container when he/she went to the toilet.
- c. If a patient did manage to provide the stool, it could not be transported immediately to the laboratory. Sometimes the delay was more than a few hours, or even overnight.

Keeping the above disadvantages in mind, it was therefore decided to proceed with the exercise by taking rectal swabs. The swabs carried stool on their tips to be sufficient for culture and isolation of the pathogens.

PLATE I

TRIPLE SUGAR IRON AGAR (TSI) SLANTS. A IS UNINOCULATED.
B IS INOCULATED WITH S. TYPHIMURIUM. NOTE THE REACTIONS - AN
ALKALINE SLANT (PINK) INDICATING LACK OF LACTOSE FERMENTATION,
THE BLACK INDICATES H₂S PRODUCTION, AND THE YELLOW BUTT IS DUE
TO ACID PRODUCTION. 8

PLATE II

SURFACE OF DEOXYCHLOLATE CITRATE AGAR (DCA) WITH A 24 HOUR GROWTH OF S. TYPHIMURIUM SHOWING PALE, NON-LACTOSE FERMENTING COLONIES. THE BLACK PIGMENT IN SOME OF THE COLONIES INDICATES HYDROGEN SULPHIDE PRODUCTION.

RESULTS**(i) Nosocomial gastroenteritis**

One thousand and one hundred patients were enrolled in the study using the above procedure in a 6 month period (April - October 1989).

330 (30.0%) patients were identified as having developed nosocomial gastroenteritis. Pathogens were cultured from 39 out of 330 of the nosocomial gastroenteritis patients (11.8%). Twenty nine (8.7%) were Salmonella species. Of the 29 Salmonella species, 22 (75.8%) were S.typhimurium, 5 (17.2%) S.havana, and 2 (6.8%) were S.brandenburg.

(ii) Community acquired infections

Seven hundred and seventy patients were enrolled during the six month period of the study within less than 24 hours of admission to POW or AOW ; 540 of these were under 5 years of age. Rectal swabs from these patients were cultured as above. Pathogens were isolated from 44 (5.7%) of these patients. Twenty five

(3.2%) were Salmonella species. Of the twenty five Salmonella species 16 (64.0%) were S.typhimurium, 3 S.brandenrup, 1 S.derby, 2 S.enteritidis, 2 group C₁, and 1 group C₂ (Table 2). These were Salmonella infections acquired in the community. Eleven out of 25 patients (44.0%) presented with diarrhoea as the main complaint while 14 (56.0%) were admitted for other conditions and did not complain of diarrhoea at the time of admission. That is in the community at least 1.8% (14/770) were silent carriers of salmonellae. Nine out of 770 (1.2%) were carrying S.typhimurium, three yielded S.brandenrup and two S.group. C₁ in their rectal swabs. Table 2 shows comparison of Salmonella species isolated from nosocomial and community acquired infections.

Table. 2.

Comparison of Salmonella species isolated from Nosocomial and Community acquired infections (N = 1100)

<u>Salmonella</u> spp.	Nosocomial N=330	Community N=770
S.typhimurium	22 = 6.7%	16 = 2.1%
S.branderups	2 = 0.6%	3 = 0.39%
S.havana	5 = 1.5%	0 = 0 %
S. group C ₁	0 = 0 %	2 = 0.26%
S.group C ₂	0 = 0 %	1 = 0.12%
S.enteritidis	0 = 0 %	2 = 0.26%
S.derby	0 = 0 %	1 = 0.12%
Total	29 = 9%	25 = 3%

When analysed, (Table 3) this data gives an Odds Ratio of 3.37. Thus the risk of S.typhimurium infection among nosocomial gastroenteritis patients is significantly higher than in patients with community acquired infections.

Table 3.

Risk of S.typhimurium among nosocomial and community acquired infections.

	N = 330	N = 770	N = 1100
S.typhimurium	Nosocomial	Community	Total
+	22	16	38
-	308	754	1062
<u>Total</u>	<u>330</u>	<u>770</u>	<u>1100</u>

Odds Ratio = $\frac{22 \times 754}{16 \times 308} = 3.37$ (Risk of nosocomial infection relative to community acquired infection)

95% CI for OR = 1.74 - 6.50

$\chi^2_1 = 13.24, P < 0.001$)

This risk ratio is highly significant.

Table 4.

Sex distribution of Salmonella infections
(comparison of nosocomial and community acquired
infections)

	Nosocomial	Community	Total
Males	16	13	29
Females	13	12	25
Total	29	25	54

Odds Ratio = 1.13. (95% CI = 0.38 - 3.32)

($\chi^2_1 = 0.01$, NS) NS = Not significant

The sex distribution of the nosocomial and community acquired infections is shown in Table 4. It demonstrates that there was no significant difference between the two sexes.

Table 5 shows the age distribution. Maximum number of cases occurred during the first 5 years of life both in nosocomial and community acquired infections. Fifty five per cent (16/29) of nosocomial and 72.0% (18/25) of community acquired infections were before the age of 5 years. The prevalence of community acquired Salmonella infections among under fives was 18/540 (3.33%) and among those aged five and above it was 7/230 (3.04%), there being no evidence of an age related risk of community acquired infections. However the incidence of nosocomial Salmonella gastroenteritis was lower in those below 5 years, compared with those aged 5 and above 6.7% (16/239) and 14.3% (13/91) respectively. This difference was significant ($\chi^2_1 = 4.61, p < 0.05$), and it suggests that patients aged 5 years and above are at a greater risk of acquiring nosocomial Salmonella infections than those below 5 years.

Table 5.

**Age distribution of Salmonella positive cases
(Nosocomial and community acquired infections).**

<u>Age in years</u>	<u>Nosocomial</u>	<u>Community</u>	<u>Total</u>
0 - 1	9 (31.0%)	7 (28%)	16
2 - 3	6 (20.7%)	9 (36%)	15
4 - 5	1 (3.4%)	2 (8%)	3
6 - 10	3 (10.3%)	2 (8%)	5
11 - 20	2 (6.9%)	1 (4%)	3
21 - 30	4 (13.8%)	1 (4%)	5
31 - 40	4 (13.8%)	3 (12%)	7
<u>Total</u>	<u>29</u>	<u>25</u>	<u>54</u>

(iii) **NOSOCOMIAL SALMONELLA INFECTION IN HIV
POSITIVE CASES.**

During the study period 112 HIV positive patients were enrolled. Seventy nine of these had rectal swabs taken on admission or within less than 24 hours of admission, while 33 of the HIV positive patients had

been longer than 72 hours in the hospital with acquired immunodeficiency syndrome (AIDS). Salmonella were not isolated from any of the 79 HIV positive patients with or without diarrhoea on admission, while 9 (27.2%) of 33 patients with AIDS who developed nosocomial diarrhoea had Salmonella species isolated from their rectal swabs. Seven (21.2%) had S.typhimurium while S.havana and S.group C1 were isolated from one patient each. The sex and age distribution of 112 HIV positive cases is shown in Table 6. The majority of HIV positive patients were in the age group 20-40 years - the sexually active age group. The male to female ratio was not significantly different. Table 7 shows the 9 HIV positive positive patients who acquired nosocomial salmonella infections. There was only one child under one year of age. This was a case with S.havana; a resident Salmonella in the nursery at the time. The others were adults.

Table 6:

Age and sex distribution of HIV positive cases
(N=112).

<u>Age in years</u>	<u>Males</u>	<u>Females</u>	<u>Total</u>
0 - 1	2	0	2
2 - 3	1	2	3
4 - 5	2	0	2
6 - 10	0	0	0
11 - 20	1	3	4
21 - 30	25	20	45
31 - 40	17	16	33
> 40	12	11	23
<u>Total</u>	<u>60</u>	<u>52</u>	<u>112</u>

Table 7.

**Age distribution of HIV positive nosocomial
Salmonella gastroenteritis cases.**

<u>Age in Yrs</u>	<u>HIV positive (N=9)</u>	<u>Spp.of Salm.</u>
0 - 1	1 (11%)	S.havana
2 - 3	0 (0%)	
4 - 5	0 (0%)	
6 - 10	1 (11%)	S.typhimurium
11 - 20	1 (11%)	S.group C ₁
21 - 30	3 (33%)	S.typhimurium
31 - 40	2 (22%)	S.typhimurium
> 40	1 (11%)	S.typhimurium
<u>Total</u>	<u>9</u>	

4.2b A STUDY OF NOSOCOMIAL SALMONELLA SEPTICAEMIA

(i) **Materials and Methods**

In the Laboratory reports for blood cultures were scrutinized daily. In a six month period (July-Dec.1989), 179 positive cultures for S.typhimurium were identified. Patients with positive blood culture reports were followed in the wards, identified, consent obtained to join the study and were interviewed, using the same questionnaire (Appendix I). Patients who had been in the hospital longer than 72 hours for complaints other than fever on admission were identified as nosocomial septicaemia, while those who were admitted with febrile illness and had their blood culture positive for S.typhimurium were infections taken as community acquired.

ii) **Results**

Table 8 identifies the Salmonella species isolated from cases of septicaemia.

One hundred and fifty eight (158/179 = 88%) patients were symptomatic prior to admission, while 21 (21/179 = 12%) acquired their infection in the hospital. Eighteen (18/21 = 86%) of the nosocomial patients were infected with S.typhimurium and 3 (14%) with S.typhi, while 89% (140/158) of those with community acquired infections had S.typhimurium isolated. Those who were infected with Salmonella while in hospital for other complaints were undergoing treatment for conditions shown in Table 9. All these patients were in hospital for conditions which necessitated their stay over a long period. The mean duration of admission for nosocomial cases prior to onset of symptoms was 11.9 days (Range = 2 - 65 days. SD = 12.5 days).

