THE PREVALLENCE OF *SALMONELLA*, *SHIGELLA* AND *YERSINIA ENTEROCOLITICA* IN ADULT DIARRHOEAL OUT-PATIENTS IN SOME HOSPITALS OF ADDIS ABABA.

A Thesis

Presented to the

School of Graduate Studies

Addis Ababa University

In partial Fulfilment
of the Requirements for the Degree of
Master of Science in Zoology

---

by

Mogessie Ashenafi

May, 1983

ACKNOWLEDGEMENTS

My indebtedness to Dr. Messele Gedebou, my advisor, for his willful help, constant guidance and encouragement I gratefully received in the preparation of this dissertation is immeasurable.

I am obliged to thank the office of the Dean of the School of Graduate Studies for making the course of this investigation smooth and feasible.

I am deeply thankful to the co-operative and understanding staff of the Bacteriology Department of Central Laboratory and Research Institute(C.L.R.I) in general and Ato Afeworki Gebreyohannes, head of the department, in particular,for the material an technical help, as well as the instructive advice I got during the study.

I would also like to express my deep gratitude to Woizero Mulu Bekele, my wife, for typing the manuscript and, most of all, for the unfailing encouragement I received all through the course of this study.

Due thanks go to Dr. Berhanu A.Gashe for his advice, Ato Haile Dadi for the unreserved technical assistance and concern, Wt. Wozenet Theodros and Ato Assefa Areda for the material assistance and encouragement and Sister Yamrote Fisseha for co collection of specimens.

The financial assistance made by the Swedish Agency for Research Co-operation with Developing Countries(SAREC) in carrying out this study is gratefully acknowledged.

T A B L E OF C O N T E N T S PAGE

LIST OF TABLES

TABLES PAGE

ABSTRACT

Ninety strains of Shigella and forty-five strains of Salmonella were isolated from 1000 adult diarrhoeal out-patients from three hospitals and three clinics in Addis Ababa. No Yersinia enterocolitica was isolated.

Out of the ninety Shigella strains, 57(63.3%) were Sh.flexneri, 17(18.9%) Sh.dysenteriae, 9(10%) Sh.boydii and 7(7.8%) Sh.sonnei. And among the forty-five Salmonella strains, 17(37.8%) belonged to Group C, 13(28.9%) were Group B strains, 9(20%) were S.typhi,4(8.9) belonged to Group D other than S.typhi and Groups A and E had one isolate each(2.2%).

The standardized agar disk diffusion technique was used with disks of ampicillin, carbenicillin, chloramphenicol, cephalothin, gentamicin, kanamycin, polymyxin B, streptomycin, sulphadiazine, tetracycline, and trimethoprim-sulphamethoxazole.

All Shigella strains were sensitive to cephalothin(95.6%), gentamicin(100%), kanamycin(100%), polymyxin B(100%)and trimethoprim-sulphamethoxazole(100%). Sensitivity to ampicillin (47.7%), carbenicillin(50%), streptomycin(36.7%), sulphadiazine (34.4%) and tetracycline(31.1%) was very low. Only 15 strains were sensitive to all 11 drugs.

Ninety-three percent of Sh.flexneri,70.6% of Sh.dysenteriae, 44.4% of Sh.boydii and 71.4% of Sh.sonnei were resistant to one or more drugs. About 82% of all Shigella strains were resistant to one or more drugs. Multiple drug-resistance was detected in 82.4% of Sh.flexneri, 70.6% of Sh.dysenteriae, 33.3% of Sh.boydii and 57.1% of Sh.sonnei. About 74% of Shigella strains were

multiply resistant. Eighteen different resistance patterns were detected, of which 26.7% were simultaneously resistant to ampicillin, carbenicillin, chloramphenicol, streptomycin, sulphadiazine, and tetracycline. This pattern was the commonest in Sh. flexneri (31%) and Sh. dysenteriae (29%).

All Salmonella strains were sensitive to gentamicin, polymyxin B and trimethoprim-sulphamethoxazole. All strains of Groups A, E and S. typhi were sensitive to all drugs. A total of 31 strains (68.9%) were sensitive to all drugs.

Over 85% of Group B strains were sensitive to all drugs. Group C strains were the least sensitive. Ampicillin, carbenicillin, cephalothin, chloramphenicol, streptomycin, sulphadiazine and tetracycline were mostly ineffective against these strains, while only 53% of them were sensitive to Kanamycin.

About 15% of Group B, 64.7% of Group C, and 25% of Group D strains were resistant to one or more drugs. The most frequent was resistance to eight drugs (17.8%). Of the 45 Salmonella strains, 26.7% were multiply-resistant. In all, seven different resistance patterns were noted. The commonest pattern was seen in 47% of Group C strains: ampicillin/carbenicillin/chloramphenicol/kanamycin/streptomycin/sulphadiazine/tetracycline.

The findings in this study were compared with reports from Ethiopia and elsewhere. Based on this study and other reports from Ethiopia, it is recommended that the aetiology of diarrhoea in all ages be determined, regional reference laboratories be established, the public be educated on personal hygiene and environmental sanitation, chemotherapy against diarrhoeal diseases be discouraged and strict antibiotic policy be instituted in hospitals and nationally.

I. INTRODUCTION

1. Nature of diarrhoeal infections.

1.1. Definition

In the second and third centuries A.D., diarrhoea was defined as "consisting of the discharge of undigested food in a fluid state,(such as) when the heat does not digest the food nor convert it into its proper chyme, but leaves its work half finished" (Phillips,1975,cited by Plotkin et al,1979). More recently, diarrhoea has been defined as an increase in the frequency,fluidity or volume of bowel movements relative to the usual habit of each individual(Plotkin et al,1979).

1.2. Global importance

It has been suggested that 200 million children and adults are afflicted with acute diarrhoea on any given day and, in developing countries, acute gastroenteritis complicated by malnutrition constitutes a major cause of illness at all ages and kills millions of infants annually(Evans,1979). In whatever way it is measured, the incidence rate or the consequences, diarrhoeal diseases are the major health problem in tropical and developing countries(Cutting, 1979). The increasing need to study the aetiology of diarrhoea in different parts of the world is clearly manifested in the concern shown by WHO(WHO,1980a). A global Diarrhoeal Diseases Control(CDD) programme has been launched by WHO in 1978, as part of the Organization's commitment to primary health care and to the achievement of "Health for All by the year 2000" -the main objective of CDD being decreasing diarrhoeal disease-related mortality and

morbidity. Bacterial agents included in the programme are vibrio cholerae and related vibrios, Escherichia coli, Salmonella Shigella, Campylobacter and Yersinia enterocolitica.

1.3. Status in Ethiopia.

Despite the frequent occurrence of diarrhoea among the Ethiopian population, the pattern of diarrhoeal aetiology has not been determined, due to serious limitations of bacteriological diagnostic facilities in most hospitals. As a result, laboratory requests for diagnoses of diarrhoea are made only for parasities. A positive result is followed by treatment against the parasites identified. A negative result is generally taken as indicative of a non-parasite but bacterial or viral infection. However, this is often followed by antibiotic treatment for bacterial aetiology without bacteriological diagnosis(Personal communications with some physicians).

In Ethiopia according to the Epidemiology Division, Ministry of Public Health, enteric infections are not only widely spread in the general population but also occur in epidemic forms. These diseases are among the eight leading major communicable diseases, exceeded only by venereal diseases and helminthiasis. Between 1974 and 1976, there was an average of 146,640 reported cases annually throughout the country(Ministry of Health,1977).

2. Aetiological agents of diarrhoea.

The various aetiological agents of diarrhoea are discussed by different workers(Sack et al, 1971; Plotkin et al, 1979; WHO Scientific Working Group, 1980a,1980b,1980c,1980d, 1980e; Stintzing, 1981; and Swaminathan et al,1982). Among these

the most important are V.cholerae, Vibrio parahaemolyticus, E.coli, Rotavirus and other viral diarrhoeal agents, Entamoeba histolytica and Giardia lamblia. There are some helminths which also cause diarrhoea: Trichuris trichiura, Strongyloides stercoralis and Trichinella spiralis.

A variety of other organisms has been implicated as occasional causes of diarrhoea. These include Proteus, Providencia, Citrobacter, Klebsiella, Enterobacter, Edwardsiella tarda, Bacillus cereus and algae.

At present, the aetiology of diarrhoeal infections in Ethiopia is incomplete. In his series of works on infant diarrhoea, Stintzing(1981) reported that not a single Salmonella strain was isolated. Wallace(1976) reported a 1% isolation of Salmonella(all S.typhi)from over 700 stool specimens in Addis Ababa. Salmonella was also isolated from the very few stool specimens received over several years in the bacteriology laboratory of the Tikur Anbessa Hospital (Messele Gedebou and Alebachew Tassew,1981).

The frequencies of isolation of Shigella species in Addis Ababa were recently reported(Afeworki Gebreyohannes and Yetnebersh Limeneh, 1980; Messele Gedebou and Alebachew Tassew, 1982). Wallace(1976)believed that Shigella are commonly isolated in Ethiopia(11% of 700 specimens). On the other hand, Stintzing(1981) has claimed that Shigellae are not important aetiological agents in infant diarrhoea.

Yersinia enterocolitica seems to have a very low frequency of occurrence in children in Ethiopia. Stintzing(1981) reported

only one isolate from over 1000 children with diarrhoea. However, E.coli and Campylobacter fetus subspecies jejuni have been reported to be the most important aetiological agents of diarrhoea in Ethiopian infants(Stintzing,1981). There has not been any report of isolation of V.cholerae in this country since the last epidemic in 1970. However, Afeworki Gebreyohannes and Tewfik Yusef(1979) reported a non-cholera Vibrio from an adult with severe cholera-like diarrhoea.

3. Purpose of the investigation.

The patterns of aetiology of diarrhoea in adults have not been studied in Ethiopia(Personal communication with Messele Gedebou). The general purpose of this investigation, therefore, is to attempt to partly elucidate this pattern by determining the prevalence of Shigella, Salmonella and Yersinia enterocolitica in acute diarrhoea of adults in various hospitals in Addis Ababa. It is also to determine the drug-sensitivity patterns of the isolates.

The investigation is restricted to these organisms mainly because of technical and time limitations. Also, Shigella and Salmonella are believed to be among the most important pathogens that are frequently isolated in adult diarrhoea(Personal communication with Heads of Microbiology Laboratories of Central Laboratory and Research Institute and Tikur Anbessa Hospital). In addition to these, Yersinia enterocolitica is included because there is a need to determine its magnitude of infection in various parts of the world (WHO Scientific Working Group,1980a).

II L I T E R A T U R E R E V I E W

A. Salmonella

1. Taxonomy

1.1. General characteristics

The genus Salmonella is composed of Gram-negative bacteria that conform to the definition of the family Enterobacteriaceae and the tribe Salmonellae. Urease is not produced, sodium malonate is not utilized, gelatin is not liquefied, and growth does not occur in medium containing potassium cyanide. Lysine, arginine, and ornithine are decarboxylated. Acid is produced in Jordan's tartrate medium. Dulcitol is fermented and inositol is utilized by numerous strains. Sucrose, salicin, raffinose and lactose are not fermented(Edwards and Ewing,1972). The various biochemical characteristics of Salmonella are described and tabulated in the manual of Edwards and Ewing(1972).

1.2. Serogroups and serotypes.

About 2000 serotypes of Salmonella are known(WHO Scientific Working Group,1980a). Partial and complete identification of such serotypes is carried out by the use of absorbed antisera containing appropriate antibody. The somatic antigen provides the basis for primary separation into serogroups. There are several such groups, labelled A-Z(Kauffmann,1966).

A strain can be assigned to one or another of these groups by the use of appropriate group-antisera, and can further be serotyped by specific absorbed O and H antisera.

