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REVIEW ARTICLE

SHIGELLOSIS IN ETHIOPIA: REVIEW OF STUDIES CONDUCTED SINCE 1974

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ABSTRACT: This is a review of the work done on shigellosis from 1974 to 1992 and its very recent situation in Ethiopia. Shigellosis is a highly infectious disease of worldwide significance. Its incidence has always been the top in tropical and subtropical regions, where the general poor living condition is a prevailing phenomenon. Because of poor quality of drinking-water supplies, large quantity of domestic house flies, lack of well established sewage system, and too much crowdedness of living quarters, it was found evident for shigellosis to be one of the major health problems in developing countries like Ethiopia. It was, thus, relevant to look into the status of the disease shigellosis in Ethiopia starting from the period of 1974, since there had not been enough work done as a laboratory based evidence of this disease before this period. The works of Afeworki Gebre-Yohannes, conducted between 1974 and 1992 could be considered as the first few attempts to indicate the prevalence of the major subgroups and subtypes of *Shigella* species isolated from Ethiopian patients with bloody diarrhoea. In addition to their prevalence in developing countries, *Shigella* strains are also well noted to be notorious due to their ability to develop multi-drug resistance. Researchers like Afeworki Gebre-Yohannes and others have also continuously addressed this point throughout the past years, with which they were able to indicate the resistance patterns of all the various groups, subgroups and subtypes of the *Shigella* strains and the emergence of new single- or multiple-drug resistant features among the Ethiopian isolates. The molecular characterization of the most prevalent subgroup and subtypes of the *Shigella* strains was also seriously addressed by Afeworki Gebre-Yohannes and his group for the first time in Ethiopia starting from 1974. Plasmid-mediated single- and multi-drug resistant patterns of these strains were also analyzed up to the current period. Similarly, characterization of some virulence factors for these *Shigella* stocks have been continued using the haemagglutination and iron-acquiring mechanisms of their strains.

Key words/phrases: Antibiotic resistance; Ethiopia; Prevalence; *Shigella*.

INTRODUCTION

In developing countries, diarrhoea is among the leading causes of childhood morbidity and mortality. An estimated one billion episodes and 3.3 million deaths occur each year among children under five years of age. Overall,

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these children experience an average of 2.6 episodes of diarrhoea per child per year (Bern *et al.*, 1992). About 80% of deaths due to diarrhoea occur in the first two years of life (Snyder and Merson, 1992). In Africa, it has been estimated that every child has five episodes of diarrhoea per year and that 800 000 children die each year from diarrhoea and dehydration (Urio *et al.*, 2001). According to Wittenberg (1998, cited in Urio *et al.*, 2001), infective diarrhoea is predominantly a disease of poverty, overcrowding and environmental contamination. He noted that within the southern African subcontinent, large-scale epidemics involving *Shigella dysenteriae* type 1 and *Vibrio cholerae* had occurred.

Shigellosis is a highly infectious disease of worldwide significance. Its incidence is highest in tropical and sub-tropical regions, where the general living standards are usually poor. The poor hygienic quality of drinking water, the high number of domestic house flies, the unavailability of sewage disposal system and the close proximity of living quarters are among the major factors that enhance the spread of shigellosis. It is evident that shigellosis is, therefore, a major health problem of developing countries like Ethiopia.

Shigella species are the responsible agents for a substantial proportion of cases of bacillary dysentery, resulting in endemic and sporadic or epidemic type of disease in the developing countries. Shigellae cause diarrhoea by the penetration and destruction mechanisms of the colonic epithelial cells. This capacity has been depicted by the 120-140 Mda plasmids, which carry the genetic determinants that encode epithelial cell invasion by each of the four *Shigella* species. It has been repeatedly recorded that diarrhoeal diseases are important causes of high morbidity and mortality, especially in preschool age children. Together with plague, cholera and influenza, bacillary dysentery has been one of the great scourges of mankind. Considering the magnitude of the problem in Africa, about a decade ago, availability of information on its incidence, prevalence and epidemiology was relatively scarce. This was because facilities for its studies were not yet adequate at that time. Thus attempts to make such information available, as initiated by Afeworki Gebre-Yohannes and few other investigators, were definitely crucial and needed for public health and medical practitioners' use in Africa, at large, and Ethiopia, in particular (Afeworki Gebre-Yohannes and Yetnebersh Limeneh, 1980; Afeworki Gebre-Yohannes and Dekker, 1981; Afeworki Gebre-Yohannes, 1983, 1984; Afeworki Gebere-Yohannes and Eyassu Habte-Gabr, 1984a,b; Afeworki Gebre-Yohannes and Drasar, 1987, 1988a-c). The long-term *Shigella* carrier state, though exceptional, is well

known to exist in many places around the world. For example, a study in Guatemala had shown that children with chronic, recurrent shigellosis were dangerous sources of infection (Gangarossa *et al.*, 1970; Mata *et al.*, 1970; Mendiazabal-Morris *et al.*, 1971; Reller *et al.*, 1971).

In many developing countries, *S. flexneri* was observed to be the most dominant serogroup among the four of those associated with clinical shigellosis and even more than the *S. dysenteriae* strains. This is contrary to the situations in the developed countries whereby *S. flexneri* has given way to *S. sonnei,* the dominant serogroup causing shigellosis in such countries, (Stypulkowska-Misiurewicz *et al.*, 1971; Rosenberg *et al.*, 1977; Piechaud *et al.*, 1978). However, even up to recent periods of shigellosis investigation, both *S. dysenteriae* and *S. flexneri* have been of special concern to developing countries like Ethiopia where they have been the most prevalent serogroups.

In Ethiopia, few studies had been conducted on some aspects of shigellosis including identification of the etiologic strains by using standard biochemical and serological methods. They had also gone further into looking at the properties of the prevalent agents such as their susceptibility against the various commonly used antimicrobial agents. The aim of this review was, therefore, to update the achievements obtained so far by different investigators around this important epidemiological health problem in the country.

All those studies conducted at different times by investigators of the Ethiopian Health and Nutrition Research Institute (EHNRI) concentrated around the epidemiological status, including the prevalence and antibiogram-related properties of the etiologic agents of shigellosis in Ethiopia. All the strains used for these studies were obtained from endemic cases of shigellosis and from some sporadic incidences as well as various epidemic outbreaks occurring in the country. These strains were among those isolates collected starting from 1974 onwards and stocked in deep-freeze at $-70^{\circ}C$.

The following specific activities were, thus, performed on each of these stocked strains:

1. Specific *Shigella* serotypes were identified biochemically and serologically;
2. Antimicrobial sensitivity testing was carried out on all the collected isolates;

3. The level of transmissible drug resistance in individual *Shigella* serotypes was determined by direct and triparental crosses in broth mating; and
4. Plasmid DNA, from representative samples of wild type *Shigella* serotypes and their *Escherichia coli* K12 counterparts, was extracted according to the mini-preparation method of Birnboin and Doly (1979). The molecular weight of individual plasmid species was determined by agarose gel electrophoresis. Resistance plasmids were characterized by incompatibility testing and, in selected instances, by restriction enzyme analysis.

THE BIOLOGY OF *SHIGELLA* SPECIES

In general, the genus *Shigella* has closer relation in its DNA sequence to *Escherichia*, especially more with that of Enteroinvasive *E. coli* (EIEC) strains, and they both cause not much distinguishable illnesses. All shigellae are members of the family Enterobacteriaceae and are Gram negative rods, non-motile, oxidase-negative, non-lactose fermenters (except for *S. sonnei* which is weakly positive) and non-gas producers from fermentable carbohydrates (except certain type of *S. flexneri*).