Among these cases of septicaemic salmonellosis there was no statistically significant difference in the proportion of infections due to S.typhimurium between nosocomial or community-acquired infections, (86% and 89% respectively).

Table 10 compares the age between patients who were symptomatic prior to admission with S.typhimurium septicaemia (community acquired infections) and those who acquired it while in the hospital (nosocomial infections). In the nosocomial group 72% and in the community 96% were children under the age of 5 years.

Table 8

Comparison of Salmonella species isolated from cases of septicaemia (Hospital and community acquired).

<u>Salm.Spp</u>	<u>Nosocom. (N=21)</u>	<u>Comm. (N=158)</u>
S.typhimurium	18 (86%)	140 (89%)
S.enteritidis	0	1
S.aberdeen	0	6
<u>S.typhi</u>	<u>3</u>	<u>11</u>
<u>Total</u>	<u>21</u>	<u>158</u>

TABLE 9

**Presentation of underlying conditions of
nosocomial Salmonella septicaemia.**

<u>Underlying conditions</u>	<u>No. of cases</u>
Measles with bronchopneumonia	7
Marasmic kwashiorkor	3
Marasmus	2
Nephrotic syndrome	1
Ventral septal defect and mitral stenosis	1
Pulmonary tuberculosis	2
Severe burns and measles	1
Bronchopneumonia	1
<u>Sickle cell anaemia with osteomyelitis</u>	<u>3</u>
<u>Total</u>	<u>21</u>

Table 10.

Age distribution of patients with S.typhimurium septicaemia (Nosocomial and community acquired).

Age in Yrs	Nosocom	Community
0 - 1	8 (44.4%)	97 (69.2%)
2 - 3	4 (22.2%)	23 (16.4%)
4 - 5	1 (5.5%)	15 (10.7%)
6 - 10	2 (11.1%)	3 (2.1%)
11 - 20	1 (5.5%)	2 (1.4%)
21 - 30	0 (0%)	0 (0%)
31 - 40	0 (0%)	0 (0%)
> 40	2 (11.1%)	0 (0%)
<hr/> Total	<hr/> 18	<hr/> 140

The male : female ratio was 1 : 1 for both the groups.

4.2c **A STUDY OF CONTAMINATION OF THE HOSPITAL
ENVIRONMENT WITH SALMONELLAE.**

Materials and Methods

(i) Stools from staff

This exercise was carried out in wards where nosocomial Salmonella infection occurred. Nursing staff were requested to provide a specimen of stool. Thirty two nurses complied.

(ii) Swabs from hands of staff

Ten nurses agreed to have their hands swabbed before and after their round of babies in the Nursery. A total of 40 swabs were cultured.

(iii) Swabs from the environment

A total of 110 swabs were cultured on different occasions. These were taken in the Nursery and POW from floors, wash basins, mops, kitchen sinks, baths and

toilets. Also swabs were taken from sterilizing lotions, pantry sinks, soap bars, milk, cots, mattresses cultured.

Results and observations

All cultures above in (i), (ii), and (iii) were negative, except in the Nursery. One milk packet when tested grew S.havana. On a repeat exercise the milk did not show any contamination. A bucket containing disinfectant, used for keeping milk bottles also grew S.havana. Staff were using hands to remove the milk bottles from the disinfectant solution. This practice was discontinued, and long forceps (tongs) were provided to remove the bottles. The infected solution was immediately discarded and replaced by fresh disinfectant. On repeat cultures of the fresh solution no Salmonella was isolated. It was observed that in the Nursery the same rectal thermometer was being used for taking temperature from all the babies. In-between each baby it was only being cleaned with dry

gauze. Samples of the disinfecting solution (Hibitane) when cultured were negative for salmonellae. This practice was changed as soon as it was realized that it was totally wrong, immediately enough thermometers were provided, one per baby with individual disinfecting container for each thermometer. Since then, at least from the Nursery, the Salmonella has disappeared. Use of shared hand towels was also discouraged, disposable paper towels were provided for the Nursery staff.

4.2d A STUDY OF CLINICAL AND EPIDEMIOLOGICAL FEATURES
OF SALMONELLA TYPHIMURIUM INFECTIONS.

This part of the study was undertaken to identify the pattern of clinical presentation, the group of vulnerable patients and the possible sources of infection with a view to early clinical and environmental intervention

Materials and methods.

Study sample size

150 patients made up of the following :

- (i) 50 S.typhimurium culture positive cases.
- (ii) 100 controls i.e. there were two controls for each S.typhimurium culture positive case.

(i) Case Group

Total number of patients followed were fifty. For each case two controls were enrolled. Study period

was 4 months (September - December 1989). Laboratory reports were scrutinized every morning. All those reports that were culture positive for S.typhimurium were included in the study and followed to the ward. A questionnaire (Appendix I) was completed after obtaining verbal permission from the patient or parent/guardian in the case of a child. For each positive case the patient's age, sex, and history was obtained along with environmental data, water supply, milk supply, type of diet, disposal of sewage, and contact with animals. Clinical presentation and nutritional status were also recorded.

Patients were reviewed twice weekly. Their clinical care remained the responsibility of the admitting doctor.

(ii) **Control Group**

The environmental data of 100 controls, matched for age and sex, admitted consecutively to the wards including paediatric and adult emergency wards at KNH

were compared with the study patients.

Stools from controls were cultured for Salmonella. Stools for culture were also requested from the parent, relative or guardian who stayed with or visited the patient. Polypots were also given to the patient's parent/guardian to request stools for culture from other members of the family who were at home.

RESULTS**(a) Bacteriological findings**

Fifty cases who had positive cultures for S. typhimurium, from laboratory results (Table 11) were followed up in the wards. Majority (54%) were blood culture isolates, while 34% were from stools only. Three (6%) were positive from both stool and blood culture of the same patients. Two (4%) were from pus aspirates, one from a knee joint and one from shoulder joint of children : both were sicklers. Only one patient had S. typhimurium isolated from the urine.

Control group consisted of one hundred patients. No pathogens were isolated from stools of any of these controls.

Table 11.

Isolation of S.typhimurium from various clinical specimens.

Fifty patients whose laboratory results were positive for S.typhimurium were as follows:

Blood culture positive only	=	27 (54%)
Stool culture positive only	=	17 (34%)
Stool + Blood	=	3 (6%)
Aspirate (pus)	=	2 (4%)
<u>Urine only</u>	=	<u>1 (2%)</u>
<u>Total</u>	=	<u>50</u>

Age and sex distribution of patients is shown in Table 12. Most (72%) of the patients infected with S.typhimurium were under five years of age. There was no difference in the sex distribution, the male : female ratio was 1 : 1.

TABLE 12.

Age and Sex distribution of Clinical cases with S.typhimurium infection

Age in Yrs	Males	Females	Total	Percent
0 - 1	10	12	22	44
2 - 3	4	5	9	18
4 - 5	3	2	5	10
6 - 10	4	2	6	12
11 - 20	1	1	2	4
21 - 30	0	1	1	2
31 - 40	1	1	2	4
> 40	2	1	3	6
Total	25	25	50	100

(b) **Community aquired infections.**

Seventy six percent (38/50) of patients with S.typhimurium infection were symptomatic prior to admission. Forty eight per cent (24/50) of the patients came directly from the Western Province, 30% (15/50) from Central Province, 10% (5/50) from Eastern Province, and 12% (6/50) were from in and around Nairobi areas (Table 13).

Table 13

Area of origin of patients and controls			
	Cases(N=50)	Controls(N=100)	Total
Western province	24	35	59
Central province	15	25	40
Eastern province	5	10	15
<u>Nairobi</u>	<u>6</u>	<u>30</u>	<u>36</u>
<u>Total</u>	<u>50</u>	<u>100</u>	<u>50</u>

On statistical testing against control group home locations, a home location of Western province was

statistically significant ($P < 0.001$) in the acquisition of community acquired Salmonella septicaemia.

(c) **Risk factors of patients with S.typhimurium infections.**

Table 14 shows the statistical analysis of community acquired S.typhimurium (38 patients) versus controls, using odds ratios and the chi square statistic, for the various risk factors.

Seventy six percent (29/38) of patients who acquired their infections in the community were drinking untreated water from a river, lake or well. Thus piped water was available to only 24% (9/38) of infected patients compared to 80% (80/100) of controls. Pit latrines were used by 92% (35/38) of patients and their families while only 45 percent (45/100) of the controls used pit latrines. Untreated cow's milk was taken by 71% (27/38) cases while only 18% (18/100) controls drank untreated milk.

Domestic animals in order of frequency were

chickens, cows, goats, dogs, pigs and cats. These animals were kept by 81% (31/38) of the patients families while in the control group only 46 percent (46/100) of the patients and their families had contact with animals.

Diets of maize porridge, vegetable stews, banana, pawpaw were similar in both patients and control groups. Isolation of S.typhimurium for Nairobi patients was significantly less frequent than among patients from other parts of Kenya. In community acquired S.typhimurium infections versus controls (Table 14), the following risk factors showed significant odds ratios : residence outside Nairobi (7.71); untreated water (12.89); pit latrines (14.26); domestic animals (5.20); chickens only (2.78); cows plus chickens (2.03); and untreated cows milk (11.28).