About 98% of all Salmonella strains isolated fall into the first 8 O groups, A to E_4 (Freeman,1979).

2. Nutritional and other growth requirements.

Salmonella species have simple nutritional requirements, growing readily on the usual nutrient media. In synthetic media, an ammonium salt and glucose, pyruvate, lactate, etc, are adequate sources of nitrogen and carbon. The great majority of strains do not require vitamins or amino acids, but some strains of the typhoid bacillus require added tryptophan. The optimum temperature is 37°C, but growth occurs at a reasonable rate at room temperature. They are facultative anaerobes, growing equally well under either aerobic or anaerobic conditions.

Salmonella can be isolated using differential and selective media of various types(Edwards and Ewing,1972). Since they are not fastidious they can be grown on ordinary media.

3. Epidemiology of Salmonella infections.

3.1. Sources of infection.

In discussing the sources of the commonly occurring serotypes of Salmonella in man, it is necessary to consider first the role of the carrier and then the animal reservoir of the bacteria.

Some convalescents, as well as persons without history of symptoms, may excrete Salmonella for long periods. The long-term carrier state(six months or more) following disease, occurs more often than is commonly realized(Eisenberg et al,1955). Many asymptomatic excretors of Salmonellae have been discovered through routine examination of faecal specimens from food handlers(Savage,1956). Infants frequently become long-term carriers and may harbour the bacteria for longer periods than older persons. Further, they transmit infection to other

members of the family(Szanton,1957).

Active cases of salmonellosis in man are sources of contamination and transmission to other human beings and to lower animals. Infection of one person by another may occur in a variety of ways. Transmission may be direct by means of the faecal-oral route, it may be air-borne(Black et al,1960) or it may be indirect by means of foods (Chapman,1980;Gunn and Bullon-Loarte,1979;Gupta et al,1980), towels,contaminated toilet seats, and many other objects(Committee on Salmonella,1969).

The wide spread distribution of Salmonellae in animals used for food frequently results in contamination of human foods derived from these animals. Contaminated poultry, meat, egg products, and pork and beef products are associated with outbreaks of salmonellosis(Committee on Salmonella,1969). Raw milk(Vogt et al,1981) and contaminated water(Koplan et al,1978) are also found to cause salmonellosis.

The frequency with which Salmonellae are isolated from domestic fowls indicates that these animals probably constitute the largest single reservoir of these bacteria(Hobbs,1961; Falade and Ehizokhale,1981).

Salmonellosis in cattle is caused primarily by serotypes Dublin and Typhimurium, but more than 75 other serotypes have been encountered, the most common of which are Newport, Enteritidis, Anatum, Heidelberg and Panama(Committee on Salmonella,1969).

Salmonellae are most frequently associated with enteritis in swine(Cazbon et al,1978; Sharma and Pathak,1974).

Salmonella infections are quite common in horses(Committee on Salmonella,1969), dogs(Keyhani,1978; Britt et al,1978 and rate(Bezjak and Thorburn,1981). Salmonellae are also isolated from forest mammals(Kourany et al,1976).

It is well known that cold-blooded animals may be infected with Salmonella. Turtles and snails(Freeman,1979) and fish (Janssen and Meyers,1968) may harbour Salmonella.

3.3. Incidence of infection in adults.

Salmonellosis of man is an important infectious disease in the world. Its prevalence can only be estimated at this time, and it is certain that reported infections represent but a minority of the total number, because the fatality-rate is low and the illness frequently mild and self-limited.

Although much of the currently available information has come from North America and the United Kingdom, where surveillance reports are published regularly(WHO Scientific Working Group, 1980a), reports from the developing countries are not completely lacking.

In Kenya, Wamola(1980) attempted to elucidate the bacterial stool pathogens between 1975 and 1978 and found that the isolation rate of Salmonella was 24-35%. In Algeria, Zoukh and Pattevin (1978) made a survey of enteropathogenic enterobacteria from 1974 to 1977 and drew the conclusion that among Salmonella, S.typhi was the predominant type. Salion(1979) isolated 186 strains between 1965 and 1976 and demonstrated 41 serotypes not previously isolated in Upper Volta. In Dakar, Senegal, Lafaix et al (1978) studied over a 10-year period (1966-76) 1335 strains, of which S.typhi was the dominant strain followed

by S.typhimurium.

Excretion of bacterial enteropathogens by Vietnamese refugees in Hong Kong was surveyed in 1979 and the prevalence rate of Salmonella was 13.5%(Anton et al,1981). In Taiwan, Tsing et al(1981) determined 31 serotypes from 227 strains of Salmonella and indicated that S.derby was the most frequent. Allos(1978) studied a total of 13,409 specimens between 1973 and 1976 in Iraq and found that prevalence of Salmonella was 10.85%). In India, an outbreak of food-poisoning affecting 62 personnel following lunch in a community mess was caused by a rare Salmonella serotype(Gupta et al,1980).

In Mendoza,Argentina Curi de Montburn et al(1981) described 6 outbreaks and 59 sporadic cases occurring between 1972 and 1976. In both cases, S.typhimurium was found to be the predominant isolate. Koplan et al(1978) reported that a church gorup attending a rural camp developed gastrointestinal illness caused by S.arechevalta. Roof-collected rain water, contaminated by bird faeces, was the source of infection. In Peru, Gunn and Bullon-Loarte(1979) reported an extensive outbreak in which 545 students of the University of Trujillo were seriously affected by S.thompson.

4. Pathogenic mechanisms.

Salmonella infection in man is almost always acquired by ingestion of the microorganism. The incubation period varies between 8 and 72 hours and duration of illness is between 5 and 7 days(Plotkin et al,1979). The number of viable bacterial organisms which must be given to healthy adult males to produce

diarrhoea was determined to be 10^5(Dupont and Hornick,1973).

Strains of Salmonella typhimurium were studied by Gianella et al(1973) in the ligated rabbit loop model to determine the mechanism whereby bacteria, which invade the gastrointestinal mucosa, evoke fluid secretion and the observation suggested that mucosal invasion may be a necessary factor for intestinal fluid loss in salmonellosis. In rats Salmonella produces an ileocaecitis, while in primates diffuse colitis is seen in addition to ileitis. In monkeys, with experimentally induced diarrhoea due to S.typhimurium, morphological changes have been seen in the colonic and ileal mucosa but not in the jejunal mucosa; those with severe diarrhoea, however, exhibited fluid and electrolyte transport abnormalities in the jejunum, ileum and colon(WHO Scientific Working Group,1980a).

In other studies, Koupal, and Deibel(1975) characterized an enterotoxin factor isolated from culture of Salmonella enteritidis to possess various properties similar to both the heat-stable and heat-labile enterotoxin of E.coli.

S.typhimurium(and presumably other serotypes associated with diarrhoea) can elaborate enterotoxins which promote watery diarrhoes. Strains associated with diarrhoea affect both the large and small bowel but typically remain localized in the bowel wall. In contrast, S.typhi and S.paratyphi cause generalized systemic infection involving the entire reticulo-endothelial system and bacteraemia is a cardinal feature of enteric fevers(Levine,1979).

5. Susceptibility to antimicrobial agents.

Outbreaks of infection to drug-resistant organisms are an increasing problem in both the developing and the developed countries. Ill-advised treatment of uncomplicated human salmonellosis with antimicrobial agents, and the intensity of human exposure to antibiotics, undoubtedly play a role in the increase of antimicrobial resistance. Much of the increasing use of antibacterial drugs is unnecessary(Simmons and Stolley, 1974). In many occasions they are employed without any clinical or laboratory examination. Rational therapy is possible only if culture data are available. Antibiotics are also used inappropriately in viral infections, which do not require such treatment, and in the many diarrhoeal diseases in which they are ineffective. In Salmonella gastroenteritis these drugs prolong the period of excretion(Dixon,1965). There is often prophylactic application without a rational basis, as in mass chemoprophylaxis against cholera. Other factors, such as pressure exerted by patients and their relatives and promotion by the pharmaceutical industry, also contribute to the rapidly growing use of antibiotics(WHO,1978).

The indiscriminate use of antimicrobial agents and the general intensity of exposure of food-producing animals to antibiotics probably have comparable importance. Nearly half of all of the antibiotics produced in the US is used in animals and this is related to the risk of human infection with multi-drug resistance strains of Salmonella of animal origin(Bennett, 1980).

Considerable variation is found in the incidence of antibiotic resistance in Salmonellae in reports from different countries.

In their study of 754 Salmonella isolates from 46 states in the US, Ryder et al (1980) documented a continuing increase in antibiotic resistance. Resistance to one or more drugs had significantly increased within eight years (21% to 31%). The increase in frequency of resistance was significant for streptomycin, sulphonamides, ampicillin and kanamycin and the frequency of strains resistant to six or more antibiotics increased greatly (0.8% to 5%).

In Kenya Wamola and Mizra, tested (1981) 88 strains of S. typhimurium and 18 of S. typhi for their sensitivity to antimicrobial agents and most of the S. typhimurium strains were found to be resistant to ampicillin and sulphadiazine. There was also increased resistance to trimethoprim-sulphamethoxazole.

In Algeria, 91% of most Salmonella serotypes showed resistance to 3-7 antibiotics. However, all typhoid and paratyphoid bacilli, except one S. typhi bearing an R-plasmid to 6 antibiotics, were sensitive (Mered et al, 1977).

In a survey carried out in Hong Kong in 1973-1974, Im et al (1978) indicated that more than 90% of 105 S. johannesburg strains were resistant to ampicillin and trimethoprim-sulphamethoxazole; and over 60% were resistant to chloramphenicol, sixty percent were triply resistant to chloramphenicol, ampicillin and trimethoprim-sulphamethoxazole. In a later study in the same country, changing patterns of drug resistance were also described by Chau et al (1982). The resistance pattern

ampicillin/streptomycin/tetracycline/chloramphenicol/ kanamycin/sulphadiazine, predominant between 1973 and 1975, was changed to ampicillin/streptomycin/chloramphenicol/ kanamycin/sulphadiazine during 1976-1979. These patterns were found to be plasmid-mediated.

In Italy, Bassetti et al(1978) reported that of 52 Salmonella strains, 56% were resistant to chloramphenicol, 64% to kanamycin, 52% to trimethoprim-sulphamethoxazole, 89% to ampicillin, 89% to streptomycin and 95% to carbenicillin. Later, in 1979, Gaffodio et al found that 83 out of 100 strains of Salmonella were resistant to one or more antibiotics and 57 were multi-resistantewith R factors.

In Canada, Grant and DiMambro(1977) reported that 12.4% of 589 human strains of Salmonella were resistant to chloramphenicol and ampicillin and detected plasmids with resistance patterns of chloramphenicol/kanamycin/streptomycin/tetracycline or chloramphenicol/tetracycline.

In Thailand, Leksomboon et al(1981) reported that 40% of Salmonella isolates were resistant to more than one antibiotic.

In India, Panhotra et al(1980) isolated 111 strains of S.typhimurium and found 98% resistance to streptomycin, 97% to chloramphenicol, 96% to tetracycline, 97% to ampicillin, 97% to kanamycin, 99% to sulphadiazine and 96% to trimethoprim-sulphamethoxazole, and all but one showed multiple drug-resistant resistance. In a different area of the same country, Koshi (1981) reported an alarming increase in multi-drug resistance from 38.9% in 1978 to 83% in 1977 and 95.5% in 1980.

In an eleven-year study of drug-resistance in Salmonella in the Netherlands, Manteen et al(1971) showed that 40% of S.typhimurium were resistant to tetracycline. Chloramphenicol resistance remained very low and ampicillin resistance decreased.