The genus *Shigella* is subdivided into only four groups of species on the basis of their biochemical and serological reactions:

Group A, *S. dysenteriae*, with 10 distinct serotypes all of which do not ferment mannitol;

Group B, *S. flexneri,* with 6 serotypes, all fermenting mannitol;

Group C, *S. boydii*, with 15 sertotypes, all fermenting mannitol; and,

Group D, *S. sonnei*, only with a single mannitol-fermenting serotype.

Shigella dysenteriae type 1 holds a special place in public health problems because it causes especially severe infections that may occur in explosive epidemics and even pandemics (Bernand *et al.*, 1990; Abizai *et al.*, 1994; Tamirat Abebe, 2002). These organisms harbor an R plasmid encoding resistance to several antibiotics (Afeworki Gebre-Yohannes and Drasar, 1989a, b, 1990a, b; Bernand *et al.*, 1990). The virulence factors present in all virulent *Shigella* strains with their roles in the pathogenesis of the organisms can be summarized as shown in the Table 1 below (Venkatesan *et al.*, 1988; Abizail *et al.*, 1994).

Table 1 The various virulence factors of *Shigella* strains and their possible role in pathogenesis.

Virulence Factors	Possible Role in Pathogenesis
IpaD	Adherence
IpaB, C	Invasion, escape from phagocytic vesicle
Mxi protein	Excretion Ipa A – D
IcsA	Intracellular spread (actin polymerization)
Olm	Mount along action filaments
Shiga toxin (StxA, StxB)	A-B Toxin, A subunit cleaves 235 rRNA; responsible for HUS.
LPS O antigen	Inflammation
Vac C	Regulates ICS gene expression
Vac B	Post transcriptional control of *ipa* and *ics* A expression
Kcp A	Regulates *ics* A expression
Fur	Regulates expression of StxA/StxB and iron acquisition genes
Vir F	Regulator of Vir B expression
Vir B	Histone like protein, regulation of Vir F expression

The Infectious Cycle of the *Shigella* Strains in Epithelial Cells and the Role of its Inflammation in Facilitating Diarrhoeal Disease Progression

Transmission of shigellosis is through oral route. The organisms progress along the intestinal tract until they reach the mucosal surface of the colon and rectum. They eventually invade these surfaces and result in causing inflammation and tissue destruction. Finally, this, in turn, facilitates the diarrhoeal disease progression. *Shigella* invades the epithelial lining through its apical surface very efficiently, and there are essentially two possible ways for the bacteria to reach the baso-lateral pole of the epithelium (Tamirat Abebe, 2002):

1. Crossing the epithelium covering the lymph nodes associated with the mucosa, or
2. Taking advantage of the inflammation that destabilizes the epithelial integrity to translocate and reach the sub-epithelial tissue.

The pathogenesis of *Shigella* is attributed to the organism's ability to invade, replicate and spread intracellularly within the colonic epithelium. The invasion of host cells by *Shigella* strains is a complex multi-factorial event in which many bacterial proteins are involved. These proteins are some of the several group plasma-encoded protein types called invasion plasmid antigens (*Ipa*A, *Ipa*B, *Ipa*C and *Ipa*D) (Venkatesan *et al.*, 1988). These are virulence factors involved, with the indicated roles in pathogenesis, as shown in the above-summarized note (Table 1). These antigens help in the entry of the *Shigella* strains into epithelial cells by subverting the integrated defense barrier through multi-step processes. This proceeds by inducing mucosal inflammation, responsible for the major tissue destruction, which in turn facilitates invasion at the early stage of the process (Venkatesan *et al.*, 1988; Philippe *et al.*, 1999). These virulence

factors participate in the entry of the organism into non-phagocytic cells, in the escape of the bacterium into the cytoplasm, in the intracellular multiplication and in the cell-to-cell spread.

This entry of the bacterium into the non-phagocytic cells involves a macropinocytic event requiring a massive cytoskeletal rearrangement. Upon contact with the target cells, *Shigella* secretes the *Ipa* proteins that bring the cytoskeletal rearrangement by first eliciting membrane ruffles at the cell-bacteria interaction site. Within a few minutes, cell entry process is efficiently acquired, lysis of the phagocytic vacuole occurs and is followed soon by intense bacterial multiplication. The non-motile bacterium then acquires the ability to spread inside the first cell and gain access into adjacent cells without further contact with the extracellular medium (Philippe *et al.*, 1999). The ability to lyse the vacuole is linked to the expression of *Ipa* proteins by the bacterium. *Ipa*B is the main effecter, but according to recent evidence, *Ipa*C may also contribute to the vacuole lysis process. Then the bacterium escapes from the lysed vacuole and moves along stress fibers that radiate from adhesion plaques to the nucleus. The molecular basis of this movement is still unknown. In epithelial cells, the organelle-like movement is hard to detect but instead, the bacteria moves in the cytoplasm by an actin-based movement (Barzu *et al.*,1997).

As soon as contact occurs between this moving organism and the inner surface of the cytoplasmic membrane, a protrusion is formed, which is eventually phagocytosed by adjacent cells. Expression of cadherins is a prerequisite to the phagocytosis of the protrusions by adjacent cells. The bacteria are then trapped inside a pocket surrounded by a double membrane, which eventually is lysed by a factor, 57-kDa protein, encoded by the *Ipa* genes called *ics*B. In the context of the epithelial cells, the invasive phenotype of *Shigella* can be considered as an efficient means of intracellular colonization by the pathogen. In this way the cell-to-cell spread process is achieved by the bacterium after its efficient entry into the non-phagocytic cells encompassing intracellular growth and intracellular motility.

The cell penetration, replication and spread of the bacteria within the human colonic epithelium are essential steps in the pathogenesis of shigellosis. *S. flexneri* invades the colon through lymphoid follicle-associated M-cells. Once in the mucosa, the *Shigella* organism is phagocytosed by the resident macrophages and escapes from phagosome into the cell cytoplasm. This bacterium triggers apoptosis in macrophages both *in-vitro* and *in-vivo*

including the release of one of the main pro-inflammatory cytokines, interleukin-1 (IL-1). So both the cell invasion and macrophage killing by apoptosis are most prominent features in the development of the disease shigellosis. The *Ipa*B is the only factor of the four proteins secreted by the *Shigella* species before cell invasion that is necessary and sufficient for initiating the macrophage apoptosis (Andrea and Arturo, 1997). The inflammation elicited by the shigellae is another central component of the pathogenic process of shigellosis like dysentery (Andrea and Arturo, 1997). The ability to induce macrophage cell death is described by all invasive clinical isolates belonging to the different *Shigella* species, which signifies that macrophage apoptosis is significant in the rectal mucosa of dysenteric patients (Andrea and Arturo, 1997; Barzu, *et al.*, 1997).

Thus, shigellosis is characterized by a strong inflammatory response in the colon, where resident macrophages engulf the bacteria and the infected macrophages release IL-1, a cytokine that is primarily responsible for inflammation during dysentery. This release of IL-1 is tightly linked to *Shigella* induction of apoptosis in macrophages (Andrea and Arturo, 1997). The binding of *Ipa*B to *IL*-1B converting enzyme (ICE), or an ICE-like protease, in *S. flexneri*-infected macrophages, constitutes an essential event in the molecular mechanism of *Shigella*-induced apoptosis. Only ICE processes *IL*-1B, one of the two types of *IL*-1s, to its active form. ICE (caspase 1) is, in turn, a member of a growing family of cysteine proteases. During apoptosis, the cell nucleus becomes small and condensed, features that make it distinct from normal nuclei.