Table 14

**Risk factor analysis of community acquired
S.typhimurium versus controls**

Risk factors	Comm	Cont	OR	95% CI	X21	P.value
Outside Nairobi	36/38	70/100	7.71	1.74-34.12	8.12	<0.005
Untreated water	29/38	20/100	12.89	5.27-31.52	38.72	<0.001
Pit lat. use	35/38	45/100	14.26	4.11-49.43	23.18	<0.001
Domestic animals	31/38	46/100	5.20	2.09-12.91	12.73	<0.001
Chickens only	15/38	19/100	2.78	1.22-6.31	5.16	<0.02
Chickens + cows	7/38	10/100	2.03	0.71-5.80	1.11	N.S
Untreated cows milk	27/38	18/100	11.18	4.70-26.61	32.90	<0.001

Key

Comm = Community cases Cont = controls NS = Not significant
Lat = Latrines.

(d) HOSPITAL ACQUIRED INFECTIONS

Twelve patients (12/50 = 24%) acquired their S.typhimurium infection while undergoing treatment for other conditions. Three patients were in hospital for treatment of measles and malnutrition. Three were being treated for malignant diseases with chemotherapy. Two acquired their infections while on treatment for joint swellings and septic arthritis, both of these were sicklers. Two were in hospital for cardiac problems. Two of the other patients were being treated one each for spinal injuries and acute renal failure. All these patients had been in the hospital for longer than three days and therefore they were considered to have acquired their infections nosocomially (Table 15).

Table 15

**Underlying conditions in patients with hospital
acquired infections**

<u>Underlying conditions</u>	<u>No</u>
Measles + malnutrition	3
Malignant diseases	3
Septic arthritis + sickle cell disease	2
Rheumatic heart disease + congestive cardiac failure	2
Spinal injury + paraplegia	1
Renal failure	1
<u>Total</u>	<u>12</u>

(e) **Clinical presentation of cases.**

Clinical presentation of 50 patients is shown in Table 16. In majority of the patients the presenting complaint was fever (84%), diarrhoea (56%), malnutrition (40%), and cough (36%). Bone and joint infections (4%), jaundice (4%) and CNS involvement (2%) was seen only in few cases. There were 2 cases with sickle cell disease; these were diagnosed on haemoglobin electrophoresis. Severe protein energy malnutrition (PEM) was seen in 20 (40%) of patients. Some of these were children who were recovering from measles and bronchopneumonia.

Table 16

**Clinical presentation among 50 cases of
S. typhimurium infections**

<u>Clinical presentation</u>	<u>No. of cases</u>	<u>Per cent</u>
1. Fever	42	84
2. Diarrhoea	28	56
3. Malnutrition	20	40
4. Cough	18	36
5. Bone & joint sepsis	2	4
6. Jaundice	2	4
7. CNS involvement	1	2

(f) **Family contacts of cases.**

In the family contact study, patients relatives were requested to bring in stool specimens from other members in the household and from themselves. They were given stool containers to collect the specimens. Only five families complied. A total of 13 stools specimens were received. When cultured all were negative for Salmonella.

(g) **Outcome**

There were 6 patients with S.typhimurium septicaemia (12%) who died. All were children under one year of age. Known complications that occurred were: three suffered from severe malnutrition and marasmus; one developed S.typhimurium meningitis, and two died of bronchopneumonia and measles.

Using the same methods of analysis as for Table 14 above, various risk factors about all cases have been compared with controls (Table 17).

Risk factor analysis of all S.typhimurium cases versus control (Table 17), the following risk factors showed significant odds ratios : residence outside Nairobi (3.14); untreated water (12.67); pit latrines (19.15); domestic animals (5.35); chickens only (2.84); chickens plus cows (3.16); untreated cows milk (10.63).

Table 17

Risk factor analysis of all S.typhimurium cases versus controls.

	Cases	Cont.	OR	95%CI	X ² ₁	P.value
Outside Nairobi	44/50	70/100	3.14	1.21-8.16	4.98	<0.05
Untreated water	38/50	20/100	12.67	5.62-28.57	41.75	<0.001
Pit latr	47/50	45/100	19.15	5.59-65.63	31.71	<0.001
Domestic animals	41/50	46/100	5.35	2.35-12.16	16.2	<0.001
Chickens only	20/50	19/100	2.84	1.34-6.05	6.59	<0.005
Chickens+ cows	13/50	10/100	3.16	1.27-9.8	55.40	<0.01
Untreated cows milk	5/50	18/100	10.63	4.82-23.45	37.20	<0.001

Key

OR = Odds ratio CI. = Confidence interval
 Latr = Latrines.

4.3 DISCUSSION

From the above results it is clear that children under five years of age were particularly affected with S.typhimurium infections (72% of the total). Similar findings have been reported in earlier studies from KNH (Wamola & Mirza 1981, Mirza and Wamola 1989). Mortality is higher in this age group than others. The clinical presentations of fever, diarrhoea, septic arthritis, jaundice and malnutrition described here in the Kenyan child have also been reported in the Rwandese children (Lepage et al. 1984 and 1987). From Ibadan, Nigeria S.typhimurium was reported to account for over 70 per cent of all Salmonella isolates in blood (Asmiru and Osoba 1986). The poorly nourished, weaned child is highly vulnerable to all the gram negative infections including infections with S.typhimurium. In this study PEM due to marasmus and marasmic kwashiorkor was seen in 40% of children, a similar finding in an earlier study from Kenya (Nesbitt and Mirza 1989) which dealt with Salmonella septicaemia

in the Kenyan child. The association of Salmonella infection with sickle cell disease is well recognized (Huckstep 1962; Mirza and Nsanzumuhire 1979; Zarkowski et al. 1986). In this study there were 2 (4%) patients who were sicklers. These were from Western Province where a 28 per cent prevalence of sickle cell trait has been demonstrated, one of the highest in Kenya (Foy and Kendall 1974, Shiroya 1987). In sickle cell disease, frequent capillary thrombosis is associated with infection and autosplenectomy. This reduces resistance and predisposes the patient to invasion by Salmonella. Once Salmonella gain access in the blood stream, they localize in areas of ischaemic necrosis which result from the sickling process in capillaries. Ischaemia of bone marrow may also lower local resistance and permit the growth of Salmonella with resultant Salmonella osteomyelitis.

Risk factors included residence outside Nairobi, untreated water, pit latrines, association in the home environment with domestic animals especially chickens

and cows, and drinking untreated cows milk (Table 17).

There were limitations to contact tracing as only 5 families complied to bring in stool specimens, and they did so once only. As carriers are intermittent excretors, multiple cultures were required to identify infection. This was not possible due to lack of compliance, as 150 stool polypots were given out, only 13 returned with the specimen. To follow up contacts of Salmonella infection more resources and staff are needed which were limited in this study.

Although no Salmonella were isolated from the environment at the time of investigation, the environmental sampling should not be ignored as faecal soiling with Salmonella of a ward is not unusual especially in maternity, paediatric, psychiatric and geriatric wards (Abbot et al. 1980).

All swabs from the hands of Nursery staff were also negative although hands are known to be the most important vehicle in spread of person to person infection in hospitals, (Ojajarvi 1978).

4.4 CONCLUSIONS

From the above data, it is evident that both community acquired or hospital acquired S.typhimurium infections are quite common at KNH. There is need for intervention both at community level and at hospital level.

4.5 RECOMMENDATIONS

A. In the community

1. Provision of piped water for domestic use for all the people of Kenya.
2. Education in the rural areas on the hazards of drinking unboiled milk and water.
3. Improvements in environmental hygiene and education on proper disposal of rubbish and excreta, by burning or burying etc.
4. Education on personal hygiene, with emphasis on washing of hands before eating or preparing food, and after having been to toilet.
5. A separate enclosure for domestic animals - away

from the main house-hold.

B. In the Hospital

1. Early recognition of "at risk" patients, provision of isolation facilities, adequate and appropriate drug supplies are essential.
2. Education of staff both medical and paramedical, on the role of hands as vehicles of infection and therefore the need for hand - washing on certain occasions (CDC 1975); e.g.
 - a. before and after aseptic procedures such as catheterisation, intravenous infusions, and dressing changes;
 - b. before and after surgical procedures;
 - c. before entering and leaving isolation rooms;
 - d. before and after contact with excretions such as faeces and urine or secretions from wounds or infections;
 - e. after using toilet; handling bedpans or urine bottles;

- f. before giving injections to patients, delivering food, and any other patient care activity which involves close patient - to - nurse contact.
- 3. Public health measures for hospital cleanliness and general hygiene.

CHAPTER 5

THE DEFINITION OF THE MAJOR PHAGE TYPES OF SALMONELLA
TYPHIMURIUM STRAINS ISOLATED AT KNH FROM VARIOUS
PATHOLOGICAL CONDITIONS.

5.1 INTRODUCTION

The emergence of salmonellosis as a world wide problem, the complexity of the problem and the certainty that there is a strain diversity within each serotype necessitated the development of special methods of strain identification for epidemiological purposes. Among such methods that of bacteriophage typing emerged as the one giving at the same time the most reliable results and the maximum amount of strain differentiation (Felix 1956, Callow 1959).

Bacteriophages are viruses that parasitize bacteria. These viruses incorporate themselves within the bacterial cell, multiply and eventually destroy the cell. Fig.3 is a diagrammatic illustration of a Bacteriophage.