B. Shigella

1. Taxonomy.

1.1. General characteristics.

The genus Shigella is composed of Gram-negative non-motile bacteria that conform to the definition of the family Enterobacteriaceae and the tribe Escherichieae. With the exception of certain biotypes of Shigella flexneri 6, visible gas is not formed from fermentable carbohydrates. Compared with Escherichia, Shigella are less active in their utilization of carbohydrates. Salicin, adonitol, and inositol are not fermented. Strains of Shigella sonnei ferment lactose upon extended incubation, but other species do not utilize this substrate in conventional media. Lysine is not decarboxylated, the majority of strains do not possess a demonstrable arginine dihydrolase system, and ornithine is decarboxylated only by Sh.sonnei and Sh.boydii 13(Edwards and Ewing,1972).

1.2. Serogroups and serotypes

The genus Shigella is divided into 4 serogroups namely; Sh.dysenteriae(A), Sh.flexneri(B), Sh.boydii(C) and Sh.sonnei (D). There are 10 serotypes within Sh.dysenteriae, 6 within Sh.flexneri, 15 within Sh.boydii and only one within Sh.sonnei.

2. Nutritional and other growth requirements.

Shigella are aerobic or facultatively anaerobic, and their optimum temperature for growth is 37^{O}C. They will grow on ordinary nutrient (beef extract) media. In synthetic media, nicotinic acid is apparently required by some strains (Freeman, 1979).

Shigella can be isolated and grow on the same types of primary media used for Salmonella(Edwards and Ewing 1972).

3. Epidemiology of Shigella infections.

3.1. Sources of infection.

Infections caused by Shigella are probably far more common than are generally recognized. Attacks of severe illness grade off into mild and almost trivial attacks of simple diarrhoea. Probably, the most important single reservoir of infection is the human carrier, either convalescent or with an inapparent infection. The spread of the disease is due to the more or less direct transfer of the specific Shigella strain from infected intestinal discharges to the alimentary tract of another individual. Polluted water can be the medium of transmission (Hughes et al,1975;Weissman et al,1976; Katzenelson et al, 1976). Improper disposal of excreta, permitting dissemination by flies (Bidawid and Edeson,1978) and the contamination of food by chronic carriers and convalescents(Donaldo and Gangarosa, 1969) appear to be the most important factors in the spread of bacillary dysentery. Shigella was isolated from lettuce-leaves sold in local markets in Malaysia. The lettuce was grown in areas where night soil was habitually used(Chapman,1980). In Nigeria, isolation of Shigella from snails was reported(Obi and Nzeako,1980). Recently cases of shigellosis were found to be common in San Fransisco in people who adapted an "alternative life style" and practiced orogenital and oral-anal sexual contact(Dritz et al,1977) and in the United States among sexually transmitted diseases considered as special

problems are those caused by Shigella(Holmes and Puziss,1980).

3.2. Incidence of infection in adults.

In less-developed areas of the world, shigellosis has its peak attack rate in toddlers and pre-school children. It is believed that repeated clinical and sub-clinical infections in childhood make indigenous adults resistant with the prevalent endemic strain. Introduction of a new serotype, however, in an ecology where personal hygiene is sub-optimal due to lack of water, poor sanitation and no health education, can result in high attack rates in adults as well as children(Levine,1979). This was seen in Central America(Gangarosa et al,1970) when Sh.dysenteriae 1(the shiga bacillus)was introduced into susceptible populations. Recently, between 1975 and 1976, there was an epidemic incidence of shiga dysentery in Mogadishu(Nuti et al,1977).

There are various reports on Shigella isolates from humans. Wamola(1980) reported that Shigella species were the commonest stool pathogens(51-54%) in Kenya between 1975 and 1979. In Algeria, between 1974 and 1977, 847 strains of Shigella were examined and a high frequency of Sh.flexneri was noted (Zoukh and Poittevin,1978).

In south Vietnam, Ai.et al(1975) isolated 981 Shigella strains from 13,943 diarrhoeal patients during the period 1969-1973. In Hanoi, Vietnam, 300 Shigella strains, predominantly Sh.flexneri, were isolated during the period of 1974-1976(Ngu,1977). In Malaysia, Kan and Chan(1980)

isolated 241 Shigella strains from 19,883 cases of diarrhoea from 1974 to 1978. In a study of Vietnamese refugees in Hong Kong, Anton et al(1981) isolated 1.3% Shigella from 228 cases. In Bangladesh, out of 3,550 patients examined between December, 1979 and November, 1980, 11.6% of the isolates were Shigella (Stoll et al,1982). In Ethiopia, Afeworki Gebreyohannes and Yetnebersh Limeneh(1980) reported an isolation of 165 Shigella strains between 1974 and 1978. Messele Gedebou and Alebachew Tassew(1982) also isolated 105 strains of Shigella between 1975-1980. In both reports the frequency of Sh.flexneri was the highest, followed by Sh.dysenteriae, Sh.boydii and Sh.sonnei.

4. Pathogenic mechanisms.

Following ingestion of Shigella, the organisms reach the intestinal lumen where they proliferate. The fundamental virulence characteristic of Shigella is its ability to invade epithelial cells and subsequently multiply therein(Plotkin et al, 1979). After invasion of the mucosa, the proliferating Shigellae elaborate an enterotoxin which is believed to have some role in initiation of diarrhoea.

The predominant site of mucosal invasion and pathology include the terminal ileum and the length of the colon. Shigellae, multiplying in the intestinal mucosa and lamina propria, stimulate a striking infiltration of polymorphonuclear leucocytes into the areas of infected bowel. Death of colonic mucosal cells results in micro-ulcers through which red blood cells and plasma proteins leak into the bowel lumen. The colonal mucosa at this time is swollen, covered with mucus and

marked by haemorrhages(Levine,1979).

The incubation period in acute onset of diarrhoea is 1-3 days and duration of illness is between 2 and 20 days (Plotkin et al,1979), and according to DuPont and Hornick(1973) the infective dose of this pathogen is 10^1-10^2.

5. Susceptibility to antimicrobial agents.

In many countries a high incidence of antibiotic resistance has been observed in Shigella. Ninety percent of strains of Sh.sonnei in the United Kingdom were resistant to ampicillin (Davis,1970). In 1973,48.54% of strains of the same species were resistant to tetracycline and streptomycin in New York City(Neu et al,1975).

In India, streptomycin resistance was found in 29.3% of Shigella strains(Rao et al,1979). In South Vietnam, however, streptomycin was one of the most effective antibiotics, although the Shigella isolates were highly resistant to tetracycline and chloramphenicol (Ai et al,1975; Mutanda et al,1981). In Hanoi, Vietnam, a high incidence of resistance to ampicillin and chloramphenicol was detected(Ngu,1977).

Shigellae are generally resistant to most commonly-used antibiotics and are also known to develop multiple drug-resistance. Multiple resistance is believed to be received from E.coli already present in the patient's intestinal tract. In vitro demonstrations of transfer of resistance between E.coli and Shigella under conditions that allow cell contact were documented by various workers(Mitsuhashi,1969; Watanabe,1963; Watson,1967).

Watanabe(1963) and Mitsuhashi(1969) found that this type of multiple drug-resistance was mediated by cytoplasmic DNA particles, which replicated autonomously and more rapidly than host chromosomes. Plasmids code for a variety of factors and are by far the major sources of acquired antibiotic resistance in Enterobacteriaceae(WHO,1978).

Multiple drug resistance was first noted in Japan in the early 1950's, and by the end of 1967, about 30% of Shigella strains isolated in Japan were resistant to two or more drugs (Falkow,1975).

In 1968-1970, an epidemic strain of Sh.dysenteriae 1 showed multiple drug-resistance to tetracycline, chloramphenicol, streptomycin and sulphonamides in Central America(Gangerosa et al, 1970). An additional resistance to ampicillin was later reported from Mexico City in 1972(Olarte et al,1976) and from Bangladesh in 1974(Rahaman et al,1974). During 1973-1976, Shigella strains isolated in Somalia showed multiple drug resistance to as many as 7 drugs (Mero,1976). Multiple resistance to 5 drugs and R-factors were also reported in India(Kaliyugaperunal et al,1978; Paniker et al,1978) and increase in multiresistant Shigellae was noted in South Vietnam(Ai et al,1975).

In Ethiopia, multiple drug-resistance and R-factors were reported by Messele Gedebou and Alebachew Tassew(1979) and a comparative drug resistance within Shigella strains by Afeworki Gebreyohannes and Yetneberesh Limeneh(1980) and Messele Gedebou and Alebachew Tassew(1982).

C. Yersinia enterocolitica.

1. Taxonomy.

1.1. General characteristics.

The genus Yersinia is currently placed in the family Enterobacteriaceae and comprises three species, namely Y.pestis, Y.pseudstuberculosis and Y.enterocolitica(Mollaret and Thal,1974). Yersinia enterocolitica is a Gram-negative rod and facultatively anaerobic. I_t is motile when grown at 22-25^{O}C but non-motile at temperature above 30^{O}C. N_o gas and H_2s are produced. Urease is positive but phenylalanine is negative. Oxidase, lysine decarboxylase, arginine dihydrolase, esculin and citrate(Simmons') are negative. Indol is variable. Catalase, methyl red and ornithine decarboxylase are positive. Carbohydrates are attacked fermentatively. Y.enterocolitica ferments glucose, galactose, mannitol, sucrose, sorbitol and cellobiose. It does not ferment rhamnose, adonitol, dulcitol, raffinose and lactose.

1.2. Serogroups and serotypes.

The serological classification of Y.enterocolitica is presently extended to 23 serogroups and 31 serotypes based on their O(somatic)and H(flagellar)antigens(Swaminathan et al,1982).

2. Nutritional and other growth requirements.

Yersinia enterocolitica is not a nutritionally fastidious organism when grown at 28^{O}C , but may require thiamine, alanine, methionine, cysteine, glutamic acid, pantothenic acid and niacin (Burrows and Gillette,1966). The p^H range for survival and growth of Y.enterocolitica appears to be 4.6-9.0, with an optimum range of 7.0-8.0(Swaminathan et al,1982). The growth temperature

range of this microorganism in nutrient broth is 1-40^{0}C (Sutherland and Varnam,1977).

Cultural methods for the isolation of Y.enterocolitica from clinical and environmental specimens parallel those commonly used for Salmonella and Shigella ie.enrichment in liquid media, isolation of pure culture on plating media, and identification of isolates by biochemical and serological tests. Since colonies of Y.enterocolitica on conventional enteric media tend to be very small after 24-48 hours,they may be easily overlooked, particularly in the persence of large numbers of other enteric bacteria. However, Schiemann(1979)formulated a new agar medium, cefsulodin-irgasan- -novbiocin(Cin)agar, and this was reported to be by far the best medium for the recovery of Y.enterocolitica(Head et al,1982).

3. Epidemiology of Y.enterocolitica infection.

3.1. Sources of infection

It appears that the animal kingdom is a significant reservoir of Y.enterocolitica(Swaminathan et al,1982). The swine has been implicated as a major reservoir of Y.enterocolitica serotypes involved in human infections. Several investigators have isolated Y.enterocolitica from pigs(Toma and Deidrick, 1975; Pedersen,1976). Dogs have also been incriminated as potential reservoirs(Pedersen and Winbald,1979; Wilson et al, 1976). Isolation of Y.enterocolitica from small rodents has also been reported(Kapperud,1975,1977). Other animals from which strains of Y.enterocolitica have been isolated include cows,horses,sheep and monkeys(Swaminathan et al,1982;

Nilehn,1969).

Several investigators noted that water may be a reservoir for Y.enterocolitica and that infection due to Y.enterocolitica may be transmitted by consumption of contaminated water(Toma and Deidrick, 1975;Kapperud,1977;Capriolo et al,1978; Schiemann,1978). Y.enterocolitica has been isolated from raw milk(Hughes,1979) and from pasteurized milk(Hughes,1980). Swaminathan et al(1982) cited various workers who isolated Y.enterocolitica from chicken,pork and beef samples.