In the presence of the cell-death message that is received from the invading shigellae, the macrophages release massive amounts of IL-1B (activated by the IpaB), which initiate a strong inflammation representing the starting point of the disease (Barzu *et al.*, 1997). The role of inflammation in facilitating the disease progression is shown by the paradoxical effect of causing severe damage to the mucosa. It also allows further bacterial invasion before inflammation extending beyond areas of mucosal invasion and by complications reflecting uncontrolled local and systemic inflammation, such as toxic megacolon, colonic perforations, pseudo-leukemoid syndrome, and haemolytic-uremic syndrome (Josette *et al.*, 1999).

Iron Limitation, Acquisition and other Protein Binding Properties as Virulent Factors of Shigellae Strains Isolated from Diarrhoeal Patients

The ability of pathogens to obtain iron from transferrin, ferritin,

haemoglobin and other iron-containing proteins of their host is central to whether they live or die. To combat invading bacteria, animal hosts go into an iron-withholding mode and also use a protein (Nramps 1) to generate reactive oxygen species in an attempt to kill the pathogens. Some invading bacteria respond to this effect by producing specific iron chelators called siderophores that remove the iron from the host protein sources. Others rely on direct contact with host iron proteins, either abstracting the iron at their surface or, as with haeme, taking it up into the cytoplasm. The expression of a large number of genes (>40 in some cases) is directly controlled by prevailing intracellular concentration of Fe (II) via its complexing to a regulatory protein (the *fur* protein or equivalent). This biochemistry of the bacterial cell can then accommodate the challenges from the host. Agents that interfere with bacterial iron metabolism may prove extremely valuable for chemotherapy of diseases (Ratledge and Dover, 2000).

The low free iron concentration in body fluids creates bacteriostatic conditions for many microorganisms and is, therefore, an important defense factor of the body against invading bacteria. Pathogenic bacteria have developed several mechanisms for acquiring iron from the host's source. Siderophore-mediated iron uptake involves the synthesis of these low molecular weight iron chelators, which compete with the host iron-binding glycoproteins such as lacotferrin (LF) and transferrin (TF) for the iron molecule. Without such mediation of siderophores, other alternative ways of inducing iron uptake include the possession of outer membrane protein receptors that actually recognize the TF- or LF-iron complex molecules. This results in the internalization of the iron, and the use of haeme-compounds released into the circulation after lysis of erythrocytes (Otto *et al.,* 1992). Iron limitation, a condition encountered within mammalian hosts, induces the synthesis of a number of proteins in pathogenic *Shigella* species. These include several outer membrane proteins, shiga toxins, and other proteins involved in the biosynthesis and transport of high affinity iron-binding compounds like siderophores. Although siderophores play a major role in the virulence of some bacterial pathogens, they are not so essential for the virulence of *Shigella* species (Pyne, 1989). Since *Shigella* species invade and multiply within host cells, alternative iron acquisition systems, such as the ability to utilize haem-iron, permit growth of the intracellular bacteria. Virulent *Shigella* also posses a cell surface haem-binding protein, whose synthesis correlates with infectivity and virulence. But this protein is not involved in iron acquisition; rather, it may allow the bacteria to coat themselves with haem compounds, thus enhancing their ability to interact

with target host cells (Pyne, 1989). As in *Yersinia* spp. and *E. coli*, *Shigella* strains also grow on haem or haemoglobin as the sole iron source while there is variation in the type of their siderophores produced. The *Shigella* spp., that multiply within the colonic epithelial cells and produce dysentery, produce two types of siderophore-mediated iron transport system, as in *E. coli,* and produce diseases ranging from mild diarrhoea to septicaemia and meningitis. Thus, some *Shigella* spp., like *E. coli* produce the catechol siderophore enterobactin, while the second type, the aerobactin siderophore is produced by *S. flexneri* and *S. boydii* and the third, the hydroxamate type of siderophore, is synthesized by some members of *S. sonnei* as in some clinical isolates of *E. coli* (Steven *et al.*, 1999).

The adherence of enteric bacteria to intestinal epithelial cells is regarded as a vital step in the initiation of infection and thus an important process in the pathogenesis of the disease (Qadri *et al.*, 1988; Schultsz *et al.*, 1992). Hydrophobic interaction is one mechanism among many others, like net surface charge, lectin mediated binding and fibronectin binding involved in adherence of bacteria to various substrata (Magnusson, 1980; Lindhal *et al.*, 1981; Magnusson *et al.*, 1982; Rosenberg *et al.*, 1986; Wadstrom *et al.*, 1986). It is believed that characterization of the bacterial surface hydrophobicity may be useful in understanding the adherence of microbial pathogens to intestinal membranes (Venkatesan *et al.*, 1988).

Several methods have been developed to estimate cell surface hydrophobicity. Some of these are:

1. Two-phase partition systems (Magnusson *et al.*, 1977; Edebo *et al.*, 1983);
2. Hydrophobic interaction chromatography (HIC) (Smyth *et al.*, 1978; Sherman *et al.*, 1985; Seltmann *et al.*, 1986);
3. Bacterial adherence to hydrocarbons (Rosenberg *et al.*, 1980);
4. The salt aggregation test (SAT) (Lindhal *et al.*, 1981; Ljungh *et al.*, 1982; Faris *et al.*, 1984 cited in Qadri *et al.*, 1988; Qadri *et al.*, 1988); and
5. The ability to bind Congo red (Maurelli *et al.*, 1984; Kay *et al.*, 1985; Daskaleros *et al.*, 1987; Qadri *et al.*, 1988; Schultsz *et al.*, 1992).

The binding of Congo red by bacteria is also considered as an indicator of virulence as has been suggested for smooth *S. flexneri* and *Aeromonas* spp.

Different studies have compared the binding of strains of different *Shigella* species to materials like Phenyl and Octyl Sepharose at various concentrations of ammonium sulfate, which caused the bacteria to aggregate and the binding was negligible at lower concentrations. It has also been observed that in smooth forms of *Shigella* species, like enteroinvasive *E. coli*, the binding property to those materials as congo red can distinguish between virulent and avirulent strains and also as a criterion of hydrophobicity. Congo red and other dyes have also been used as indicators of ligand-binding sites either on the cell surface or on soluble proteins.

Thus dyes like the Congo red agar are useful to distinguish the virulent from the avirulent strains equally as do the pathogenicity tests like the Sereny test method. The binding properties of the different *Shigella* species show a decline in the order of *S. dysenteriae, S. flexneri, S. boydii*, and *S. sonnei*. This shows the general agreement with the virulence of *Shigella* species and the severity of the disease these pathogens may cause. Similarly, the test of the *Shigella* species for their haemagglutinin binding properties, conducted in our laboratory recently, and, the salt aggregation test (SAT) analysis done elsewhere, showed that hydrophobicity decreases in the same order as for the Congo red binding and other surface proteins, as shown below in Table 2 (Senait Kebede *et al.*, 1999) and Table 3 (Schultsz *et al.*, 1992), respectively.

Table 2 Distribution of haemagglutination property of *Shigella* serogroups with human and guinea pig red blood cells.

Serogrpoup	None Hagg	Human RBC-A			Human RBC-B			Guinea Pig RBC		
		HA^1	HA^2	HA^3	HB^1	HB^2	HB^3	GP^1	GP^2	GP^3
A (n=11)	2	1	2	5	2	1	1	4	1	2
B (n=17)	1	-	1	10	7	2	2	6	3	5
C (n=4)	1	2	1	-	1	-	-	2	-	-
D (n=7)	-	1	1	5	2	1	1	1	1	4
Total(n=39)	4	4	5	20	12	4	4	13	5	11

HA^1, haemagglutination at low level; HA^2, at moderate level; HA^3, at strong level; Gpg^{1-3}, guinea pig red blood cells haemagglutination at low, moderate, and strong levels.