Bacteriophage typing may be used in a local context to identify the types responsible for given outbreaks of salmonellosis and to help to trace their sources. It may be employed regionally to define the type distribution in larger areas of a country, or nationally in an attempt to discover the possible channels of transfer of certain local types to other parts of the country. On an international scale such phage typing is helpful in several ways:

Because of the speed of modern travel, persons may arrive in a country while incubating a disease such as typhoid fever. The phage types with which they are infected are sometimes characteristic of the country of their origin, and any secondary cases they may cause may be rapidly identified by means of phage typing.

Bacteriophage typing being primarily an epidemiological tool is rarely performed in a routine microbiology laboratory for diagnostic purposes. The procedure is time consuming, labour intensive, and highly specialized, therefore feasible only in a

research laboratory.

During the study period S.typhimurium strains isolated from clinical material were inoculated in sterile milk and stored at -70°C . Out of this collection only 34 strains were sent to Public Health Laboratory Service (PHLS), division of Enteric Pathogens, Colindale avenue London as described in Materials and Methods below.

5.2 MATERIALS AND METHODS

One hundred and nine S.typhimurium strains were collected during the study period from various clinical specimens received in the Medical microbiology laboratory at KNH. The S.typhimurium strains were identified serologically, antibiotic sensitivity tested by disk diffusion method and documented. After identification they were heavily inoculated in 1 ml. cryovials containing sterile skimmed milk and stored at -70°c (Appendix III Formula of milk).

As phage typing facility is not available in Nairobi, 34 S.typhimurium strains were selected from the above collection with two of each type showing almost identical antibiotic sensitivity pattern and sent to PHLS, London, for phage typing.

5.3 RESULTS

The results received are shown in Table 18 together with the sensitivity pattern. Phage types 56, 193, and 208 appeared to be most common. Two of the

strains were phage type 135, one phage type 132, and one each phage type 2 and phage type 3 aerogenic. Five strains were untypable. One strain was reported to react with the typing phages but did not conform to any recognizable pattern, and it was abbreviated RDNC on the report.

TABLE 18.

ANALYSIS OF PHAGE TYPES (PT) DISTRIBUTION AND
THEIR PATTERN OF RESISTANCE.

PT.	No. of strains in each PT.	Antibiotics and number resistant								
		Am	Tet	Cot	Kan	Gen	Str	Sul	Chl	Ceft
56	10 (29.4%)	10	6	10	6	3	10	10	7	0
193	7 (20.6%)	7	2	7	7	6	7	7	5	0
208	6 (17.6%)	6	6	6	3	0	6	6	3	0
135	2 (5.9%)	0	0	0	0	0	2	2	0	0
132	1 (2.9%)	0	0	1	0	0	1	1	1	0
2	1 (2.9%)	1	1	0	0	0	1	1	0	0
3 aer.	1 (2.9%)	0	0	0	0	0	0	1	0	0
Untyp.	5 (14.7%)	5	3	5	0	2	5	5	3	0
RDNC	1 (2.9%)	1	0	1	1	0	1	1	0	0
Total	34									
Total No. resist.		30	18	30	17	11	33	34	19	0
Percent resist.		88	53	88	50	32	97	100	55	0

Key

PT = Phage Type 3 aer. = 3 aerogenic. Untyp. =
Untypable

RDNC = This culture reacts with the Typing phages,
but does not conform to arecognized pattern.

Amp = Ampicillin

Gen = Gentamicin

Tet = Tetracycline

Str = Streptomycin

Cot = Co-trimoxazole

Sul = Sulphafurazole

Kan = Kanamycin

Chl = Chloramphenicole

Ceft = Cefotaxime

5.4 DISCUSSION

These results show that the S.typhimurium isolated from the clinical material at KNH belonged to many different phage types and carried multiple drug resistance. Most common types appeared to be phage type 56 (29.4%), 193 (20.5%) and 208 (17.6%). It is interesting to note that phage type 193 (20.5%) was reported from Kigali, Rwanda (Bogaerts et al. 1985) and the authors speculated that this multiresistant strain of S.typhimurium was probably imported from neighbouring Kenya. The same multiresistant phage type was earlier reported from South Africa in a hospital outbreak of S.typhimurium (Robins - Browne et al. 1983).

Phage type 208 has also been reported from other countries Anderson et al. (1977), Table 19.

Table 19.

Phage type 208 and countries of origin

<u>Phage type</u>	<u>Country of Origin</u>	<u>Year of Isolation</u>
208	Jordan	1974
208	Kuwait	1971
208	Syria	1974
208	Turkey	1973
208	Iran	1972
208	England	1974

This geographically widespread phage type 208 S.typhimurium probably has a clonal origin which by some means as yet unknown has become distributed throughout the Middle East, Britain and now Kenya.

Phage-type 56 was the most common type in S.typhimurium strains isolated from KNH. Search through the available literature shows no evidence of any reported cases of this phage type from anywhere.

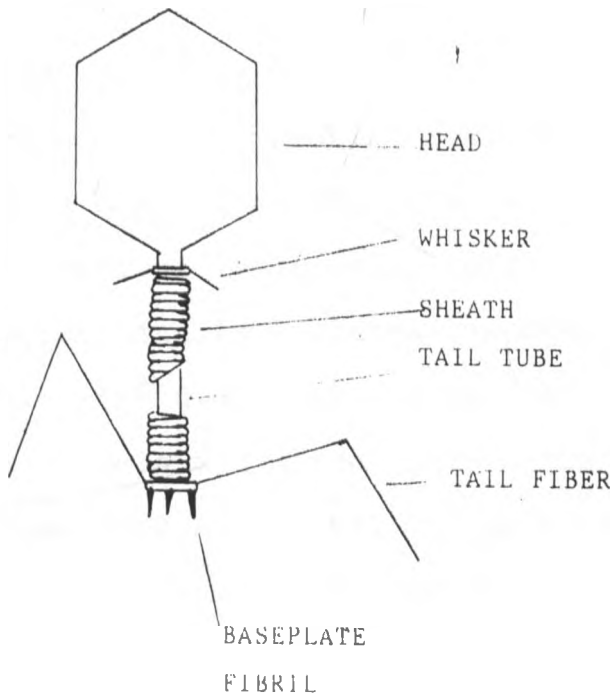
No regional or tribal relationship for the different phage types 56, 193, 208 was found, there being no significant association with any particular

area or a tribe.

5.5 CONCLUSIONS

From the phage-typing results, it is evident that multiple phage types have been isolated from the clinical material at KNH. They do not conform to any particular pattern apart from being multiresistant strains. Phage-type 56 appears to be the most common to KNH. It needs further epidemiological investigations to determine its origin - animal or man alone?.

FIG : 3 DIAGRAM ILLUSTRATING STRUCTURES OF A PHAGE



CHAPTER 6

THE STUDY OF THE ANTIBIOTIC SENSITIVITY PATTERN OF
SALMONELLA TYPHIMURIUM ISOLATES FROM CLINICAL MATERIAL
AT KNH.

6.1 INTRODUCTION

The emergence of antimicrobial resistance among bacteria at KNH is posing serious problems in antimicrobial therapy of infections. Antimicrobial resistance among Salmonella isolates has been reported in several previous studies (Say and Wamola 1974, Slack and Wamola 1977, Wamola and Mirza 1981, Mirza and Wamola 1983, Nesbitt et al. 1988). The purpose of this study was to determine and assess the sensitivity pattern of S.typhimurium, and provide guidance on the treatment.

6.2 MATERIALS AND METHODS

One hundred and nine strains of S.typhimurium isolated from clinical specimens from patients at

Kenyatta National Hospital were identified by serotyping by the methods of Edwards and Ewing (1972) and sensitivity tested by the disc diffusion method. The medium used was Diagnostic Sensitivity Test Agar (DST). The inoculum on DST was made by using one drop of an overnight broth culture, and spread by means of a dry sterile swab on DST. Commercially prepared "multodisks" in routine use in the laboratory at KNH were used with the following eight antimicrobials: ampicillin 10 mcg, tetracycline 10 mcg, cotrimoxazole 25 mcg, streptomycin 10 mcg, kanamycin 30 mcg, gentamicin 10 mcg, sulphafurazole 100 mcg, and chloramphenicol 30 mcg. Also single discs with the following antimicrobials : amikacin 30 mcg, cefotaxime 30 mcg, sulphafurazole 23.8 mcg and trimethoprim 1.2 mcg were tested on organisms which showed resistance to most of the above conventional antibiotics. The zone sizes in millimeters were compared with those of a standard sensitive control organism (E.coli NCTC 10418). The results were expressed as "sensitive" "

moderately sensitive" or "resistant" according to the criteria of Stokes and Waterworth (1972). "Moderately sensitive" strains were considered to be clinically responsive to increased antibiotic dosage and are included in the designation "sensitive" in the results. When an organism was found to be resistant to three or more of the antibiotics tested, the organism was referred to as being resistant to multiple antimicrobials.

6.3 RESULTS

Results of resistance pattern of S.typhimurium from 1970 - 1988 are shown in Table 20. From the table it is evident that resistance to ampicillin (95%), cotrimoxazole (83%), kanamycin (53%), gentamicin (32%), streptomycin (95%), chloramphenicol (88%) and sulphonamides (100%) has gone up quite significantly. It was interesting to note that 18 of the S.typhimurium strains were sensitive to cotrimoxazole, but when tested separately with trimethoprim (2.5mcg) and

sulphamethoxazole (25mcg) all were resistant to sulphamethoxazole and only 13% were sensitive to trimethoprim.