3.2. Incidence of Y.enterocolitica infections in adults.

Y.enterocolitica appears to be widely distributed in the environment and human infections due to the organism have been reported in several countries, spreading over several continents (Swaminathan et al,1982).

Y.enterocolitica is mostly isolated from infants and children and reports on adult yersiniosis are scarce.

Y.enterocolitica was first isolated from humans in Czechoslavakia in 1963(Rakovsky 1973 cited by Swaminathan et al,1982). The frequency of isolation of Y.enterocolitica from persons suffering from intestinal diseases in the southern region of west Germany was 1.5% and was second only to the incidence of Salmonella(Swaminathan et al,1982). In Canada, Caprioli et al(1978) observed that the frequency of isolation of Y.enterocolitica serotypes other than O:3 was high in adults. Martin et al(1982) reported a family outbreak of yersiniosis in Canada, where a mother and her two children sustained diarrhoea. Weissfeld and Sonnenwirth(1980) noted that incidence

of yersionosis in adult patients equalled the incidence of salmonellosis in USA. Sanadi et al(1982) reported that an attempt was made to isolate this pathogen from diarrhoeal patients in Bangladesh but no Y.enterocolitica was isolated and they believed that the pathogen is not important in tropical countries.

4. Pathogenic mechanisms of Y.enterocolitica

Y.enterocolitica causes acute gastroenteritis or enterocolitis manifested most commonly by diarrhoea,nausea, vomiting, and fever, and also causes an acute syndrome resembling appendicitis(Arvastson et al,1971). In mice infected intravenously with Y.enterocolitica, Carter(1975) observed a systemic, pyogenic infection involving the spleen, the liver and the lungs. For humans, the infective dose of Y.enterocolitica is 2.9×10^8 bacteria(Pai et al,1980).

Production of a heat-stable enterotoxin by Y.enterocolitica has been demonstrated by several investigators(Pai and Mors, 1978; Pai et al,1978). This enterotoxin is resistant to treatment at $121^{0}C$ for 30 minutes, or storage at $4^{0}C$ for 7 months and is stable in the P^{H} range 1-11(Boyce et al,1979). Thus, the enterotoxin of Y.enterocolitica may survive normal food processing and storage operations and the acid p^{H} of the stomach and it is conceivable that illness may occur as a result of consumption of preformed enterotoxin.

III. M A T E R I A L S A N D M E T H O D S

1. Collection of specimens.

Stool specimens were collected in sterile containers from diarrhoeal out-patients between November, 1982 and March, 1983. The specimens from various hospitals and clinics were collected daily using a "Cary and Blair" semi-solid transport menstrum. This menstrum was inoculated with the specimen collected on a buffer-treated swab, and the swab was left in the tube. The transport medium was designed to hold the bacterial population in the specimen more or less stationary, and to prevent, as much as possible, overgrowth of any particular microorganism (Edwards and Ewing, 1972).

2. Culture media.

'Oxoid' dehydrated powders were used to prepare MacConkey, Shigella-Salmonella(SS) and Brilliant green(BG)agar plates and selenite enrichment broth for primary inoculation. Similarly, the following were prepared for the biochemical tests: nutrient broth, triple sugar iron(TSI) agar slants, Lysine iron(LI)agar slants, urea agar slants, Simmons' citrate agar slants, sulphide-indol-motility(SIM) media, mannitol broth(1%), glucose broth(1%), nutrient agar slants, tryptic soy yeast(TSY)broth and Mueller-Hinton plates.

3. Antisera

'Difco' polyvalent and group antisera were employed for confirmation and serogrouping the biochemically-identified Salmonella. For Shigella, only group antisera were used.

4. Antimicrobial agents

BBL disks of 11 antimicrobial agents were used in the

following concentrations: ampicillin(Amp), 10ug; carbenicillin (Car),100 ug;cephalothin(Cep),30 ug: chloramphenicol(Chl),10ug; gentamicin(Gen),10 ug; kanamycin(K_{an}),30 ug;polymixin B(Pol), 300 units; streptomycin(Str),10 ug; sulphadiazine(Sul), 1mg; tetracycline(Tet),30 ug; trimethoprim-sulphamethoxazole(Tri-Sul),25 ug.

5. Isolation

The specimens were brought to the laboratory and plated on three primary media. MacConkey,SS and BG agar plates were inoculated with the swab and streaked using a sterile loop. The swab was then dipped and left in the selenite enrichment broth. All the inoculated plates and the enrichment broth were incubated at 35-37^{o}C for 24 hours. The MacConkey and SS plates were examined for non-lactose fermenting colonies while the BG agar plates for red and non-mucoid colonies. Using a sterile straight wire, a single colony of either type was picked and inoculated in about 3 ml of nutrient broth. This was incubated at 35-37^{o}C for 2 to 4 hours until growth was ascertained by turbidity. Mac and SS plates, on which small non-lactose fermenting colonies appeared were further incubated at 28^{o}C for 24 hours. The colonies were then tested biochemically for Y.enterocolitica as in Swaminathan et al(1982).

6. Biochemical identification.

Using a sterile, straight wire, the broth culture was used to inoculate the following set of biochemical tubes;

a) TSI agar: the butt was stabbed and the slant streaked. This medium was used to determine the fermentation of glucose,

lactose and sucrose as well as the production of hydrogen sulphide.

b) LI agar slant: the butt was stabbed and the slant streaked. This was used to detect the oxidative deamination of lysine on the slant and decarboxylation of lysine in the butt.

c) Urea agar slant:only slant was streaked to determine hydrolysis of urea.

d) Simmon's citrate agar slant: only the slant was streaked to check the utilization of citrate as a sole carbon source.

e)SIM medium: this medium was stabbed for possible detection of hydrogen sulphide production and motility. Production of indol was also detected by adding Ehrlich's reagent on growth on the same medium.

f) Mannitol broth(1%) was inoculated to determine fermentation of mannitol.

g) Glucose broth(1%) with inverted Durham tube was inoculated to detect fermentation of, and gas production, from glucose. All biochemical tubes were incubated at 35-37^{o}C for 18-24 hours.

To check the purity of the broth inocula, and thus reliability of biochemical tests, each broth inoculum was sub-cultured on MacConkey"check plates" and incubated at 35-37^{o}C for 18-24 hours. Cultural purity was visually confirmed.

As quality control for sterility of media for the biochemical tests, all tubes of media were pre-incubated at 35-37^{o}C for 18-24 hours.

The selenite enrichment broth cultures were streaked on the same type of agar media used for primary isolation and

incubated at 35-37°C for 18-24 hours. The type of colonies indicated above were similarly processed for biochemical characterization.

7. Serogrouping.

Isolates that met the minimal biochemical profile for Salmonella or Shigella were serogrouped by slide agglutination method as in Edwards and Ewing(1972), using antisera from ' 'Difco'. A drop of physiological saline solution was put on a clear sterile microscope slide. Culture from TSI agar slant was taken using a sterile loop and suspended in the saline solution. A drop of antiserum was put on the suspension and the slide was tilted back and forth and examined for possible agglutination. Isolates which agglutinated with Salmonella or Shigella antisera were inoculated on nutrient agar slant, incubated at 35-37°C for 18-24 hours and stored at 4°C until antimicrobial susceptibility testing was done.

8. Antimicrobial susceptibility testing

Susceptibility testing of all strains identified as Shigella or Salmonella was carried using the standardized agar disk diffusion technique(Bauer et al,1966).

Each isolate was sub-cultured on a plate of MacConkey agar and incubated at 35-37°C for 18-24 hours. From a pure culture of each isolate, 4 to 5 colonies were randomly selected and transferred to a tube containing 4 ml of TSY broth. The broth culture was standardized as in the procedure and, using a sterile cotton swab, the entire surface of a Mueller-Hinton agar plate was swabbed evenly with the broth culture. The

inoculated plate was left at room temperature for 3 to 5 minutes. With the aid of an automatic dispenser(BBL), the set of 11 sensitivity disks was placed on the surface of each Mueller-Hinton plate, and the disks were gently pressed by sterile forceps to ensure firm contact. The plates were then incubated at 35-37°C for 18-24 hours. A standard reference strain of Escherichia coli(ATCC 25922), sensitive to all the antimicrobial drugs used in this study, was routinely tested as a control. Finally, diameters of inhibition zones were measured in milimeters using a caliper. Interpretation of the measurements as sensitive, intermediate, and resistant was made according to a standard interpretative chart(Matsen and Barry,1974). The very few' intermediate' readings were considered as sensitive for a purpose of assesment of the data.

IV. R E S U L T S

1. Infection rates

One thousand diarrhoeal specimens from adult out-patients were collected from various hospitals and clinics in Addis Ababa: 253 specimens from Menelik II Hospital, 60 from Arada Clinic, 260 from Teklehaimanot Clinic, 102 from Central Command Hospital, 298 from Tikur Anbessa Hospital and 27 from the University Clinic at the Science Faculty(Table 1).

A total of one hundred thirty five strains of *Shigella* and *Salmonella* were isolated from the specimens. *Shigella* were the aetiologic agents for 9% and *Salmonella* for 4.5% of the diarrhoeal cases. No *Yersinia enterocolitica* was isolated. As shown in Table 1, *Salmonella* and *Shigella* were isolated at different rates from the various hospitals and clinics.

2. Serogroups.

Among the *Salmonella* strains, the most commonly isolated ones were those of Group C(37.8%) followed by Group B(28.9%), *Salmonella typhi*(20%) and Group D(other than *S.typhi*) (8.9%). Groups A and E had a similar prevalence(2.2%).

Serogrouping of the ninety *Shigella* isolates showed that *Sh.flexneri* was the most commonly isolated species(63.3%) followed by *Sh.dysenteriae*(18.9%), *Sh.boydii*(10%) and *Sh.sonnei*(7.8%).

3. Susceptibility to antimicrobial agents.

3.1 Salmonella

The sensitivity of all forty-five isolates of *Salmonella* is presented in Table 2. All strains were sensitive to only

three antimicrobial agents: gentamicin, polymyxin B and trimethoprim-sulphamethoxazole. Sensitivity to the remaining eight drugs was fairly high and varied between 73.3% and 82.2%. However, the frequency of sensitivity to the drugs varied for the five serogroups. The single isolates of Group A and E and all S.typhi were sensitive to the eleven drugs tested. The sensitivity of Group B was higher than 85%. All Group D isolates were sensitive to all except cephalothin(66.7%). The majority of Group C strains were not sensitive to ampicillin, carbenicillin, cephalothin, chloramphenicol, streptomycin, sulphadiazine and tetracycline. Only 53% were sensitive to kanamycin. A total of 31 strains(68.9%) were sensitive to all 11 drugs used in this study: 11 strains of Group C(35.3%), 2 strains of Group D(75%) and all the remaining strains of Group A and E and S. typhi.

Resistance of Salmonella isolates to one or more drugs is shown in Table 3. Resistance to one or more drugs was detected in 15.4% of Group B strains, 64.7% of Group C strains, and 25% of Group D strain. About 31% of all Salmonella strains were resistant to one or more drugs. The most frequent resistance was to eight drugs(17.8%), followed by to one and two drugs(4.4% each) and to three and seven drugs(2.2%each). Resistance to one or more drugs was common in Group C strains, the most frequent being to eight drugs(47%).

Multiple drug-resistance(resistance to 2 or more drugs) was detected in Group B strains(7.8%) and Group C strains (64.7%). Of the forty-five Salmonella strains, 26.7% were

multiply-resistant.

There were two resistance patterns in Group B strains, four in Group C and one in Group D(Table 4). In all, seven different patterns of resistance were noted. The commonest pattern(47%) was seen in Group C strains and this was simultaneous resistance to ampicillin, carbenicillin, cephalothin, chloramphenicol, kanamycin, streptomycin, sulphadiazine and tetracycline.