PREVALENCE OF *SHIGELLA* SEROGROUPS AND SEROTYPES IN ETHIOPIA

Two decades ago, very limited studies were reported on the genus *Shigella* in Ethiopia. Few workers, like Afeworki Gebre-Yohannes and his group, who conducted some studies between 1978 and 1982 were able to indicate the estimated incidence of *S. flexneri* in the 50-70% range and showed the common strains at their serogroup and serotype levels, as shown in Fig. 1 (Afeworki Gebre-Yohannes and Eyassu Habte-Gabr, 1984a) and Table 4 below.

Table 3 Proten-binding characteristics of *Shigella* strains.

Species types	Congo red binding (μg) 10^{10} cells[a]	Sereny's test		SAT Molarity $(NH_4)_2SO_2$ (M)
		Pcr$^+$	Pcr$^-$	
S. dysenteriae type 1 (4)	15.0	+	-	1.5
S. dysenteriae type 1 (1)	16.0	+	-	1.5
S. dysenteriae type 1 (2)	18.0	+	-	1.5
S. dysenteriae type 1 (2)	18.0	+	-	1.5
S. dysenteriae type 1 (2)	19.0	+	-	1.5
S. dysenteriae type 1 (1)	20.0	+	-	1.5
S. dysenteriae type 1 (1)	20.5	+	-	1.5
S. dysenteriae type 1 (3)	21.0	+	-	1.5
S. dysenteriae type 1 (1)	22.0	+	-	1.5
S. dysenteriae type 1 (1)	23.5	+	-	1.5
S. dysenteriae type 1 (2)	26.0	+	-	1.5
S. dysenteriae type 1 (1)	31.0	+	-	1.5
S. dysenteriae type 1 (2)	39.0	+	-	1.5
S. dysenteriae type 1 (1)	39.5	+	-	1.5
S. dysenteriae type 1 (1)	42.0	+	-	1.5
S. dysenteriae type 1 (1) (rough)	44.0	-	-	AA[c]
S. flexneri type Y (1)	6.0	+	-	2.0
S. flexneri type 2a1a3b (3)	7.5	+	-	2.0
S. flexneri type 1b,2a (3)	8.0	+	-	2.0
S. flexneri type 2a,2b (4)	10.0	+	-	2.0
S. flexneri type 2a (1)	12.0	+	-	2.0
S. flexneri type 2a,3a (2)	13.0	+	-	2.0
S. flexneri type 2a (1)	14.5	+	-	2.0
S. flexneri type 2a,1a (3)	15.0	+	-	2.0
S. flexneri type 2a (1)	30.5	+	-	2.0
S. boydii type1-6,7-11 (3)	2.0	+	-	2.5
S. boydii (1)	4.0	+	-	2.5
S. boydii type 1-6 (2)	6.0	+	-	2.5
S. boydii type 7-11 (1)	6.5	+	-	2.5
S. boydii typ 7-1,12-15(5)	9.0	+	-	1.5(1)2.5(4)
S. boydii type 12-15 (1)	10.0	+	-	1.5
S. boydii type 7-11 (1)	12.0	+	-	2.5
S. boydii type 1-6 (1)	13.0	+	-	2.5
S. boydii type 7-11 (1)	15.5	+	-	2.5
S. boydii type 7-11 (1)	20.0	+	-	2.5
S. sonnei form1 (s) (1)	0.0	+	-	3.0
S. sonnei form1 (s) (2)	2.0	+	-	3.0
S. sonnei form1 (s) (1)	4.0	+	-	3.0
S. sonnei form1 (s) (1)	5.0	+	-	3.0
S. sonnei form1 (s) (1)	9.0	+	-	3.0
S. sonnei form 2 (r) (1)	26.0	+	-	AA[c]
S. sonnei form 2 (r) (1)	27.0	+	-	AA
S. sonnei form 2 (r) (1)	32.0	+	-	AA
S. sonnei form 2 (r) (1)	33.0	+	-	AA
S. sonnei form 2 (r) (1)	33.5	+	-	AA
S. sonnei form 2 (r) (1)	34.4	+	-	AA
S. sonnei form 2 (r) (1)	41.0	+	-	AA

[a] data represent an average of three values determined on the same day;

[b] The SAT values for the smooth pigmented(Pcr+) and non-pimented(Pcr-) strain were similar;

[c] AA indicate auto-aggregation in physiological saline.

Table 4 Chronological incidence rates of the *Shigella* isolates by species, serogroup and serotype levels as obtained during the 1974 to 1985 collections.

Shigella spp.	Number of prevalent shigellae by years of study periods of shigellosis								
	Serogroup and Serotypes	1974-1978		1978-1980		1980-1982		1974-1985	
		No.	%[a]	No.	%	No.	%	No.	%
S. dysenteriae Group A	Total[b]	48	29.1	118	32.8	ND		395	26.7
	Serotype 1	32	66.7	77	65.3	ND		194	49.1
	" 2	16	33.3	30	25.4	ND		58	14.9
	" 3	ND		3	2.5	ND		106	26.8
	" 4	ND		4	3.4	ND		12	3.0
	" 6	ND		2	1.7	ND		10	2.5
	" 7	ND		2	1.7	ND		15	3.8
S. flexneri Group B	Total	81	49.1	182	50.6	350	82.0	912	61.6
	Serotype 1	ND		63	34.6	107	30.6	197	21.6
	" 2	ND		49	29.9	118	33.7	419	45.9
	" 3	ND		3	1.7	21	6.0	47	5.2
	" 4	ND		48	26.4	67	19.1	153	16.8
	" 5	ND		0	0.0	1	0.3	1	0.1
	" 6	ND		19	10.4	36	10.3	95	10.4
S. boydii Group C	Total	19	11.5	41	11.4	77	18.0	137	9.3
	Serotype 1	ND		7	17.1	13	16.9	20	14.6
	" 2	ND		1	2.4	14	18.2	15	10.9
	" 3	ND		2	4.9	4	5.2	6	4.4
	" 4	ND		10	24.4	20	26.0	30	24.4
	" 5	ND		5	12.2	21	27.3	26	22.0
	" 8	ND		6	1 4.4	19	24.7	25	18.2
	" 9	ND		1	2.4	ND		1	0.7
	" 10	ND		3	7.4	4	5.2	7	5.1
	" 12	ND		1	2.4	ND		1	0.7
	" 14	ND		1	2.4	4	5.2	5	3.7
S.sonnei Group D	Total	17	10.3	19	5.3	ND		36	2.4
	Serotype 1	ND		19	5.3	ND		19	
Grand Total		165	100.0	360	100.0	427	100.0	1480	100.0

[a] Percentage of each serotype is taken out of the total of each respective serogroup;

[b] Percentage of each serogroup is taken out of the total of each year's collection.

ND = Not done.

During that time no attempt was made to identify the prevalence of the other *Shigella* serogroups at their serotype levels. However, in later periods, many studies have tried to assess the prevalence of the *Shigella* serogroups and their serotypes as exemplified in the following chronologically presented sequence.