Analysis of multi-resistance (Fig 4) shows antibiotic resistance patterns in S.typhimurium strains. Out of the 109 strains, 96 (88%) showing resistance to more than three clinically useful antibiotics i.e. ampicillin, cotrimoxazole and chloramphenicol. Gentamicin resistance has also risen from 2% in 1972 to 32% while sulphonamide resistance is now 100%.

The resistance patterns were as follows : thirty eight (35%) strains were resistant to A,T,Cm,S,Su and C; 20 (19%) to A,Cm,S,Su,K,G,C; 15 (14%) to A,T,Cm,S,Su,K,G,C; 13 (12%) to A,K,S,Su,C; 10(9%) to A,Cm,S,Su,C; 8(7%) to A,T,Cm,S,Su and 5(4%) were resistant to sulphonamides alone.

Resistance of S.typhi strains documented over the years was compared to S.typhimurium.

Table 21 shows that Salmonella typhi as compared to S.typhimurium is still remarkably sensitive, to all the antibiotics except sulphonamides to which it is now 100% resistant.

6.4 DISCUSSION

In studies at KNH over the years (Table 20, 21), S.typhi has remained sensitive to most antibiotics, while S.typhimurium has developed multiple resistance. In 1972 (Say et al. 1972) only 2% strains of S.typhi were sulphonamide resistant, but in 1980 (Wamola et al. 1981) resistance rose to 39%. Although only 24% S.typhimurium strains were resistant to chloramphenicol in 1972, the resistance rose to 30% in 1980 (Wamola et al. 1981). It has now gone up to 88%, this is clinically highly significant, and will make successful treatment of patients suffering from S.typhimurium infections especially septicaemia & meningitis, very difficult in future, if one was to consider these "drugs of choice" in the treatment of patients.

6.5 CONCLUSIONS

Keeping in mind the resistance pattern of S.typhimurium in this study it would seem appropriate to continue to use chloramphenicol as the mainstay therapy for S.typhi, but not for S.typhimurium. Cefotaxime, or other third generation cephalosporins would be the drugs of choice for the initial therapy. Good results were obtained from the use of cefotaxime in treating multi-drug resistant S.typhimurium septicaemia in Rwandese children (Lepage et al. 1984). However these are expensive drugs and not easily obtainable, where essential drug lists are aimed at low cost treatment of common problems. There is also need for more rational and coordinated administration of antibiotics and for strict measures against their misuse. There is need for continuous surveillance of resistance in salmonellae at KNH with a view to facilitating the appropriate use of antibiotics and permitting early recognition of epidemics caused by S.typhimurium, so that control measures can be rapidly

applied. The objective should be to promote the development of a national policy for antibiotic use.

Table 20 Percentage resistance of S. typhimurium (past and present)

Antimicrobials	1970-1972	1975	1979	1981	1983	1988	1989
	Say et al. (N=74)	Slack et al. (N=181)	Mirza et al. (N=19)	Wamola et al. (N=88)	Mirza et al. (N=76)	Nesbitt et al. (N=23)	Mirza present series (N=109)
Ampicillin 10mcg	65	52	68	63	50	95	95
Tetracycline 10mcg	62	69	79	59	33	63	56
Co-trimoxazole 25mcg	26	9	5	13	8	66	83
Kanamycin 30mcg	22	4	10	3	0	10	53
Gentamicin 30mcg	2	0	5	0	0	5	32
Streptomycin 10mcg	39	49	47	25	75	68	95
Chloramphenicol 30mcg	24	NT	36	30	33	42	88
Amikacin 30mcg	NT	NT	NT	NT	NT	0	2
Cefotaxime 30mcg	NT	NT	NT	NT	NT	0	0
Trimethoprim 1.2mcg	NT	NT	NT	NT	NT	NT	87
Sulphamethoxazole 23.8mcg	NT	NT	NT	NT	NT	NT	100

NT = not tested; N = number of strains tested

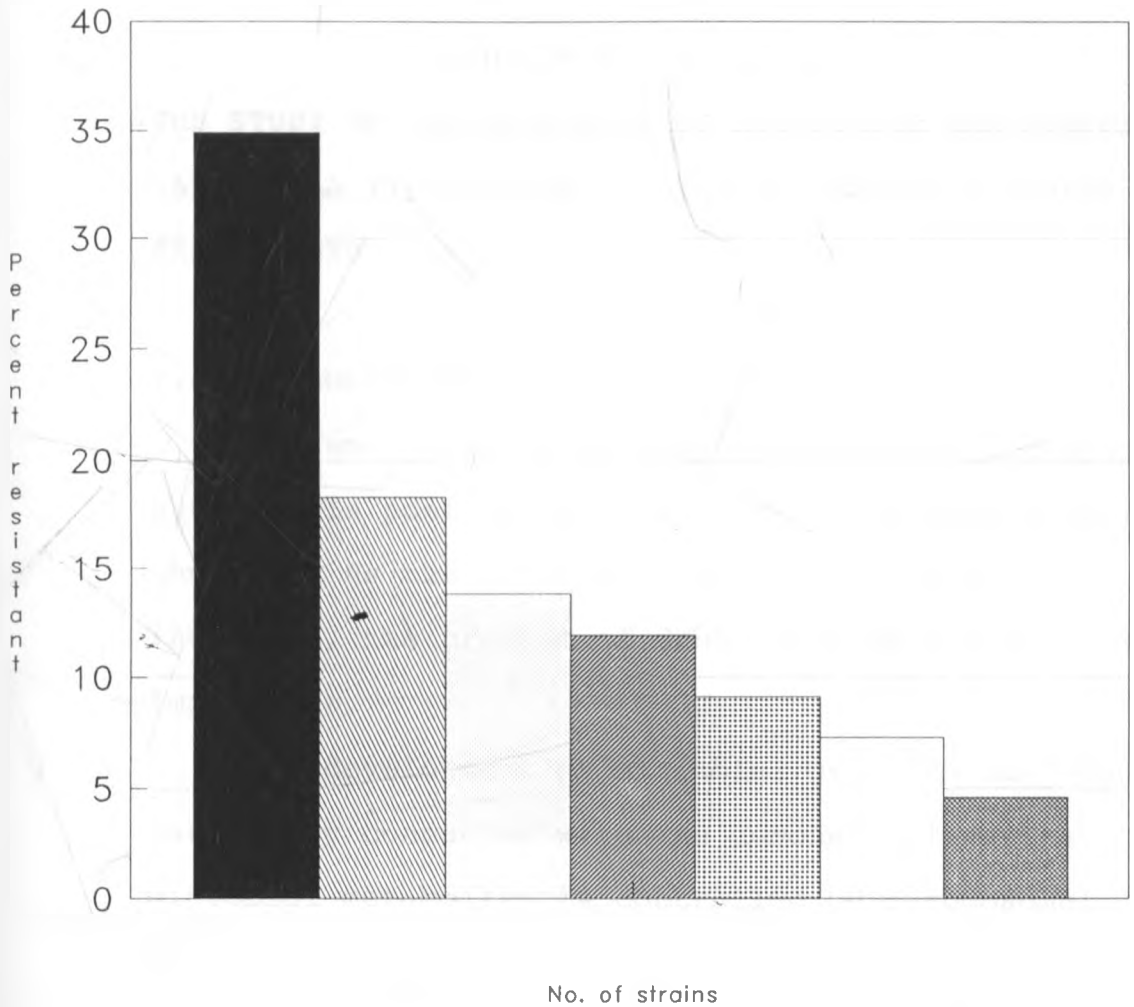
Table 21 Comparative percentage resistance of S. typhimurium and S. typhi

Antimicrobials	1972		1981		1989	
	<u>S.typhim</u>	<u>S.typhi</u>	<u>S.typhim</u>	<u>S.typhi</u>	<u>S.typhim</u>	<u>S.typhi</u>
	(N=42)	(N=106)	(N=88)	(N=18)	(N=109)	(N=8)
Ampicillin 10mcg	65	5	63	22	95	18
Tetracycline 10mcg	62	5	59	28	56	25
Co-trimoxazole 25mcg	26	0	13	6	83	17
Kanamycin 30mcg	22	0	3	0	53	0
Gentamicin 30mcg	2	0	0	0	32	0
Streptomycin 10mcg	39	8	25	33	95	17
Sulphafurazole 100mcg	4	2	60	39	100	0
Chloramphenicol 30mcg	24	1	30	0	88	0
Amikacin 30mcg	NT	NT	NT	NT	2	0
Cefotaxime 30mcg	NT	NT	NT	NT	0	0

NT = not tested; N = number of strains tested

S.typhim = S.typhimurium

Figure 4: Antibiotic Resistance Pattern
(N=109)



■ ATCmSSuK ▨ ACmSSuK □ ATCmSSuK ▩ ASSuKC
 ▤ ACmSSuK □ ATCmSSu ▧ Su

A ampicillin; T tetracycline; K kanamycin;
 Cm cotrimoxazole; S streptomycin; Su sulfa
 C chloramphenicol; G gentamicin

C H A P T E R 7

**THE STUDY OF THE MECHANISM OF ANTIBIOTIC RESISTANCE OF
SALMONELLA TYPHIMURIUM STRAINS BY PLASMID TRANSFER
EXPERIMENTS**

7.1 INTRODUCTION

The emergence of antimicrobial resistance among bacteria is posing serious problems in antimicrobial therapy. As the range and power of chemotherapy increases, the problem of drug resistance also grows in importance.