Table 1

Frequency of isolation of Salmonella and Shigella from adult out-patients in the various hospitals and clinics.

Hospital or Clinic	Number of specimens	Salmonella		Shigella		Both genera.	
		Number of isolates	%	Number of isolates	%	Number of isolates	%
Menelik II H.	253	10	4	14	5.5	24	9.5
Arada C.	60	1	1.6	7	11.7	8	13.33
Teklehaimanot C.	260	14	5.4	2.8	10.8	42	16.2
Central Command H.	102	1	1	14	13.7	15	14.7
Tikur Anbessa H.	298	18	6	25	8.4	43	14.4
University C.	27	1	3.7	2	7.4	3	11.1
All hospitals and clinics	1000	45	4.5	90	9.0	135	13.5

Table 2

Sensitivity of 45 strains of Salmonella to antimicrobial agents

Salmonella serogroups	Number of strains	Percent. susceptibile to										
		Amp	Car	Cep	Chl	Gen	Kan	Pol	Str	Sul	Tet	Tri-sul
Group A	1	100	100	100	100	100	100	100	100	100	100	100
Group B	13	100	100	100	100	100	100	100	92.3	84.6	100	100
Group C	17	35.3	47	35.3	47	100	53	100	47	47	41.2	100
Group D (other than S.typhi)	4	100	100	66.7	100	100	100	100	100	100	100	100
S.typhi	9	100	100	100	100	100	100	100	100	100	100	100
Group E	1	100	100	100	100	100	100	100	100	100	100	100
All species	45	75.6	80	73.3	80	100	82.2	100	77.8	75.6	77.8	100

Table 3

Resistance to one or more drugs and multiple-resistance of *Salmonella* isolates

Resistance to:	Percent resistance						
	Group A (1 strain	Group B (13 strains	Group C (17 strains	Group D (4 strains	S. typhi (9 strains	Group E (1	All species (45 strains)
one drug only	0	7.8	0	25	0	0	4.4
two drugs only	0	7.8	5.9	0	0	0	4.4
three drugs only	0	0	5.9	0	0	0	2.2
four drugs only	0	0	0	0	0	0	0
five drugs only	0	0	0	0	0	0	0
six drugs only	0	0	0	0	0	0	0
seven drugs only	0	0	5.9	0	0	0	2.2
eight drugs only	0	0	47	0	0	0	17.8
one or more drugs	0	15.6	64.7	25	0	0	31.1
two or more drugs	0	7.8	64.7	0	0	0	26.7

Table 4

Salmonella patterns of resistance to antimicrobial agents

Serogroup	Resistance pattern	Number of strains	%
Group B	Sul	1	7.8
(3 strains)	Str/Sul	1	7.8
Group C	Amp/Cep	1	5.9
(17 strains)	Amp/Cep/Tet	1	5.9
	Amp/Car/Cep/Chl/Str/Sul/Tet	1	5.9
	Amp/Car/Cep/Chl/Kan/Str/Sul/Tet	8	47
Group D (other than S.typhi) (4 strains)	Cep	1	25

3.2. Shigella

The sensitivity of all ninety Shigella isolates is shown in Table 5. All the strains were sensitive to kanamycin, gentamicin, polymixin B and trimethoprim-sulphamethoxazole. Most(95.6%) were sensitive to cephalothin. Sensitivity to ampicillin, chloramphenicol, streptomycin, sulphadiazine and tetracycline varied between 31.1% and 57.7%. Ampicillin, carbenicillin, streptomycin, sulphadiazine and tetracycline were mostly ineffective(<50%), while only 57.7% of the strains were sensitive to chloramphenicol. The frequency of sensitivity to ampicillin, chloramphenicol, carbenicillin, sulphadiazine and tetracycline varied for the four species. The sensitivity of Sh.flexneri to ampicillin, carbenicillin, streptomycin, sulphadiazine and tetracycline was less than 39%. Below 35% of Sh.dysenteriae were sensitive to chloramphenicol, streptomycin, sulphadiazine and tetracycline. The very low sensitivity of Sh.dysenteriae to chloramphenicol(23.5%)and of Sh.flexneri to tetracycline(21.1%) are worth noting. Only 15 strains(16.7%) were sensitive to all 11 drugs used in the investigation: 4 strains each of Sh.dysenteriae and Sh.flexneri, 3 strains of Sh.boydii and 2 strains of Sh.sonnei.

Resistance to one or more drugs in Shigella isolates is shown in Table 6. Resistance to one or more drugs was detected in 70.6% of Sh.dysenteriae, 93% of Sh.flexneri, 44.4% of Sh.boydii and 71.4% of Sh.sonnei. About 83% of all Shigella serogroups were resistant to one or more drugs. The most frequent resistance was to six drugs (27.5%) followed by

resistance to five drugs(15.3%), two drugs(13.2%)and four drugs (11.1%). Resistance to one drug and threedrugs were 8.8% and 7.7% respectively. Such resistance and frequencies varied for the different serogroups. For Sh.dysenteriae resistance to six drugs was 35.3% followed by resistance to four drugs(23.5) and to five drugs(11.8%). For Sh.flexneri the most frequent resistance was to six drugs(31.5%) followed by resistance to five drugs(19.3%) and two drugs(14%).

Multiple drug resistance was detected in Sh.dysenteriae (70.6%), Sh.flexneri(82.4%), Sh.boydii(33.3%) and Sh.sonnei (57.1%). Of the ninety Shigella strains, 74.4% were multiply-resistant.

Eighteen different patterns of resistance were observed in all: six in Sh.dysenteriae, eleven in Sh.flexneri, four in Sh.boydii and three in Sh.sonnei(Table 7). The commonest pattern for Sh.dysenteriae was Amp/Car/Chl/Str/Sul/Tet(29%). This same pattern was also the commonest for Sh.flexneri(31%).

Table 5

Sensitivity of 90 strains of Shigella to antimicrobial agents

Species	Number of strains	Percent sensitive to										
		Amp	Car	Cep	Chl	Gen	Kan	Pol	Str	Sul	Tet	Tri-sul
Sh.dysenteriae	17	52.9	52.9	94.1	23.5	100	100	100	39.4	29.4	29.4	100
Sh.flexneri	57	35.1	38.6	93.0	61.4	100	100	100	33.3	28.1	21.1	100
Sh.boydii	9	77.8	77.8	100	77.8	100	100	100	55.6	77.8	77.8	100
Sh.sonnei	7	100	100	85.7	100	100	100	100	42.9	42.2	71.4	100
All species	90	47.7	50	95.6	57.7	100	100	100	36.7	34.4	31.1	100

Table 6

Resistance to one or more drugs and multiple-resistance of *Shigella* isolates

Resistant to:	Percent resistance				
	Sh.dysenteriae (17 strains)	Sh.flexneri (57 strains	Sh.boydii (9 strains	Sh.sonnei (7 strains	All species
one drug only	0	10.5	11.1	14.3	8.8
two drugs only	0	14	11.1	28.6	13.2
three drugs only	5.9	7	0	28.6	7.7
four drugs only	23.5	10.5	0	0	11.1
five drugs only	11.8	19.3	11.1	0	15.3
six drugs only	35.3	31.5	11.1	0	27.5
one or more drugs	70.6	93	44.4	71.4	82.5
two or more drugs	70.6	82.4	33.3	57.1	74.4

Table 7

Shigella patterns of resistance to antimicrobial agents

Serogroup	Resistant pattern	Number of strains	%
	Chl/Sul/Tet	1	6
	Chl/Str/Sul/Tet	4	23
Sh.dysen-	Amp/Car/Chl/Str/Tet	1	6
teriae	Amp/Car/Chl/Str/Sul	1	6
(17strains	Amp/Car/Chl/Str/Sul/Tet	5	29
	Amp/Car/Cep/Chl/Sul/Tet	1	6
	Chl	1	2
	Sul	4	7
	Tet	1	2
	Amp/Cep	2	3
Sh.flexneri	Sul/Tet	6	10
(57strains)	Str/Sul/Tet	4	7
	Amp/Car/Str/Tet	6	10
	Amp/Car/Chl/Str/Tet	2	3
	Amp/Car/Str/Sul/Tet	8	4
	Amp/Car/Chl/Sul/Tet	1	2
	Amp/Car/Chl/Str/Sul/Tet	18	31
	Str	1	11
Sh.boydii	Str/Sul	1	11
	Amp/Car/Chl/Str/Tet	1	11
(9 strains	Amp/Car/Chl/Str/Sul/Tet	1	11
	Cep	1	14
Sh.sonnei	Str/Sul	2	29
(7 strains	Str/Sul/Tet	2	29

V. D I S C U S S I O N .

The 4.5% and 9% frequency of isolation of Salmonella and Shigella respectively, in this study indicated the aetiologic frequency of these two species in diarrhoeal out-patients attending various hospitals in Addis Ababa.

The frequency of isolation of Salmonella in this study(4.5%) was much lower than the 10.8% reported by Allos(1978) from Iraq and higher than that(1%) reported by Wallace(1976) from Ethiopia.

The 9% frequency of isolation of Shigella in this study was higher than the 7% isolation of Ai et al(1975) from South Vietnam and 1.2% isolation from Malaysia(Kan and Chan,1980) but lower than the 11.6% isolation of Stoll et al(1982) from Bangladesh and 11% of Wallace (1976) from Ethiopia.

The finding, in this study, of Group C Salmonella as the most frequent of the Salmonella serogroups is in contrast to reports from USA(Ryder et al,1980), Kenya(Wamola,1980) and Iraq(Allos,1978). In Senegal(Lafaix et al,1978) and Algeria (Zoukh and Poittevin,1978), S.typhi was the predominant strain.

The 63.3% frequency of isolation of Sh.flexneri in this study was higher than that(49%) reported by Afeworki Gebreyohannes and Yetnebersh Limeneh(1980) but lower than that (70%) of Messele Gedebou and Alebachew Tassew(1982). The same species was, however, the most frequent in all the three studies from Ethiopia. The order of frequency of isolation of the different species of Shigella was also similar in the three studies, thus indicating the prevalence of this order of aetiologic

frequency in Addis Ababa during the study periods.

Sh.flexneri was also the predominant serogroup in Kenya, Tanzania and Uganda(Mutanda et al,1979), in Vietnam(Ngu,1977; Ai et al,1975), in India(Kaliyugaperunal et al,1978) and in Malaysia(Kan and Chan,1980). In contrast, Sh.sonnei, the least frequent in this study, was the predominant serogroup in the United States(Neu et al,1975; Byers et al,1976), Sweden(Urban, 1972), United Kingdom(WHO,1978) and in the far East(Aoki,1968). Sh.sonnei was also the second predominant serogroup in East Africa(Mutanda et al,1979), Algeria(Zoukh and Poittevin,1978) and Malaysia(Kan and Chan,1980).

The position of Sh.dysenteriae as the second most frequent isolate(18.9%) in this study was similar to that in Vietnam(Ngu, 1977) and India(Kaliyugaperunal et al,1978) but was in remarkable contrast to reports from the United States(Controni et al,1978 cited by Messele Gedebou and Alebachew Tassew,1982), Sweden(Urban,1972), Far East (Aoki,1968), East Africa(Mutanda et al,1979) and Malaysia(Kan and Chan,1980) where it was rarely, if ever, isolated. In Somalia(Mero, 1976;Nuti et al,1977)and parts of India(Macaden et al,1980), however, Sh.dysenteriae was the species most frequently isolated.