For example, Messele Gedebu and Alebachew Tassew (1982) tried to assess the frequency of isolation and *in-vitro* drug sensitivity of the *Shigella* species in Addis Ababa. These investigators were able to show that, out of the 105 *Shigella* isolates studied, 70% were *S. flexneri*, 15% *S. dysenteriae,* 10% *S. boydii* and 5% *S. sonnei*. Similarly in 1985, Mogessie Ashenafi and Messele Gedebou (1985) conducted a study on adult diarrhoeal disease in

Addis Ababa and found a total of 90 *Shigella* isolates from 1000 out-patients and showed the prevalence of the different strains to be in the order of frequency of *S. flexneri, S. dysenteriae, S. boydii* and *S. sonnei.*

Despite the apparently infrequent occurrence of shigellaemia, or the paucity of reports on it, there were report of 100 cases earlier in 1962 indicating that all the 4 *Shigella* species had been incriminated, of which *S. dysenteriae* was reported to account for the majority of cases of *Shigella* septicaemia (Barrett-Connor and Connor, 1969). Polymicrobial septicaemia due to bacteria other than *Shigella* species had been relatively widely reported (Hermans and Washington, 1970; Kiani *et al.*, 1979; Mackowiak *et al.*, 1980), while there was only one earlier report from elsewhere showing polymicrobial septicaemia involving *Shigella* (Mackowiak *et al.*, 1980). In Ethiopia, a single report by Genene Bekele *et al.* (1986) had shown, from two cases, the isolation of polymicrobial septicaemia involving *Shigella* species. One of these cases was a diabetic patient with chronic renal failure admitted for dysentery and shock and whose blood culture yielded *S. dysenteriae* with other enteric pathogen. The second case was a patient admitted for abdominal cramps, diarrhea, fever and chills with *S. flexneri* grown from his blood culture. Both results had indicated that *Shigella* species can be isolated both from stool and blood samples in cases of diarrhea and septicaemic infections. It should thus be well noted by clinicians that *Shigella* could be incriminated in either monomicrobial or polymicrobial septicaemia and such awareness would be very useful for possible presumptive diagnosis, encouraging blood culture and the initiation of better empirical therapy. It should be regular practice to take blood cultures in all suspected cases of stool culture-confirmed bacillary dysentery associated with fever.

In a study conducted in a rehabilitation camp in Korem, Ethiopia, among two hundred patients with diarrhea, it was possible to determine the presence of pathogens in their stool samples (Desenclos *et al.*, 1988). A total of 42 (21.1%) of these camp residents were patients with a positive culture for the main pathogenic member of the Enterobacteriaceae, the isolation rate of which was 15.6% for *Escherichia coli*, 3.5% for *Shigella* spp. and 2.0% for *Salmonella* spp. In another study conducted from 1994 to 1996 to determine the prevalence of *Shigella* strains and *Salmonella* species among the outpatients of the Gondar College Teaching Hospital, it was found that, out of 7,993 miscellaneous specimens cultured, 147 yielded *Shigella* and 80 *Salmonella* isolates (Abreham Aseffa *et al.*, 1997). In this study, *S. flexneri* and *S. dysenteriae* were the most frequently isolated species among the

Shigella at the rate of 58.5% and 36.7%, respectively.

Another *Shigella*-related diarrhoeal case study on 700 out-patients in Addis Ababa had shown that 50 *Shigella* strains were isolated of which serogroup 'A' composed 28%, 'B' 44%, 'C' 18% and 'D' 10% (Abebe Mache *et al.*, 1997). As has repeatedly been reported, diarrhea in developing countries is an important cause of morbidity and mortality, especially among children, mainly due to poor socio-economic factors and sanitary conditions (Guerrant *et al.*, 1990). This was evidenced by a few studies in Ethiopia, too, among which the Jimma study, conducted as a cross-sectional survey to determine the prevalence of *Shigella* sero-groups and their antibiotic resistance pattern among paediatric out-patients in Southwest Ethiopia, could be cited here (Abebe Mache, 2001). The results showed that out of the 77 *Shigella* isolates, sero-group 'A' comprised 29.9%, 'B' 40.3%, 'C' 10.4% and 'D' 10.4%, which was very identical to the results of the above study report from Addis Ababa. In one of the more recent studies conducted in 1998 at a pediatrics hospital in Addis Ababa (Senait Kebede *et al.*, 1999), among the 39 clinically confirmed diarrhoeagenic children patients, it was observed that the rates of isolation of the four *Shigella* serogroups were in the order of *S. flexneri* (59%), *S. dysenteriae* (28.2%), *S. boydii* (7.7%) and *S. sonnei* (5.1%).

On the other hand, currently diarrhoea is becoming one of the major clinical problems in HIV-infected patients which indicates that there is a need to monitor the antimicrobial susceptibility patterns of enteric bacterial pathogens in order to ensure appropriate treatment and control of the HIV-associated diarrhoeal disease. Thus on the basis of this concept, a cross-sectional study was conducted from Feb-July 2001 to isolate and determine the magnitude of potential bacterial pathogens from the stool of HIV-infected and HIV-non-infected patients and their antimicrobial susceptibility patterns in Jimma Hospital, Southwest Ethiopia, (Mohammed Awole *et al.*, 2002). In this study a total of 372 consecutive HIV sero-positive and sero-negative patients presented to Jimma Hospital for different illnesses were selected for faecal sample collection and culturing for the isolation of various enteric bacterial pathogens like *Salmonella, Shigella* and *Campylobacter* species. Stool Zehl-Nielson staining was also done for the identification of *Mycobacterium* species. Among 99 HIV-infected patients with diarrhoea, 25 (25.0%) had enteric bacteria consisting of 8 (8.1%) *Salmonella*, 4 (4%) *Shigella* and 13 (13.1%) *Campylobacter* species. *Mycobacterium* species were identified in 3 (3%) of the stool specimens of these patients. Similarly, in another study which compared the prevalence of

Yersinia enterocolitica isolates to the commonly encountered enteropathogens of diarrhoeagenic bacterial groups in diarrhoeal patients of Addis Ababa, it was observed that among the stool samples of 205 patients only 3 (1.5%) were positive for *Yersinia enterocolitica*, whereas the *Salmonella* species were isolated from 22 (10.7%) patients and *Shigella* species from 12 (5.8%) (Birhanu Andualem and Aberra Geyid, 2003). This 5.8% frequency of *Shigella* isolation rate in all age groups was lower than the 11.7% isolation rate at Tikur Anbassa and Ethiop-Swedish Children's Hospital reported by Daniel Asrat *et al.* (1999).

Afeworki Gebre-Yohannes and Eyassu Habte-Gabr (1984) conducted a study to identify the common *Shigella* serotypes prevailing in Addis Ababa and in some rural areas of Ethiopia, with the hope that it would serve as a base-line for further studies on the essential properties and surveillance of *Shigella* strains up to their serotype levels. For this study, they took a total of 360 *Shigella* isolates of which 273 were from Addis Ababa and 87 from rural areas (Fig. 1). All of these isolates were obtained from the EHNRI stock collections of the bacteriological culture of stool samples collected predominantly from adult patients with clinical symptoms of shigellosis. All these isolates were obtained from the diagnostic service done during the period between January 1974 and February 1985. The isolates were biochemically identified, serogrouped and serotyped using the standard slide agglutination method with commercially available antisera. These were then stored at -70°C in TSY broth with 25% glycerol for further studies. From the results it was thus possible to observe the most prevalent *Shigella* serogroups and serotypes based on their differences in chronology and urban to rural isolation rates (Table 4 and 5, respectively).

In all the studies of 1974-1978, the serogroup *S. flexneri* was found to be the most highly prevalent followed by *S. dysenteriae*, *S. boydii*, and *S. sonnei* This was also observed in the study of 1978-1980, where *S. flexneri, S. dysenteriae*, *S. boydii*, and *S. sonnei* were isolated at a frequency of 50.6%, 32.8%, 11.% and 5.3%, respectively (Afeworki Gebre-Yohannes and Drasar, 1987) (Table 4). In another study, conducted in 1988 (Afeworki Gebre-Yohannes and Drasar, 1988), it was noted that among a total of 1002 *Shigella* strains collected between 1974 and 1985, the *S. boydii* serogroup consisted of 135 strains while the other rare serogroup member, *S. sonnei,* consisted of only 71 strains. The serotype prevalence in *S. boydii* isolates was analyzed from these stocks and it was observed that serotypes 4, 5, 8, 1 and 2 were commonly encountered in 21.9%, 19%, 18.3%, 14.6% and 10.9%, respectively, while serotypes 6, 7, 11 and 15 were not isolated at all

during these study periods (Table 4). The serogroup distribution in urban and rural areas was comparable for *S. dysenteriae*, *S. flexneri* and *S. sonnei*, while *S. boydii* was significantly more common in the urban than the rural areas. Although the total number of isolates studied was significantly smaller in the rural 87 (24.2%) than in the urban 273 (75.8%) cases, among a total of 360 strains, the percentage rates in almost all serogroups appeared to be slightly higher in the rural than the urban areas (Fig. 1 and Table 5).