The development of microbial genetics has been intimately connected with the demonstrations that microbial metabolism is controlled by chromosomal genes, and that the appearance of drug resistance in a previously sensitive microorganism is due to gene mutation, either spontaneously or physico - chemically induced. If selection pressure is exerted by the continued presence of the relevant drug, resistant mutants will outgrow the sensitive parent organisms.

This type of mechanism is well established. It accounts satisfactorily for many of the phenomenon associated with drug resistance, and has successfully indicated how the spread of resistant strains may be prevented in certain situations, e.g. the use of triple therapy (streptomycin, isoniazid and PAS) in treatment of tuberculosis. If correctly applied, regimens of combination therapy almost completely avoid the development of drug resistance. In recent years rapid growth in the number of different antibacterial drugs available has been followed by the appearance of multiple drug resistance among many organisms.

In 1959, a most remarkable cause of drug resistance was observed in Japan (cited by Watanabe in 1963). It was shown that multiple drug resistance easily passed between Shigella species and Escherichia coli when these organisms grew in mixed cultures. At the same time it was proposed that this mechanism might also occur in vivo and could account for the dramatic rise of multiple drug resistance among

Shigella species in Japan. This became known as "infectious transfer" of drug resistance plasmids by conjugation among enterobacteria from a resistant donor to a sensitive recipient. This transfer of resistance required direct contact between the donor and recipient cells and the presence of specific bacterial appendages known as pili or fimbriae, the synthesis of which is governed by the Resistance Transfer Factor (RTF).

This concept was later reported and reviewed in detail by Datta (1965), Anderson (1968) and Mitsuhashi (1969).

It is now established that a sensitive strain can acquire resistance to as many as six different antibiotics in a single step as a result of growth in mixed culture with a resistant donor (Murray and Moellering 1982). Resistance transfer can occur widely, not only within populations of organisms of the same genus and species, but also between species and within genera of different organisms. This observation clarified clinical descriptions of similar patterns of

resistance appearing in organisms of different genera and species at the same time and in the same hospital (McGowan et al. 1979, Rubens et al. 1981).

The plasmids determining such resistance can only be transferred to another cell when a second element is present "the Resistance Transfer Factor". It was suggested that the RTF promotes the passage of resistance determinant from a resistant organism into a sensitive organism, so conferring drug resistance upon the sensitive recipient. Anderson and Lewis (1965b) showed that a carrier particle (delta particle) can be identified separately from the resistance determinant. Only organisms with both delta particle and resistance determinant can confer resistance upon sensitive acceptors. Drug resistant strains which carry only resistance determinants become donors if they acquire delta particles from some other organism. Drug sensitive organisms which carry delta particles can turn R+ delta- strains into infectious donors.

Clear clinical evidence that transfer of drug

resistance occurs in vivo within the human bowel and on the skin was obtained by Petrocheilou et al.(1976), Sack (1979), Van der Waaj (1982), Bodey et al. (1982), and Locksley et al. (1982), these sites being the major reservoirs of resistant organisms. Transfer of resistance plasmids also can occur in various body fluids, such as urine, plasma and ascitic fluid, but these reservoirs do not seem to be of major clinical significance (Mendez et al. 1982). At KNH it has been persistently shown that S.typhimurium has developed multiple resistance to most antibiotics (chapter 6 Table 20), but there is no report on plasmid mediated antibiotic resistance transfer. It was therefore decided to study the mode of antibiotic resistance acquired through plasmid transfer in S.typhimurium to see if it contributed significantly to the problem and better definition of S.typhimurium profile.

7.2 MATERIALS AND METHODS

S.typhimurium strains isolated from clinical specimens in the laboratory had been preserved in milk and frozen at -700C (Appendix III).

For drug resistance transfer, 35 strains were selected randomly from the preserved pool of 109 S.typhimurium strains. These were as follows: 24 from blood cultures, 6 from CSF and 5 from stools. To confirm their viability and purity, these were thawed, subcultured on MacConkeys agar and incubated at 370C for 24 hours. All the 35 strains were viable and pure on subculture. They were then retested for their sensitivity pattern by the disc diffusion method on DST agar (Oxoid) using the same method and same antibiotic discs as in the original tests of sensitivity described in chapter 6.

Testing for drug resistant transfer, the following procedure was followed :

Salmonella isolates primarily resistant to either tetracycline, ampicillin or chloramphenicol or

resistant to either two or three of these antibiotics were tested as donors using a lactose-fermenting strain of Escherichia coli K 12 as the recipient strain. The donor and recipient strains were both inoculated into tryptose phosphate broth, and incubated at 37°C for 24 hours.

For the transfer of antibiotic resistance plasmid/s from S.typhimurium to sensitive lactose fermenting E.coli K 12, the conjugation procedure used is outlined below:-

1. Take soya broth 2ml x 2
2. Add 0.02 ml of the overnight culture of S.typhimurium and E.coli K 12 to 2ml soya broth separately
3. shake for 2 - 3 hours at 37°C in a water bath (Taiyo-Shaker).
4. Check bacterial density of culture. This should be approximately 5×10^8 /ml.
5. Mix 0.5 mls (or equal volumes) of both cultures.
6. Filter the mixture using millipore filters (Type

HA 0.45 um).

7. Take out the filter and put it on soya agar plate.
8. Incubate for 3 - 5 hours at 37°C.
9. Suspend the filter paper in 2 mls of soya broth and mix by Vortexmixer vigorously.
10. Spread the culture on MacConkeys agar plate and incubate at 37°C overnight.
11. Pick up different lactose fermenting colonies of E.coli K 12 and suspend in 1 ml soya broth, incubate at 37°C for 2 -3 hours.
12. Streak each bacterial transconjugant on MacConkeys agar plate and incubate overnight.
13. Check antibiotic sensitivity for each E.coli transconjugant using the disc diffusion method with the drugs to which the donor strains were resistant.
14. If the donor S.typhimurium carried transferable plasmids, the E.coli K 12 will show resistance to the same antibiotics to which the donor strains were resistant.

7.3 RESULTS

Results of conjugation between resistant S.typhimurium and sensitive E.coli K 12 experiments.

The antibiotic resistance pattern of 35 strains of S.typhimurium used in the conjugation experiments is shown in Fig. 5. These were S.typhimurium strains isolated from various clinical specimens as indicated in the histogram (Fig.5).

All the 35 strains studied showed that they transferred their resistance to E.coli in the transconjugant, i.e their resistance was due to plasmids. This was as far as work in our laboratory could proceed due to restricted resources. A few of the of the strains were sent to Professor Takayuki Ezaki of Gifu University, School of Medicine, Japan. He found that identification of the plasmids of S.typhimurium strains was not easy as multiple plasmids were present (Fig 6). All the plasmids were transferable to E.coli by conjugation. From his

experiments he considered that the drug resistant plasmids carried drug resistant transposons (such as Ap-transposon, Tc-transposon, Cm-transposon, and Km-transposon). Since the molecular sizes of the large plasmids of these S.typhimurium strains were different from each other, each large plasmid probably carried a different drug resistant gene (Table 22). To determine genetically whether the drug resistant plasmids of these S.typhimurium strains carried transposons or not would be very tedious work and could only be carried out in highly specialized laboratories.

7.4 DISCUSSION

The numbers of multiply resistant bacteria at KNH has not been confined to Salmonella species alone, the increase in the proportion of resistant strains of other organisms has also been noted (Mirza et al. 1981, 1982).

Once the R-factors have been acquired, the organisms are at a selective advantage and could spread with greater ease in the presence of antibiotics in the hospital and afterwards in the community outside the hospital.

It is possible that the resistant Salmonella came from animal sources, but this was not investigated.

From the above experimental work, the presence of conjugative plasmids in S.typhimurium has been demonstrated. A study as this establishes the fact that it is no longer sufficient, in the investigation of infections, especially with the enterobacteria, to identify, for example the serotype of a Salmonella, or its resistance pattern. It has become necessary that characterization should be rendered more precise by phage-typing the organisms when possible, and distinctive biochemical markers should also be sought, while routine testing of strains for drug resistance in the laboratory should be continued.

However, the simple identification of resistance markers is inadequate. The plasmids carrying them require further exploration. Because of the enormous dispersal of resistance among enterobacteria, the epidemiological studies may now need to be carried to genetic and even molecular levels