Shigella strains are generally sensitive to cephalothin, gentamicin,kanamycin. Polymyxin B and trimethoprim-sulphamethoxazole, as found in this study and reported by other workers here in Ethiopia(Afeworki Gebreyohannes and Yetnebersh Limeneh,1980; Messele Gedebou and Alebachew Tassew, 1982) and elsewhere(Panhotra et al,1980;Rao et al,1979;

Macaden et al,1980; Stoll et al,1982). The frequency of sensitivity to all the drugs in this study(16.7%) was much lower than that(30%) reported by Afeworki Gebreyohannes and Yetnebersh Limeneh(1980), whose strains were not all from Addis Ababa, and almost similar to that(15%) of Messele Gedebou and Alebachew Tassew(1982), who reported on isolates from Addis Ababa.

The frequency of resistance of the Shigella strains to one or more drugs(82.5%) was higher than that(80%) of strains from Korea(Chun and Seol,1978), United States(New York) (66%)(Neu et al,1975) and lower than that from Thailand,(100%) (Leksomboon et al,1981) and India(90.4%) (Kaliyugaperunal et al,1978).

This rate is almost similar to that(85%) of Messele Gedebou and Alebachew Tassew(1982) in isolates from Addis Ababa but much higher than that(70%) of Afeworki Gebreyohannes and Yetnebersh Limeneh (1980) in strains perhaps from various places in the country.

Of the 11 drugs tested, single resistance was found against chloramphenicol, sulphadiazine,tetracycline,streptomycin or cephalothin. This was in contrast to that of Messele Gedebou and Alebachew Tassew(1982), who reported single resistance only against streptomycin, sulphadiazine or tetracycline.. However, resistance to sulphadiazine was the most frequent(4.4%)in this study, as well as in that(10%) of Messele Gedebou and Alebachew Tassew(1982).

The resistance patterns of Shigella strains in this study were generally different from those of other reports(Neu et al,

1975; Byers et al,1976; Messele Gedebou and Alebachew Tassew, 1982). The most common resistance pattern was that of ampicillin/carbenicillin/chloramphenicol/streptomycin/ sulphadiazine/tetracycline(26.7%).

This resistance to six drugs, detected in 29% of Sh.dysenteriae, 31% of Sh.flexneri and 11% of Sh.boydii(Table 7) was similar(except the additional resistance to carbenicillin) to that of one of the strains of Sh.dysenteriae 1 involved in the 1972 dysentery outbreak in Mexico City(Olarte et al,1976).

The second common resistance pattern was ampicillin/ carbenicillin/streptomycin/sulphadiazine/tetracycline(8.9%). The two patterns accounted for 35.6% of the multiply drug-resistant Shigella. The commonest pattern reported by Messele Gedebou and Alebachew Tassew (1982) was streptomycin/ /sulphadiazine(23%). The resistance pattern found to be the commonest in this study was also reported by Messele Gedebou and Alebachew Tassew(1982), but in a much lower frequency(11%). The findings in this study, therefore, indicated that multiple drug-resistance, to five and six drugs, is on the increase.

The frequency of ampicillin resistance in this study(52.3%) was much higher than that(22%) reported by Afeworki Gebreyohannes and Yetnebersh Limeneh(1980) and that(21%) of Messele Gedebou and Alebachew Tassew(1982). This indicated a trend of increasing frequency of resistance to ampicillin in Addis Ababa. Such a trend was reported by various investigators(Byers et al,1976; Ngu,1977 Stoll et al,1982). Forty-six of the forty-seven ampicillin-resistant strains were also resistant to two or more other drugs.

The majority were resistant to three, four or five drugs; including carbenicillin, chloramphenicol, streptomycin, tetracycline, sulphadiazine and cephalothin (Table 7).

Resistance of Shigella to the commonly-used drugs is being reported from various parts of the world, indicating the need for a newer and more effective drug of choice for treatment of shigellosis (Messele Gedebou and Alebachew Tassew, 1982). The results in this study were similar to the findings of Messele Gedebou and Alebachew Tassew(1982), in that all, or nearly all, the Shigella isolates were susceptible to cephalothin, gentamicin, kanamycin and polymixin B. However, these drugs are considered clinically ineffective and hence cannot replace ampicillin as the drug of choice(Controni et al, 1978, cited by Messele Gedebou and Alebachew Tassew, 1982).

All Shigella strains in this study, and almost all reported by others, have been sensitive to trimethoprim-sulphamethoxazole (Byers et al, 1976; Macaden et al, 1980; Afeworki Gebreyohannes and Yetnebersh Limeneh, 1980; Messele Gedebou and Alebachew Tassew, 1982). Trimethoprim-sulphamethoxazole has also been clinically demonstrated to be more effective than ampicillin in the treatment of shigellosis (Chang et al, 1977; Nelson et al, 1976). The high frequency in vitro multiple drug resistance, but sensitivity to trimethoprim-sulphamethoxazole in this study supported the view of others(Messele Gedebou and Alebachew Tassew, 1982) that this drug should be the choice for treatment of shigellosis, particularly in regions with strains of high frequency of multiple drug resistance.

As in reports from several other countries(Bassetti et al, 1978, Wamola and Mizra,1981; Messele Gedebou and Alebachew Tassew, 1981) a high percentage of Salmonella strains in this study was susceptible to chloramphenicol, gentamicin,kanamycin, polymyxin B and trimethoprim-sulphamethoxazole. Despite the wide use of chloramphenicol for treatment of suspected salmonellosis in this country, there has not been any resistant strain of S,typhi in this study, or that reported by Messele Gedebou and Alebachew Tassew(1981). However, chloramphenicol resistance was detected here in Group C strains(53%) but in a much lower frequency than that(85%) reported by Messele Gedebou and Alebachew Tassew(1981).

The frequency of resistance of Salmonella strains to one or more antimicrobial agents(31.1%) in this study was much lower than that (79.4%) reported by Messele Gedebou and Alebachew Tassew (1981). A higher frequency of resistance to one or more drugs was also reported by Gaffodio et al(1979) from Italy, Panhotra et al(1980)from India, Im et al(1978) from Hong Kong and Mered et al(1977) from Algeria.

Out of the eleven drugs tested, single resistance was found only against sulphadiazine and cephalothin(2.2%) (Table 4). This was in contrast with Messele Gedebou and Alebachew Tassew (1981), where a higher percentage of resistance was reported to streptomycin and sulphadiazine(24.2%).

The frequency of multiple antimicrobial resistance of Salmonella strains in this study(26.7%) was much lower than the 47% by Gaffodio et al(1979) in Italy. 41.2% by Messele Gedebou

and Alebachew Tassew(1981) in Ethiopia, but higher than the 15% of Schroeder et al(1968)in the USA.

The resistance patterns of Salmonella strains in this study were generally different from those of other reports(Koshi, 1981; Messele Gedebou and Alebachew Tassew,1981). The most common resistance pattern of Group C strains, resistance to ampicillin, carbenicillin, cephalothin, chloramphenicol, kanamycin, streptomycin, sulphadiazine and tetracycline(17.8%), was also reported by Messele Gedebou and Alebachew Tassew(1981). However, their most common resistance pattern was streptomycin /sulphadiazine(24.2%) or sulphadiazine only (24.2%). Comparing the findings in the studies, one would note that the frequency of multiple drug-resistance in Group C strains has increased from 7.3% to 17.8%.

The attempt to isolate Y.enterocolitica was not successful in this study. A possible reason could be the limitation in the technique of isolation. As indicated earlier, all plates were routinely incubated at room temperature or at 28°C. Thus Y.enterocolitica which did not grow at 35-37°C could have been missed. Samadi et al(1982), using better isolation and enrichment techniques, had also attempted to isolate this pathogen from patients with various systems of yersiniosis and from animals believed to be natural reservoirs. They did not isolate any Y.enterocolitica.

RECOMMONDATIONS

Based on this study, the following recommendations are made.

1. Further more extensive investigations should be undertaken in order to determine the other cause of diarrhoea as well. Shigella and Salmonella obviously are not the only aetiological agents, as these organisms were not involved in about 86% of the cases. Thus other bacterial, viral and parasitic agents in diarrhoea, their seasonal prevalence and degree of importance at the different age groups should also be determined. The investigation on the prevalence of Y. enterocolitica should particularly be made using better enrichment and isolation techniques. Such studies should attempt to cover the different parts of the country.

2. Since the establishment of bacteriological laboratories in all hospitals and health centers at present seems unattainable, regional reference laboratories should be established at various places throughout the country. These laboratories should follow the patterns of aetiology of diarrhoeal disease, and the drug resistance patterns of the aetiological agents, in their respective regions and inform health personnel for the necessary measures.

3. Personal hygiene and environmental sanitation should be observed. Lack of these two factors is the main cause of diarrhoeal diseases in a developing country like Ethiopia. Personal hygiene and environmental sanitation can be attained by(a) giving appropriate health education through the various mass organizations,(b) arranging safe sewage-disposal systems

and (c) supplying easily available potable water to the population.

4. Diarrhoeal diseases in adults are generally self limited. Chemotherapy, therefore is not advisable, and treatment, when necessary, should be based on oral therapy(oral glucose-electrolyte-solution) at all ages(Cash,1979). The value of this treatment lies in its potential for wide availability, simplicity of use, safety,and low cost. If chemotherapy becomes indispensable, then culture data should be obtained before hand.

5. At present, the public has an easy access to all antibiotics and this has resulted in an indiscriminate use of these drugs. To control this drug abuse, a national policy for drug sale , where medical prescriptions are essential for the selling of drugs, should be enacted.

Afeworki Gebreyohannes and Tewfik Yusef,(1979). Non-cholera vibrio diarrhoea-a case presentation. Ethiop.Med.J. ,16:163-164.

Afeworki Gebreyohannes and Yetnebersh Limeneh.(1980). Multiple drug-resistance within Shigella serogroups. Ethiop.Med.J , 18:7-14.

Ai,N.V. , Hanh,N.D., Van,L.T., Le,N.V., and Ho,T.M.(1975). Bacteriological and epidemiological studies on Shigella infection in South Vietnam. Bull.Soc.Path.Exot., 68(3):262-266. Abstracted in Trop.Dis.Bull. , 73(4):293,1976.

Allos, G.(1978). List of Salmonella serotypes found in Iraq. Bull.Soc.Path.Exot;71(4/5):323-333. Abstracted in Trop.Dis.Bull. , 76(10):877,1979.

Anton,P. , Arnold, K., Truong, G.K. and Wong, W.T.(1981). Bacterial enteric pathogens in Vietnamese refugees in Hong Kong. Southeast Asian J.Trop.Med.Pub.Hlth. , 12(12):151-156.

Aoki , Y.(1968). Serological groups of Shigella in Japan and neighbouring countries: A review. Trop.Med., 10:116-126.

Arvastson, B. , Damgaard, K., and Winbald, S.(1971). Clinical symptoms of infection with Yersinia enterocolitica. Scand. J. Infect.Dis.,3:37-40.

Bassetti, D., Ciravegna, B., Navone, C., and Rosciolli, B.(1978). Gentamicin in the treatment of Salmonella infections. J.Int.Med.Res., 6:460-462.

Bauer, A.W., Kirby, W.M.M., Sherris,J.C. and Turk,M.(1966). Antibiotic susceptibility testing by a standardized single disk method. Amer.J.Clin.Pathol., 45:493-496.

Bennett, J.V.(1980). Antibiotic use in animals and human salmonellosis. J.Infect.Dis., 142(4):631-633

Bezjak,V and Thorburn,H.(1981). Survey of rat(Rattus norvegieus) in Kuwait for the presence of some human pathogens. 1. Isolation of Salmonella organisms. J.Kuwait.Med.Assoc., 15(4):229-234. Abstracted in Trop.Dis.Bull. 79(7):606,1982.

Bidawid,S.P., and Edeson,J.F.B.(1978). The role of non-bitting flies in the transmission of enteric pathogens(Salmonella species and Shigella species) in Beirut,Lebanon. Ann.Trop. Med.Parasitol., 72,117-121.