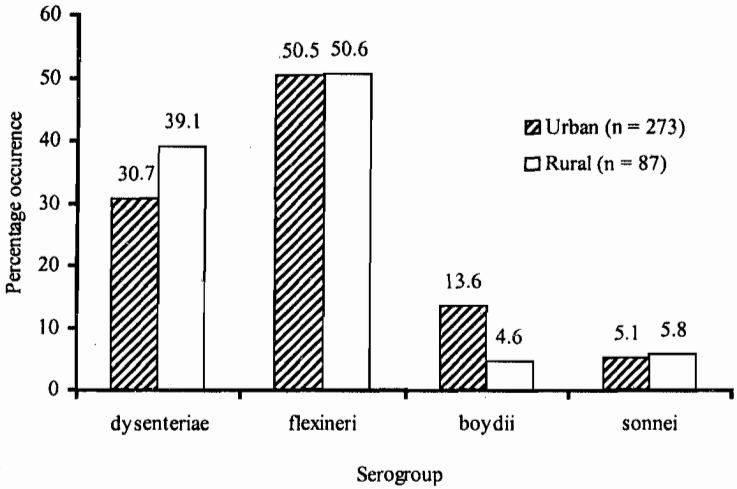

Fig. 1. *Shigella* serogroups: urban vs rural.

The predominance of *S. flexneri* in developing countries was also reflected by the study of Afewowrki Gebre-Yohannes and Eyassu Habte-Gabr (1984a) to be true in Ethiopia, too, as indicated by the yearly incidence result of its serotypes shown in Fig. 2 and 3 (Afewowrki Gebre-Yohannes, 1984). It was also shown that, as in the other developing countries, all four serogroups co-existed albeit in different proportions. *S. boydii* seemed to be among the commonest isolates in the urban centers and this, probably, indicated the importance of non-endemic strains in such centers. From the result of this study, it was also possible to observe that among the 32 known serotypes, 22 were *S. dysenteriae* serotype 1 (Shiga's bacillus) showing an incidence rate as high as 21.4% (Afeworki Gebre-Yohannes and Eyassu Habte-Gabr, 1984a). Similar high incidence rates of this serotype were also reported from many outbreaks in other countries, such as those on a coral island in the Bay of Bengal, affecting 33% of its entire population in three months (WHO, 1974 cited in Afewowrki Gebre-Yohannes and Eyassu Habte-Gabr, 1984a). Other outbreaks due to this serotype had also occurred

in Bangladesh (Rahman *et al.*, 1975), Sri Lanka (WHO, 1979 cited in Afewowrki Gebre-Yohannes and Eyassu Habte-Gabr, 1984a) and in rural Somalia (Cahill *et al.*, 1966). Among the *S. flexneri* serotypes, the findings of this study showed that type 1 was commonly obtained at a rate of 34.8% and followed by type 2 at 26.9%, whereas similar studies in other countries such as in Australia (Morahan and Hawksworth 1970) and Vietnam (Ricosse 1968) showed that type 2 serotype was reported to be the commonest of all isolates. Just as in Nigeria (Wazuzu Acholonu 1978 cited in Afeworki Gebre-Yohannes and Eyassu Habte-Gabr, 1984a), the study in Ethiopia indicated that type 4 of the *S. boydii* serotypes was found to be common with 24.4% frequency of isolation and closely followed by the serotype 5 with 22.0% (Afeworki Gebre-Yohannes and Eyassu Habte-Gabr, 1984a).

Table 5 Prevalence of *Shigella* serotype rates by urban vs rural study sites.

Serogroup	Serotype	U rban		R ural	
		N=273	100%	N=87	100%
A	1	47	17.2	30	34.5
	2	27	9.9	3	3.5
	3	3	1.1	-	-
	4	3	1.1	1	1.1
	6	2	0.7	-	-
	7	2	0.7	-	-
Total		84	30.7	34	39.1
B	1	47	17.2	16	18.4
	2	35	12.8	14	16.1
	3	1	0.4	2	2.3
	4	38	13.9	10	11.5
	6	17	6.2	2	2.3
Total	-	138	50.5	44	50.6
C	1	1.8		2	2.3
	2	1	0.4	-	-
	3	2	0.7	-	-
	4	8	2.9	2	2.3
	5	9	3.3	-	-
	8	6	2.2	-	-
	9	1	0.4	-	-
	10	3	1.1	-	-
	12	1	0.4	-	-
	14	1	0.4	-	-
Total	-	37	13.6	4	2.3
D	1	14	5.1	5	5.8

Comparing the urban/rural incidence of shigellosis, it was observed that the overall rate of *S. dysenteriae* type 1 in the urban study areas was about half that of the rural study areas. Similarly, 22 serotypes from the urban report were comparable to only 11 serotypes in the rural areas. This might indicate that more than three times as many urban isolates of *Shigella* could occur as the rural ones although the possibility of serotype importation to urban areas

could not be ruled out. The monthly *Shigella* isolation (Afeworki Gebre-Yohannes and Eyassu Habte-Gabr, 1984a; Afeworki Gebre-Yohannes and Drasar, 1987) has clearly indicated that shigellosis was a year-round incidence of diarrhoeal disease in Ethiopia. The highest incidence rates of *Shigella* during the months of June and September coincided with the beginning and end of the heavy rains in this country.

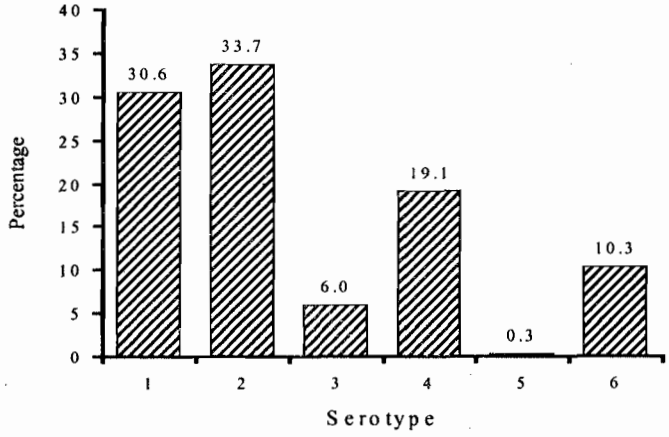

Fig. 2. Serotypes of *S. flexneri* Isolates (1978 – 1982).