7.5 CONCLUSIONS

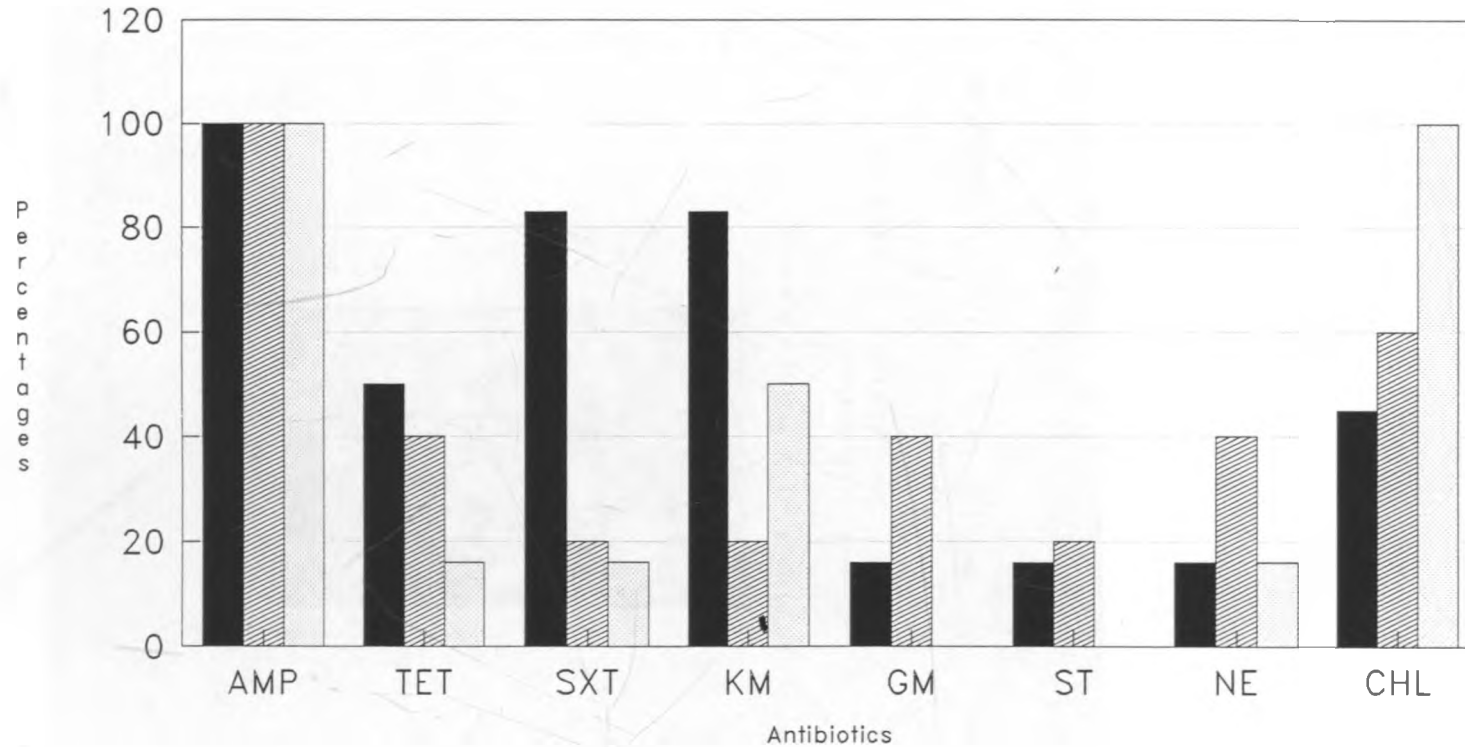
Therefore, there is an urgent need to control the spread of antibiotic resistant bacteria at KNH, by continuous monitoring the drug resistant patterns of prevailing pathogenic bacteria and the appearance of new resistance characteristic is of utmost value for the proper selection of antimicrobial agents for treatment purposes.

That there is need for dissemination of this information to the clinicians, as unawareness of local drug-resistance patterns in pathogens may lead to misuse and often overuse of antibiotics with all their harmful effects.

From the above study, there is clear cut evidence that the transferable multiple drug resistance in strains of S.typhimurium is plasmid-mediated. This kind of plasmid prevalence in clinical isolates may be the result of indiscriminate use of antimicrobial agents. Therefore there is a need for a drugs policy to be formulated urgently for KNH, help develop guide - lines to be instituted in other hospitals throughout the country.

Figure 5: Percentage Resistance of *S. typhimurium*

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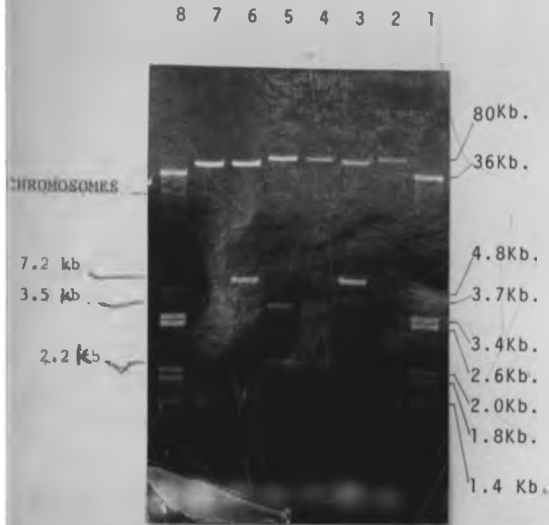


■ Blood cultures ▨ Stools □ CSF

Amp ampicillin; Tet tetracycline; NE neomy
SXT cotrimoxazole; KM kanamycin; ST strept
GM gentamicin; CHL chloramphenicol

- 130 -

FIG.6 PROTEIN PLASMID BANDINGS IN Kb. AS DEMONSTRATED BY POLYACRYMYL GEL



lane 1 and 8, Standard plasmids
 lane 2, strain 302 (Ap, Tc, ST, Cm)
 lane 3, strain 550 (Ap)
 lane 4, strain 590 (Ap, ST, Km, Gm, Ne, Cm, Sm)
 lane 5, strain 577 (Ap, Tc, ST, Sm, Cm)
 lane 6, strain 382 (Ap, Cm)
 lane 7, type strain of Salmonella typhimurium

TABLE 22

Identified plasmids of some strains of S.typhimurium, their molecular weights and patterns of phenotypic antibiotic resistance.

Strain No.	Plasmid	Phenotype
302	80 Kb	Ap, Tc, Sm, Cm
550	75 kb	Ap
	7.2 Kb	Ap
590	80 Kb)*	Ap, ST, Km, Gm,
	3.5 Kb)	(Ne), Cm, Sm
	2.2 Kb)	
577	80 Kb)*	Ap, Tc, ST, Sm,
	3.5 Kb)	Cm
382	75 Kb)*	Ap, Cm
	7.2 Kb)	

* Location of each drug - resistant gene was not determined. These plasmids were transferred to E.coli and the E.coli strain became resistant to the drugs described above, except Neomycin.

Ne = Neomycin, Ap = Ampicillin, Sm = Streptomycin, Km = Kanamycin, Tc = Tetracycline, Gm = Gentamicin, Cm = Chloramphenicol, ST = Sulphamethoxazole - Trimethoprim, Kb = Kilobase.

CHAPTER 8

GENERAL DISCUSSIONS AND RECOMMENDATIONS.

Disease syndromes caused by Salmonella species continue to be important, as evidenced by a major outbreak of infection due to multiresistant S.typhimurium in 1985 at KNH, Nairobi, which necessitated the closure of POW, the main wards for Paediatric admissions (Mirza and Wamola 1989).

Salmonella infections show a strong relationship to age. Children under 5 years of age have the highest rates of infection with peak incidence in infants and neonates. Several explanations have been proposed for the high infection rates in infants. First infections in infants may be more severe than in adults and thus more frequently brought to medical attention. Secondly infants are also immunologically immature, and their microflora and its attendant local protective factors are not fully developed. The younger the infants, the more susceptible they are to haematological spread and

localisation of infection (Saphra and Winter 1957., Bennett et al. 1959., MMWR 1984). Also in early infancy the blood - csf - barrier is evidently more easily crossed than at a later age predisposing them to meningitis. Although it has been shown that there is no transplacental transmission of Salmonella from the mother to the foetus by Netter (1950), it is possible that the infant may acquire infection by direct contact with the organism during delivery or a few days later after delivery from an infected mother. The role of the mother in transmission of infection to her newborn infant need further studies.

It is observed in Fig.7 that there is a seasonal pattern of S.typhimurium infections. The greatest number of isolates were reported to occur in the period July -October with a peak around August -September, while S.typhi and other salmonellae remained more or less static throughout the year. These are winter months in Kenya. This time of the year also coincides with the Meningococcal meningitis outbreaks (Mirza and

Wamola 1980). The relationship to climatic conditions is well documented in Meningococcal meningitis in Africa. (Lepeyssonnie 1963, Belcher et al. 1977). Climatic association of S.typhimurium isolates during the same period of the year as Meningococcal meningitis is probably coincidental. However energetic epidemiological surveillance with a high standard of laboratory support is needed to monitor S.typhimurium infections throughout the country to confirm the seasonal association of S.typhimurium outbreaks around the same period as Meningococcal meningitis, and devise possible intervention measures for both.

Salmonellosis as an important opportunistic infection in patients with AIDS is well recognized (Nadelman et al. 1985., Fischl et al. 1986., Cellum et al. 1987). In this series there were 33 HIV positive patients who developed nosocomial gastroenteritis. Nine (27.2%) had Salmonella species isolated from their rectal swabs; 7 (21.2%) of these were due to S.typhimurium. While none of the 79 HIV positive

patients had Salmonella isolated from their rectal swabs on admission. Because Salmonella infection has been associated experimentally (Davies 1976) and clinically (Sarma et al. 1977) with deficits of cell-mediated immunity (CMI), the occurrence of S. typhimurium in HIV positive hospitalised patients is not surprising. CMI is important in controlling Salmonella infection, as the organism can live intracellularly for long periods inspite of humoral immunity. Increased incidence of Salmonella infection in patients with AIDS represents an increased susceptibility to infection as a result of defects in both CMI and humoral immunity caused by HIV (Simberkoff et al. 1984, .Polsky et al. 1986). Thus prolonged stay in hospital of HIV positive patients renders them at greater risk of acquiring nosocomial Salmonella infection.

Of one hundred and seventy nine patients with Salmonella septicaemia, 21 (12%) acquired their infection in the hospital while being treated for

conditions shown in Table 9. S.typhimurium was isolated in highest numbers from both community acquired (89%) and hospital acquired (86%) septicaemic cases. (Table 8). Prolonged stay in hospital predisposed patients to nosocomial septicaemia. In these cases the mean duration of stay in hospital prior to onset of symptoms was 11.9 days (Range 2 - 65 days SD = 12.5 days).