Black, P.H., Kunz.L.J. and Swartz,M.N.(1960). Salmonellosis-A review of some unusual aspects.N.Engl.J.Med.,262:811

Boyce,J.M., Evans,D.J.,Jr., Evans,D.G.and DuPont.H.L.(1979). production of heat-stable,methanol-soluble enterotoxin by Yersinia enterocolitica.Infect.Immun., 25:532-537.

Britt,D.P.,Cole,T.A., and shipp,C.R.(1978). Salmonellae from dogs in Vom,Northern Nigeria. Trop.Anim.Hlth.Prod.,10(4): 215-218. Abstracted in Trop.Dis.Bull., 76(2):147,1979.

Burrows,T.W. and Gillette,W.A.(1966). The nutritional requirements of some Pasteurella species. J.Gen.Microbiol., 45:335-345.

Byers,P.A.,DuPont,H.L., and Goldschmidt,M.C.(1976). Antimicrobial susceptibility of Shigella isolated in Houston,Texas, in 1974. Antimicro.Agents Chemother.,9:288-291.

Capriol[illegible],T., Drapeau.A.J. and Kasatiya, S.(1978). Yersinia enterocolitica:serotypes and biotypes isolated from humans

and the environment in Quebec ,Canada. J.Clin.Microbiol.8: 7-11.

Carter,P.B.(1975). Pathogenicity of Yersinia enterocolitica for mice. Infect.Immun., 11:64-170.

Cash,R.A.(1979). Oral therapy for diarrhoea. Trop.Doctor.9:25-30

Cazbon,E.P.I., Berment,M.P. and Superead,N.(1978). Salmonella infection in market swine,Trindad and Tobago Bull.Pan.Amer. Hlth.Org., 12(1):51-54.

Chang,M.J., Dunkel,L.M., Reken,D.V., Anderson,D.,Wong,M.L. and Feigin,R.D.(1977). Trimethoprim-sulphamethoxazole compared to ampicillin in the treatment of shigellosis. Pediatrics., 59:726-729.

Chapman,S.J.(1980). The occurrence of enteric bacteria on lettuce leaves sold in local markets in Penang,Malaysia. Med.J.Malaysia,35(1):7-8. Abstracted in Trop.Dis.Bull., 78 (12):1073.1981.

Chau,P.Y., Ling.J., Threlfall,E.J. and Im,S.W.K.(1982). Genetic instability of R.plasmids in relation to the shift of drug resistance patterns in Salmonella Johannesburg. J.Gen. Microbiol., 128(2):239-245.

Chun, D. and Seol,S.Y.(1978). Drug resistance and R plasmids of Salmonella and Shigella in Korea. Trop.Med.20:123-129

Committee on Salmonella.(1969). An evalutation of the Salmonella Problem. National Academy of Science. Washington,D.C.

Curi de Montburn, S.E. Ciccarelli, A.S,De Ampuero,S.,Fernandez, R.A. and Benite,M.A.(1981). Infections due to Salmonella of various species in Mendoza, Argentina. Revista Argentina Microbiol.,13(1):23-30. Abstracted in Trop.Dis.Bull.,

79(3):232,1982.

Cutting, W.A.M.(1979). Diarrhoeal disease in the Tropics (Editorial) Trop.Doctor, 9:2-3.

Davis,J.R.(1970). Antibiotic resistance in Shigella sonnei. Lancet. 2:1157-1159.

Dixon,J.M.S.(1965). Effects of antibiotic treatment in duration of excretion of Salmonella typhimurium by children, Brit,Med.J 4:1343-1345.

Donaldo,J. and Gangarosa,E.J.(1969). Food-borne Shigellosis. J.Infect.Dis., 119:666-668

Dritz,S.K., Ainsworth, T.E., Garrard, W.F., Back, A., Palmer, R.D., Boucher, L.A. and River, E.(1977)Patterns of sexually transmitted enteric diseases in a city. Lancet, 2:639-642.

DuPont,H.L. and Hornick, R.B.(1973). Clinical approach to infectious diarrhoea. Medicine,52:265.

Edwards, P.R. and Ewing. W.H.(1972). Identificatior of Enterobacteriaceae 3rd Ed. Minneapolis, Minn.:Burgess publishing company.

Eissenberg, G.M., Palazzolo, A.J. and Flippin,H.F.(1955). Clinical and microbiological aspects of salmonellosis. A study of ninety five cases in adults and children. N.Engl.J. Med..253:90.

Evans,N.(1979). Bacterial toxins and diarrhoea. Trop.Doctor.9 :10-15.

Fálade,S. and Ehizokhale, M.U.M.(1981). Salmonella and Escherichia coli strains isolated from poultry in Ibadan,Nigeria. Anim.Hlth.Prod.Africa,29(1):99-101. Abstracted in Trop. Dis.Bull.,79(5):435,1982.

Falkow,S.(1975). Infectious multiple drug resistance. Pion limited. London.

Freeman,B.A.(1979). The enteric Bacilli. In Burrow's Textbook of Microbiology 21st ed.W.B. Saunders Company. Philadelphia, London,Toronto.pp.518-551.

Gaffodio, A.M., Martinette, P.,Mentero Quintero, N. and Angetti, A.(1979). Transmissible antibiotic resistance in minor strains of Salmonella recently isolated in the Piedment region, Italy. Boll.1st Sieroter Milan.58(3):224-234. Abstracted in Biol.Abstr., 70(10):6601,1980.

Gangarosa, E.J., Pierra, D.P. Mata, L.J. Morris, C.M. Guzman, G., , and Reller, L.B.(1970). Epidemic Shiga Bacillus dysentery in Central America : II. Epidemiological [illegible] studies in 1969. J.Infect.Dis.,122:181-190.

Gianella,RA., Formal, S.B. Damonin, G.J. and Collins, E.(1973). Pathogenesis of Salmonellosis: studies of fluid secretion, mucosal invasion and morophologic reaction in the rabbit ileum. J.Clin.Invest., 52:441.

Grant,R.B. and Di Mambro,C,(1977). Antimicrobial resistance and resistance plasmids in Salmonella from Ontario,Canada,Can.J. Microbiol., 23(9):1266-1273.

Gunn,R.A. and Bullon-Loarte,F.(1979). Salmonella enterocolitis: report of a large food borne outbreak in Trujillo,Peru. Bull.Pan Amer.Hlth.Org.,13(2):162-168.

Gupta,B.R., Singh,M.P., Verma,J.C. and Uppal,P.K.(1980). Isolates of Salmonella(3,10:r:-) from cases of human food poisoning. Indian J.Med.Res.,71:175-177.

Head,C.B., Whitty, D.A. and Ratnam,S.(1982). Comparative study of selective media for recovery of Yersinia enterocolitica. J.Clin.Microbiol.16:615-621.

Hobbs,B .C.(1961). Public health significance of Salmonella Carriers in Livestock and birds. J. Appl.Bacteriol.,24: 340.

Holmes,K.K. and Puziss,M.(1980). Recommendations of the study group for research and training in sexually transmitted diseases. J.Infect.Dis. 142(4):639-642.

Hughes,J.M., Merson,M.H., Carun.G.F. and McCabe,J.(1975). Outbreak of water borne disease in the United States. J.Infect.Dis., 132(3):336-339.

Hughes, D.(1979). Isolation of Yersinia enterocolitica from milk and a diary farm in Australia. J.Appl.Bacteriol., 46:125-130.

Hughes D(1980). Repeated isolation of Yersinia enterocolitica from Pasteurizedmilk in a holding vat at a dairy factory. J.Appl.Bacteriol,,48,383-385.

Im,S.W.K., Yam.W.C. and Teoh-Chan,C.H.(1978). Drug resistance and R plasmids in Salmonella johannesburg isolated in Hong Kong. Asian.J.Infect.Dis.2(3):215-220. Abstracted in Trop. Dis.Bull.,76(6):534,1979.

Janssen,W.A. and Meyers,C.D.(1968). Fish:serologic evidence of Infection with human pathogens, Science,159:547-548.

Kaliyugaperunal,V., Gupta,U and Mohaptra,L.N.(1978). Antimicrobial drug resistance and R factors in Shigella. Indian J.Med.Res.,68:220-224.

Kan,S.K.P. and Chan.M.K.C.(1980). Annual and geographical

distribution of Shigella serotypes in Sabah, Malaysia from 1974 to 1978. Med.J.Malaysia,35(1):9-13. Abstracted in Trop.Dis.Bull.,78(12:1013.,1981.

Kapperud,G,(1975). Yersinia enterocolitica in small rodents from Norway,Sweden and Finland, Acta Pathol,Microbiol,Scand. Sec,B.,83:335-342.

Kapperud,G.(1977). Yersinia enterocolitica and Yersinia-like microbes isolated from mammals and water in Norway and Denmark. Acta Pathol.Microbiol.Scand. Sec,B,85:129-135.

Katzenelsen,E,,Buium.I. and Shural, I.(1976). Risk of communicable disease infection associated with waste water irrigation water in agricultural settlemsnt. Science.194: 944-946.

Kauffmann.F(1966). Kauffmann-white schoma, in: The Bacteriology of Enterobacteriaceae, ChapIII,pp.149-226. Williams and Wilkin Ce;Baltimore.

Keyhani,M.(1978). Studies on concurrent salmonellosis in dogs and man. Pahlavi Med.J.,9(2):193-199. Abstracted in Trop.Dis. Bull, 76(2):147,1979.

Koplan,J.P., Deen,R.D., Swanston,W.H. and Teta,B.(1978). contaminated roof-collected rain water as a possible cause of an outbreak of Salmonellosis. J.Hyg.(Camb),81:(112): 303-309.

Koshi,G.(1981). Alarming increase in multidrug resistant Salmonella typhimurium in Southern India. Indian J.Med. Res.,74:635-641.

Koupal,L.R. and Deibel,R.H.(1975). Assay,characterization and localization of an enterotoxin produced by Salmonella. Infect.Immun.,11:14.

Kourany,M., Bowdre,L. and Jerrer, A.(1976). Panamian forest mammals as carriers of Salmonella. Amer.J.Trop.Med.Hyg., 25(3):449-455

Lafaix,C., Castets, M., Denis,F. and Diop-mar,I.(1978). salmonellosis in Dakar: bacteriological, clinical, epidemiological and therapeutic aspects. 10 years' records. Med.Trop.,39(4):369-379. Abstracted in Trop.Dis.Bull.,77(6) :442,1979.

Leksomboon,U., Esheverria,P., Suvongse,C. and Duangmani,C.(1981). Viruses and Bacteria in pediatric diarrhoea in Thailand; A study of multiple antibiotic resistant enteric pathogens. Amer.J.Trop.Med.Hyg.,30(6):1281-1290.

Levine,M.M.(1979). Shigella and Salmonella diarrhoeal disease. Trop.Doctor, 9:4-9.

Macaden,R.,Gokul,B.N.,Pererira, P and Bhat,P,(1980). Bacillary dysentery due to multi-drug resistant Sh.dysenteriae type 1. Indian J.Med.Res., 71:178-185.

Manteen,A.,Guinee,P.A.M., Kampelmacher,E.H. and Voogd,C.E.(1971). An eleven-year study of drug resistance in Salmonella in the Netherlands, Bull.Wld.Hlth.Org., 47:85-93.

Matsen,J.M.,Barry,A.L.(1974). Susceptibility testing: diffusion test procedures. In: Manualof Clinical Microbiology. 2nd ed.(ed.E.H.Lenette,E.H.Spaulding and J.P. Truant). P.421.Washington, D.C: America Society for Microbiology.

Martin,T.,Kasian,G.F.,Stead,S.(1982). Family outbreak of

Yersiniosis. J.Clin.Microbiol. 16(4):622-626.