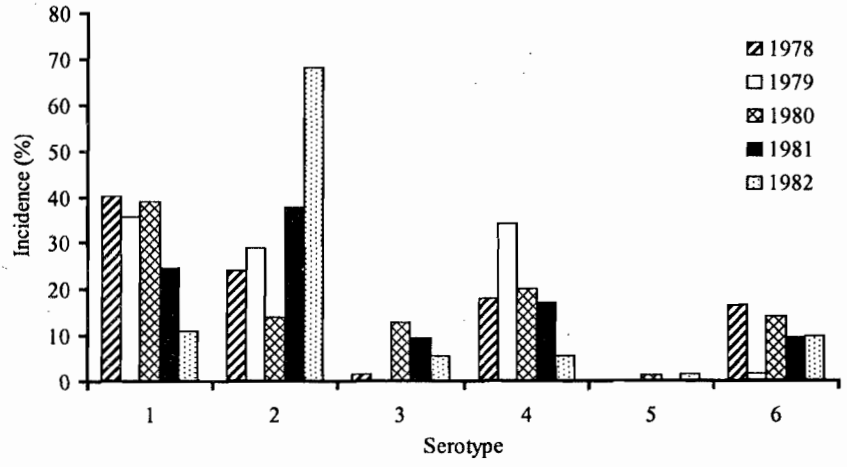

Fig. 3. Yearly incidence of the *S. flexneri* serotypes included in the study (1978-82).

In another similar study, the same group of investigators (Afeworki Gebre-Yohannes and Drasar, 1987), took 945 *Shigella* strains from their stocks of

January 1978 to December 1985, identified them at serotype level and analysed their annual distribution over an eight-year period, and mapped the seasonal distribution of shigellosis in Ethiopia. During this study, it was observed that *S. dysenteriae* and *S. flexneri* had 23.3% and 56.3% isolation rates, respectively. From this observation, the annual serotype fluctuation, as shown in Fig. 4 and 5 (Afeworki Gebre-Yohannes and Drasar, 1987), was very marked in *S. dysenteriae* serotype 1 followed by the serotype 2 until 1984, the period when serotype 3 emerged to be the predominant strain. By 1985, the isolation rates of serotypes 1, 2 and 3 became comparable, while serotypes 4, 6 and 7 were not commonly encountered. Similarly, the results on serotype fluctuation of *S. flexneri*, (Fig. 6 and 7) (Afeworki Gebre-Yohannes and Drasar, 1987), indicated that, up to 1981 serotype 1 was dominant followed by types 2 and 4. After 1981, type 2 became the most commonly encountered serotype in all the study areas. Serotype 5 was observed, for the first time, in 1981. During 1981 to 1985, type 6 accounted for about 10% of the yearly isolation (Afeworki Gebre-Yohannes and Eyassu Habte-Gabr, 1984a; Afeworki Gebre-Yohannes and Drasar, 1987).

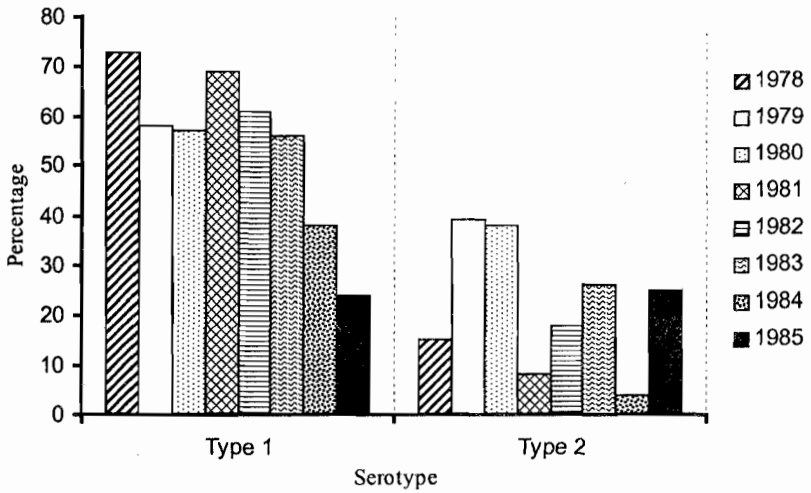

Fig. 4. *S. dysenteriae* serotypes 1 and 2: yearly fluctuations (1978-85).

In all the earlier studies on the prevalence of *Shigella*-associated diarrhoeal diseases, it was possible to confirm that, as in other developing countries, *S. flexneri* was the dominant serogroup causing shigellosis in Ethiopia, too. Its isolation rates, together with that of *S. dysenteriae*, accounted for about 80% of the total shigellosis-causing agents of the country. It is clear, therefore, that the epidemiology of shigellosis in Ethiopia has been mainly determined by these two species. At the same time the high endemic nature of *S.*

dysenteriae serotype 1 (Shiga's bacillus) had clearly been demonstrated by these works, although a relatively decreased result was shown to exist for this strain after 1981. As a result, it was assumed that this might have been replaced by the dominance of serotype 3 since then. Among the *S. flexneri* serotypes, type 1 was dominant until 1981. After this, however, type 2 became the most dominant strain in many study areas, which could be associated with drug resistance problems related to *S. flexneri* serotypes.

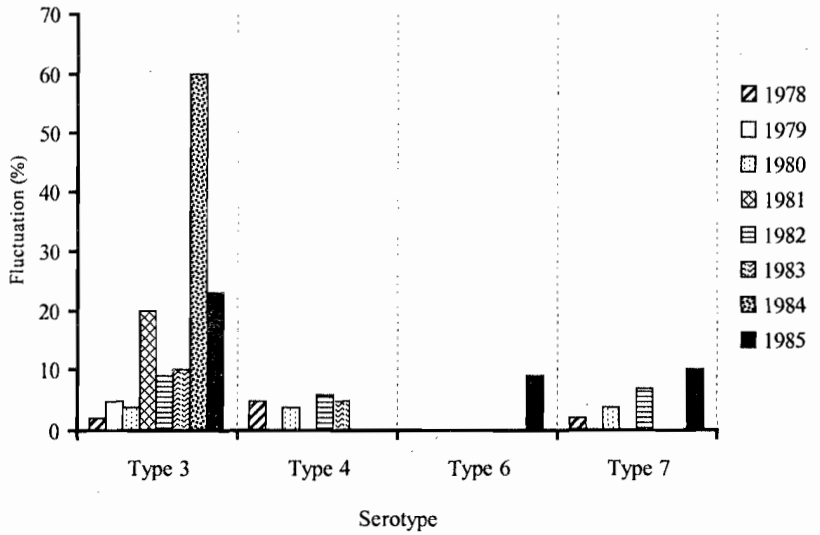

Fig. 5. *S. dysenteriae* types 3, 4, 6 and 7: yearly fluctuations (1978-85).

The monthly isolation of *Shigella* species was found to be comparatively more common during the months of April to June, where prevalence of >12% was recorded. This was a bit less (8-10%) during the months of March, July and September (Afeworki Gebre-Yohannes and Drasar, 1987). The changes of having less prevalence of the general *Shigella* isolates (<6%) in the months of August and November to January were shown to be true within the groups of *S. flexneri* than those of *S. dysenteriae*. In this study, too, like the studies elsewhere, it was shown that similar patterns of two peaks of seasonal distribution were observed, one in June and the other in September (Afeworki Gebre-Yohannes, 1980). These two peaks overlapped with the beginning and end of the heavy rainy season in Ethiopia (Afeworki Gebre-Yohannes and Drasar, 1987). Another significant observation was that seasonal fluctuation was noted more in *S. flexneri* than in *S. dysentreriae*. In Hungary (Rundai *et al.*, 1981, cited in Afeworki Gebre-Yohannes and Drasar, 1987), where *S. sonnei* was the dominant

serogroup, the yearly July-September peak was associated with increased *S. sonnei* isolation and not with that of *S. flexneri* (which was distributed evenly in every season).