In this series approximately 72% of the patients infected with S.typhimurium were children under the age of five years. Highly significant risk factors in a case control study of clinical and epidemiological features of S.typhimurium infections were : Residence outside Nairobi, Untreated water, Use of Pit latrine, Presence of domestic animals in the compound including chickens and cows, and drinking of untreated cows milk.

Because of repeated outbreaks of S.typhimurium infections at KNH, attempts to correlate them with epidemiological studies on the phage types, 34 strains were sent to the Reference Laboratory for Enteric

Pathogens at Colindale, London. The results were surprising. It was not just one phage type as reported from other hospital outbreaks (Datta 1962., Anderson 1968., Robins-Brown et al. 1983), but several different phage types were isolated as shown in Table 18. The predominant phage types reported were 56 (29.4%), 193 (20.5%) and 208 (17.6%). Probably there is a pool of different S.typhimurium strains existing in the community or in the hospital which give rise to continuous outbreaks of infection with different phage-types simultaneously. Type 56 which was isolated in highest numbers seems to be unique to Kenya. This needs further studies including studies in animals for "finger printing" the various isolates of S.typhimurium so that they may be distinguished from or identified as part of an epidemic strain.

Antimicrobial sensitivity tests (Table 20) show that since 1970, there has been a striking increase in the multiple resistance acquired by S.typhimurium to most of the antimicrobials tested including

chloramphenicol. The few strains tested for plasmid profile also show that this resistance is plasmid mediated and is not due to a single plasmid, but each strain may be carrying several resistant plasmids including transposons. Further studies are needed in this field to elucidate the particular plasmid/s involved in the transfer of resistance of a particular drug. This work as indicated by Prof. Ezaki of Gifu University Japan is very highly specialized and involved, and could only be followed in a research laboratory geared to this type of work.

Recommendations

a. Future research efforts in the area of nosocomial gastroenteritis and other infections need to address a number of important issues :

- (i) There is need for continuous surveillance to obtain better data on the incidence of and risk factors for nosocomial gastroenteritis and other nosocomial infections.

- (ii) Investigations of epidemic and endemic infections are needed to identify vehicles of transmission.
- b. There is an urgent need to set up a surveillance programme in the hospital to continuously monitor the drug resistance patterns of the prevailing pathogenic bacteria and the appearance of new resistance characteristics.
 - (i) The information about resistance pattern of the pathogenic bacteria should be disseminated on monthly bases to KNH medical staff, administration and the hospital pharmacy, and to other hospitals in the country. This would provide a guideline for the proper selection of antimicrobial agents for therapeutic purposes.
 - (ii) For current and projected future use there is need for the hospital pharmacy to be guided on purchase of drugs. The formulary should be restricted to minimum number of agents needed for most effective therapy, and elimination of duplicative agents. The rule for selection should

be of least expensive, most effective agent from a given class of agents.

c. There is need to control spread of epidemic resistance through the hospital by identification of reservoirs :

(i) Colonized or infected patients.

(ii) Environmental contamination..

d. Control spread of transmission by :

(i) Improved hand-washing and asepsis

(ii) Isolation of colonized and infected patients.

(iii) Elimination of any common source if known.

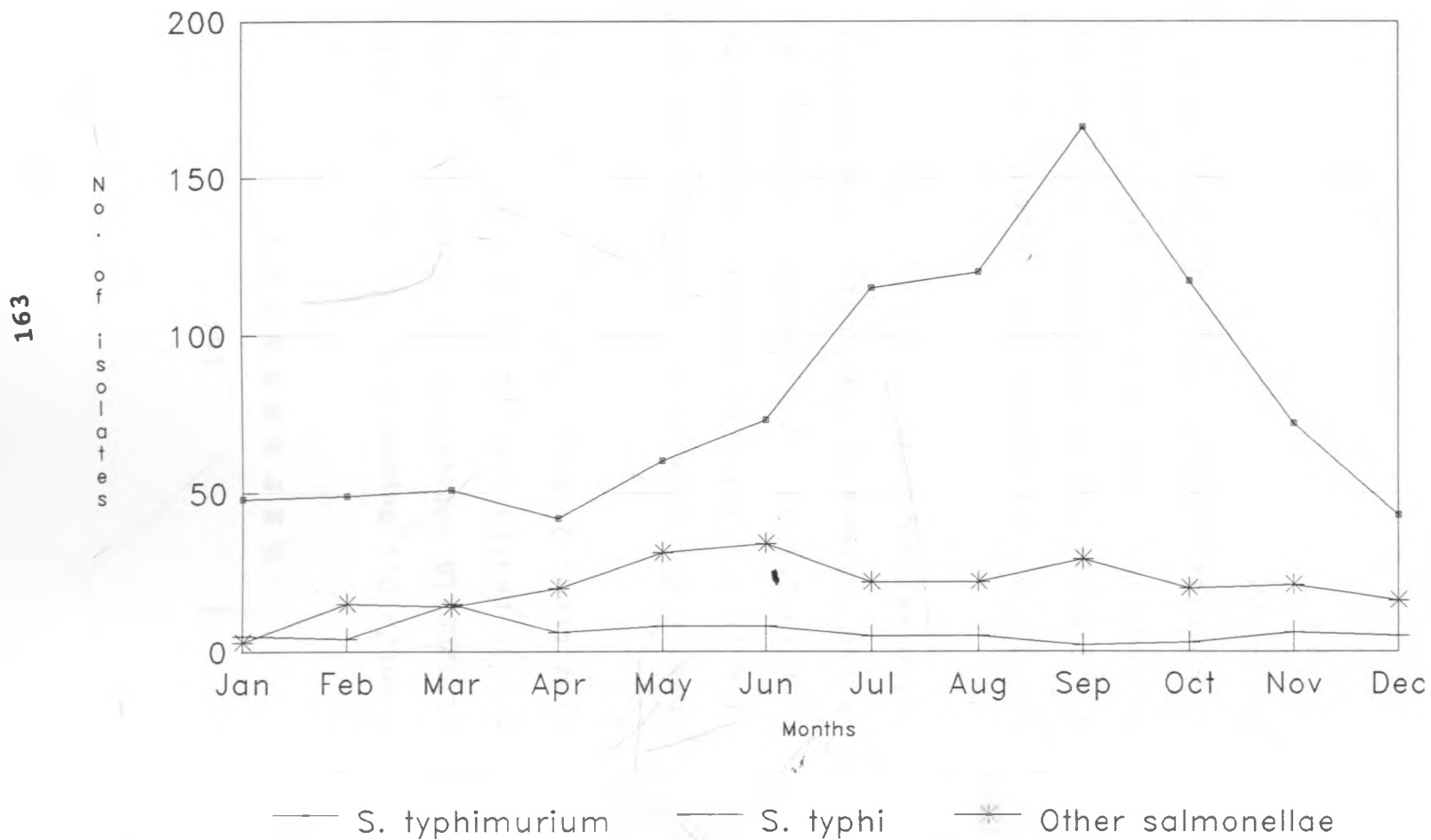
(iv) Closure of a unit to new admission if necessary.

(v) Control of antibiotic use by restriction or elimination.

This would provide more appropriate antibiotic use and better health care for patients.

e. For prevention of transmission of infection, there is need for health care education of personnel working both in the hospital and community.

Figure 7: Monthly Incidence of Salmonella (Mean 1984–1989)



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APPENDIX I

STUDY : THE PROFILE OF S.TYPHIMURIUM AT KNH

DATE OF ADMISSION _____ DATE DISCHARGED _____

NAME _____ HOSP/OP.NO. _____

RESIDENCY ADDRESS (NRB) SPECIFY AREA

AGE _____ SEX _____ TRIBE _____

HOME ADDRESS _____

DAYS OF ONSET OF ILLNESS _____ NAIROBI _____ OUTSIDE

NAIROBI

SPECIFY WHERE _____

PRESENTING COMPLAINT	DURATION	DEVELOPMENT OF COMPLICATIONS
_____	_____	_____
_____	_____	_____
_____	_____	_____

ADMITTING DIAGNOSIS _____

SOURCES OF DRINKING WATER _____

SOURCES OF DRINKING WATER_____

SOURCES OF WASHING AND BATHING WATER_____

SOURCES OF MILK SUPPLY_____BOILED_____

FRESH_____

PASTEURISED_____

CONTACT WITH DOMESTIC ANIMALS_____

SPECIFY TYPE OF ANIMAL_____

METHOD OF SEWAGE DISPOSAL_____

TYPE OF LATERINES USED_____

DIET_____

NUMBER OF PEOPLE IN THE HOUSE_____

TREATMENT BEFORE ADMISSION

TREATMENT AFTER
ADMISSION

APPENDIX II

PATIENTS CONSENT

I.....OF P.O.

BOX.....

AND OF BEING SOUND MIND, AFTER HAVING FULLY UNDERSTOOD

THE NATURE AND PURPOSE OF THIS STUDY FOLLOWING

DR.....EXPLANATION

HEREBY AGREE TO BE RECRUITED/HAVE MY WARD/CHILD

RECRUITED IN THE SAID STUDY.

SIGNED.....

WITNESS.....

APPENDIX III

FORMULAR OF PRESERVATION OF MILK FOR SALMONELLA

20 GMS	-	Powdered Milk
20 mls	-	Glycerine
200 mls	-	Distilled water

Mix above thoroughly. Dispense 1 ml Cryovials and autoclave at 121⁰C for 15 minutes only. Keep at 4⁰C in the fridge.