Mered,B., Poittevin,F and Louli,B.(1977). Surveillance of drug resistance in pathogenic enterobacteria isolated in Algeria: 1.A study of the antibiotic resistance of Salmonella responsible for typhoid and paratyphoid fevers and of other Salmonella species during 1973-1974. Arch.Inst.Pasteur Alger., 52:17-36. Abstracted in Biol.Abs.70(10):Ab.62937,1979.

Mero,E.(1976). Resistance to antibiotics of Shigella strains isolated in Somalia. Bull.Wld.Hlth.Org., 54:473-474.

Messele Gedebou and Alebachew Tassew,(1979). Antibiotic susceptibility patterns and R factor among Salmonella and Shigella isolates. Abstract. Ethiop.Med.J., 17: 99-100

Messele Gedebou and Alebachew Tassew(1981). Antimicrobial resistance and R factors of Salmonella isolates from Addis Ababa. Ethiop.Med.J.19:77-85.

Messele Gedebou and Alebachew Tassew (1982). Shigella species from Addis Ababa:frequency of isolation and In vitro drug sensitivity J.Hyg.Camb., 88:47-55.

Ministry of Health(1977). Report on Major communicable diseases and vaccination activities in Ethiopia 1974-1976.

Mitsuhashi,S.(1969). The R factors. J.Infect.Dis., 119:89-100

Mollaret, H.H and Thal,E.(1974) Yersinia. In:Bergey's Mannuals of Determinative Bacteriology, 8th edn,ed.Buchanana,R.E.and Gibbons,N.E.pp.330-332. Baltimore.

Mutanda, L.N., Kaviti,J.N. and Wamola,I.A.(1979). Patterns of Shigella species and serotypes in East Africa. East Africa Med.J.56(8):381-387.

Mutanda.L.N., Kjibriya,G, and Mansur,M.N.(1981). Patterns of Shigella,flexneri serotypes and drug resistance is Dacca. Indian J.Med.Res.73:8-12.

Nelson,J.D.,Kusmiesz.H.,Jackson,L.H. and Woodman.E.(1976). Trimethoprim-sulphamethoxazole therapy for shigellosis. JAMA.:235:1239-1243

Neu,H.C.,Cherubin,C.E.,Lango, E.D. and Winter,J.(1975). Antimicrobial resistance of Shigella isolated in New York City in 1973. Antimicrob.Agents Chemother,7:833-839.

Nilehn, B.(1969). Studies in Yersinia enterocolitica with special reference to bacterial diagnosis and occurence in human acute enteric disease. Acta.Pathol.Microbiol.Scand. Suppl.206:1-48.

Nuti,M.,Harare,O.M., and Mero,E.(1977). On a Shigella dysenteriae type 1 epidemic in Mogadishu. Ann.Scalvo.,17(2): 209-218. Abstracted in Trop.Dis.Bull. 76(8):738,1979.

Ngu,V.V.(1977). Observations in Shigella isolates in the Bach Mai Hospital(Hanoi) in the course of 3 years(1974-1976). Santé Publ.,20(4):461-464. Abstracted in Trop.Dis.Bull. 76(9):815, 1979.

Obi,S.K.C. and Nzeako,B.C.(1980). Salmonella,Arizona,Shigella and Aeromonas isolated from the snail Achatina achatina in Nigeria. Antonie Van Leeuwenhoek, 46:475-481.

Olarte,J.,Filloy,L. and Galindo.E.(1976). Resistance of Shigella dysenteriae type 1 to ampicillin and other anti-bacterial microbial agents:strains isolated during a dysentery outbreak in a hospital in Mexico city.J.Infect.Dis.,133: 572-575.

Pai,C.H.,Mors,V.,Toma,S.(1978). Prevalence of enteroxigencity in human and non-human isolates of Yersinia enterocolitica. Infect.Immun.,22:334-338.

Pai,C.H.,Mors,V., and Seemayer,T.A.(1980). Experimental Yersinia enterocolitica enteritis in rabbits. Infect.Immun., 28:238-244.

Panhotra,B.R.,Agrwal,K.C.,Graq,R.K.,Mahanta.J.,Goyal.A.K. and Ayyagari,A.(1980). Multidrug resistant Salmonella typhimurium in Chandigarh. Indian J.Med.Res.,72:169-173.

Paniker,CK.J., Vimola,K.N., Bhat,P. and Stephen,S.(1978). Drug resistance shigellosis in South India. Indian J.Med.Res. 413-417.

Pedersen,K.B.(1976). Isolation of Yersinia enterocolitica from Danish swine and dogs. Acta.Pathol.Microbiol.Scand.Sect.B., 84:317-318.

Pedersen,K.B., and Winbald,S.(1979). Studies on Yersinia enterocolitica isolated from swine and dogs. Acta.Pathol. Microbiol.Scand.Sect.B., 87:137-140.

Plotkin,G.R.,Kluge,R. and Waldman.R.H.(1979). Gastroenteritis: etiology, pathopysiology and clinical manifestations. Medicine,58:(1):95-114.

Rahman,M.M.,Huq,I.,Dey.C.R.,Khibriya,A.K.and Curlin.G.(1974). Letter: Ampicillin resistant shiga bacillus in Bangladesh. Lancet,1:406-407.

Rao,R.S.,Sanyal,S.C and Sen, P.C.(1979). Antibiotic resistance in Shigella. Indian J.Microbiol.,19(3):99-102.

Ryder,R.W.,Blake,P.A.,Murlin,A.C.Carter.G.P., Pollard,R.A. Merson,M.H.,Allen.S.D. and Brenner,D.J.(1980). Increase in antibiotic resistance among isolates of Salmonella in the United States,1967-1975. J.Infect.Dis.,142(4):485-491.

Sack.R.B.,Gorbach,S.L.,Banwell,J.G.,Jacobs,G.,Chatterjee,B,and Mitro.R.C.(1971). Enterotoxigenic Escherichia coli isolated from patients with severe cholera-like disease. J.Infect.Dis. 123(4):378.

Salion,P.(1979). First demonstration in Upper Volta of 41 serotypes of Salmonella isolated at the Muraz Center. Bull.Soc.Path. Exot,72(3)193-198. Abstracted in Trop.Dis.Bull.,77(8): 638.1981.

Samadi,A.R.,Wachsmuth,K.,Huq,M.I.,Mahbub.M. and Agbonlaher.D.E. (1982)An attempt to detect Yersinia enterocolitica infection in Dacca,Bangladesh. Trop.Geog.Med.,34(2):151-154.

Savage.W.(1956). Problems of Salmonella food poisioning.Brit. Med.J.,2:317.

Schiemann.D.A.(1978). Isolation of Yersinia enterocolitica from surface and well waters in Ontario. Canadian J.Microbiol., 24:1048-1052.

Schiemann.D.A.(1979). Synthesis of selective agar medium for Yersinia enterocolitica. Canadian J.Microbiol.,25:1298-1304.

Schroeder,S.A.,Terry,P.M. and Bennett,J.V.(1968). Antibiotic resistance and transfer factor in Salmonella, United States 1967.JAMA,205:87-90.

Sharma,S.K. and Pathak,R.C.(1974). Studies of some epidemiological and public health aspects of salmonellosis

Indian J.Publ.Hlth., 12(4):171-173, Abstracted in Trop.Dis. Bull., 73(6):471, 1976.

Simmons,H.E. and Stolley,P.D.(1974). Trends and consequences of antibiotic use in the United States.JAMA., 227:1023-1028.

Stintzing.G.(1981).Paediatric diarrhoea in a developing community(Addis Ababa,Ethiopia). Stockholm.

Stoll,B.J.,Glass,R.I.,Huq,M.I.,Khan.M.V.,Banu.H.and Holt.J(1982) Epidemiologic and clinical features of patient infected with Shigella who attended a diarrhoeal disease hospital in Bangladesh,J.Infect.Dis., 146(2):177-183.

Sutherland,J.P. and Varnam.A.H.(1977). methods of isolation of potential importance of Yersinia enterocolitica in foods stored at low temperatures. J.Appl.Bacteriol.43:13-14.

Swaminathan,B.,Harmon,M.C. and Mehlmann,I.J.(1982). A review: Yersinia enterocolitica. J.Appl.Bacteriol., 52:151-183.

Szanton,V.L.(1957). Epidemic salmonellosis:A 30-month study of 80 cases of Salmonella oranienburg infection. Pediatrics. 20:794.

Tsing.H.C.,Chao.S.H. and Kuo.J.L.(1981). Salmonellae in Tan-Shui River. Chinese J.Microbiol.Immunol.,14(3):59-67. Abstracted in Trop.Dis.Bull. 79(3)232.1982.

Toma ,S. and Deidrick,V.R.(1975). Isolation of Yersinia enterocolitica from swine. J.Clin.Microbiol.,2:478-481.

Urban,T.(1972) Transferable multiple drug resistance of Shigella strains isolated in Sweden. Scand.J.Infect.Dis.,4:221-227.

Vogt,R.L.,Hakey,A. and Allen.J.(1981). Salmonella enteritidis serotype derby and consumption of raw milk.J.Infect.Dis., 144(6):608.

Wallace,C.K.(1976). Approach to enteric infections. Ethiopian Med.J.,15:29.

Wamola,I.A.(1980). Bacterial stool pathogens in Kenyatta National Hospital. East African Med.J.57(12):867-871.

Wamola,I.A., and Mizra,N.B.(1981). Problem of Salmonella infection in a Hospital in Kenya. East Africa Med.J.58(9): 677-683.

Watanabe,T.(1963). Infective heredity of multiple drug resistance in bacteria. J.Bacterial.Rev.,27:87-115.

Watson,C.E.(1967). Infectious drug resistance in shigellosis in Cape Town.S.Afr.Med.J., 41:728-731.

Weissfeld,A.S. and Sonnenwirth,A.C.(1980). Yersinia enterocolitica in adults with gastrointestinal disturbances:Need for cold enrichment,J.Clin.Microbiol., 11,196-197.

Weissman,J.B.,Graun,G.F.,Lawrence,D.N.,Pollard,R.A.,Saslom.M.S. and Gangarosa, E.J.(1976). An epidemic of gastroenteritis traced to a contaminated public water supply. Amer.J. Epidemiol.,103:391-398.

W.H.O.(1978). Surveillance for the prevention and control of [illegible] health hazards due to antibiotic-resistant enterobacteria. WHO Tech.Rep.Ser.,No 624.

W.H.O.(1980). Research in bacterial enteric infections WHO/CDD/ BEI/ 80.1. (unpublished documents).

W.H.O. Scientific working Group(1980a). enteric infections due to Campylobacter, Yersinia. Salmonella and Shigella. Bull. Wld.Hlth.Org., 58(4):519-537.

W.H.O. Scientific Working Group(1980b), Cholera and other

vibrio associated diarrhoeas. Bull.Wld.Hlth.Org., 58(3): 353-374.

W.H.O. Scientific Working Group(1980c). Rotavirus and other viral diarrhoeas. Bull.Wld.Hlth.Org., 58(2):183-198.

W.H.O. Scientific Working Group(1980d). Escherichia coli diarrhoea. Bull.Wld.Hlth.Org., 58(1):23-36.

W.H.O. Scientific Working Group(1980e). Parasite-related diarrhoeas. Bull.Wld.Hlth.Org., 58(6):819-830.

Wilson,M.D., McCornick,J.B. and Feeley, J.C.(1976). Yersinia enterocolitica infection in a 4-month-old infant associated with infection in household dogs. J.Pediatrics. 89:767-769.

Zoukh,K. and Poittevin,F.(1978). Evolution of the distribution of enteric pathogenic enterobacteria serotypes isolated in Algeria from 1974 to 1977. Arch.Inst.Pasteurn Alger., 53:59-72. Abstracted in Trop.Dis.Bull.78(12):1075,1981.