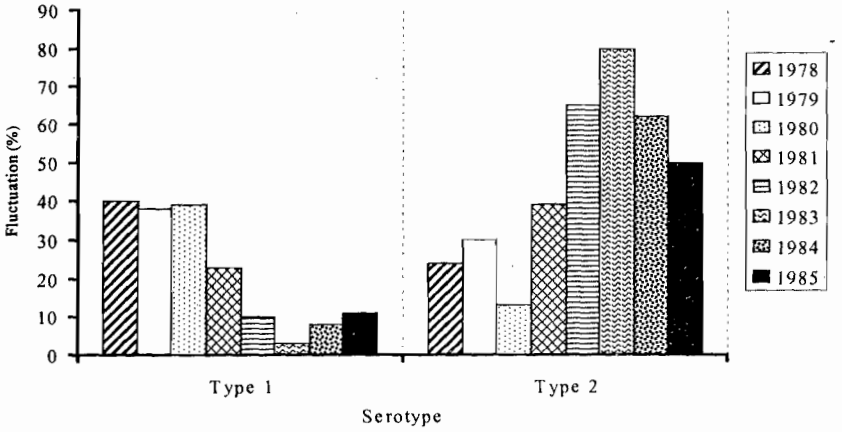

Fig. 6. *S. flexneri* serotypes 1 and 2: yearly fluctuations (1978-85).

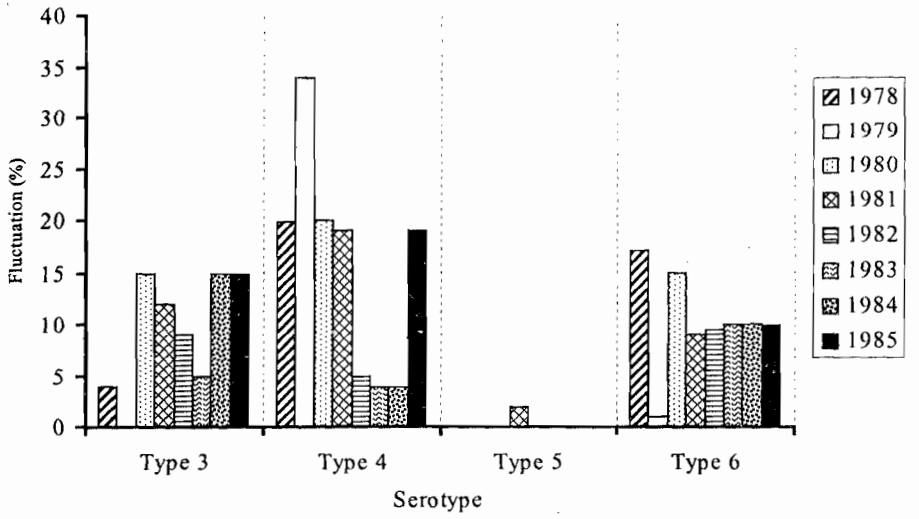

Fig. 7. *S. flexneri* serotypes 3, 4, 5 and 6: yearly fluctuations (1978-85).

Drug Resistance Pattern in *Shigella* pecies

In addition to their prevalence in tropical and subtropical regions, *Shigella* spp. are notorious for their multiple drug resistance properties. In 1968-70, a single strain of *S. dysenteriae* serotype 1, which produced resistance to chloramphenicol (C), tetracycline (T), streptomycin (S) and sulphonamides

(Su), caused extensive epidemics in Central America (Mata *et al.*, 1970, Gangarose *et al.*, 1970, Mendizabal-Morris *et al.*, 1971, Reller *et al.*, 1971) and to a lesser extent in Mexico (Olarte *et al.*, 1971). A similar outbreak of shigellosis due to ampicillin-resistant *S. dysenteriae* type 1 was also reported from Mexico (Olarte *et al.*, 1971, 1976) and Bangladesh (Rahman *et el.*, 1974) in 1972 and 1974, respectively. On the other hand, in Somalia, Mero (1976) recorded a multiple drug resistance property of this same organism against as many as 7 drugs.

After these original reports from Central America and Mexico on the emergence of multiple drug resistant *Shigella* species, many other reports on shigellosis were also repeatedly showing the occurrence of drug resistant *Shigella* species to most of the commonly used antibiotics. These included the strains of *S. dysenteriae* type 1, which had consistently been associated with unusual multiple drug resistance to antibiotics such as trimethoprim-sulphamethoxazole (Sxt), kanamycin (Kmn), gentamycin (Gmn), and nalidixic acid (Na). The Central American epidemic due to *S. dysenteriae* type 1 showed a TCSSu pattern (Reller *et al.*, 1971) while that in Bangladesh had a TCASSu pattern (Rahaman *et al.*, 1975). Similar results had also been obtained from the study in Mexico City (Olarte *et al.*, 1976). Frost *et al.* (1981) had characterized plasmid X-carrying *S. dysenteriae* type 1 in Central Africa that encoded resistance to TCASSu.

Shigella flexneri, the most dominant serogroup associated with clinical shigellosis in developing countries, has also been shown in various studies elsewhere to have diverse sensitivity patterns to commonly used drugs showing its far reaching clinical and epidemiological significance. These reports also included the repeatedly emerging multiple-drug resistance patterns of the various serotypes within this serogroup. Multiple drug resistance in specific *S. flexneri* serotypes had been reported from other countries (Afeworki Gebre-Yohannes and Yetnebersh Limeneh, 1980). TCASSu pattern, shown by *S. flexneri* type 2a strains, was observed, for example, in Cape Town (Watson, 1967). Lewis (1967) reported *S. flexneri* type 2a with TCASSuNa pattern from an outbreak in a mental hospital. In Brazil it was also reported that multiple drug resistance pattern to eight antibiotics, TCAKFNSSu, was observed in 2 out 16 strains of *S. flexneri* type 2 (Piechaud *et al.*, 1974), whereas the TCASSu pattern was seen in *S. flexneri* type 2 and 6 in France (Szturm-Rubinsten *et al.*, 1969). In Sweden *S. flexneri* isolates frequently were associated with resistance to 3 or more drugs (Hansson *et al.*, 1981).

Shigella boydii and *S. sonnei* are not commonly isolated in many developing countries, which may be due to less adequate laboratory follow up of these species. *S. boydii* had never been a dominant species in any country (Afeworki Gebre-Yohannes and Drasar, 1988a). However, it is unique among the shigellae in that multiple drug resistance is a comparatively limited property (Nowortyta, 1972, Afeworki Gebre-Yohannes and Yetnebersh Limeneh, 1980). Whether or not this is due to its low isolation rate and, therefore, lower contact with other resistant bacteria, still remains to be seen. Nowortyta (1972) reported the TSu pattern for the *S. boydii* serotypes in Poland. On the other hand, *S. sonnei* was the dominant species in the developed countries, where it was found with significant resistance to useful drugs like co-trimoxazole (Finlayson, 1984). In a study of 590 *S. sonnei* isolates from 21 countries in the 5 continents, it was found that SSu was the most commonly (60%) observed pattern (Sztum-Rubinsten *et al.*, 1974). Furthermore, the resistance pattern to 7 drugs, TCAKSSuSxt, was also reported for *S. sonnei* strains from Spain (Loppz-Brea *et al.*, 1983).

In Ethiopia, there has been ample clinical indication of multiple drug resistance within *Shigella* species (Afeworki Gebre-Yohannes and Yetnebersh Limeneh, 1980). This knowledge of their drug-resistance properties had well attracted the interests of many researchers like Afeworki Gebre-Yohannes and his groups. For example, Messele Gedebou and Alebachew Tassew (1982), in their study of the frequency of isolation of *Shigella* strains in Addis Ababa and their *in vitro* drug sensitivity pattern, found out that all or most of the *Shigella* strains they isolated were susceptible to cephalothin, gentamicin, kanamycin, polymyxin B and trimethoprim-sulphamethoxazole. Frequencies of susceptibility to ampicillin, carbenicillin and chloramphenicol were 79%, 80% and 75%, respectively. Only 37%, 23% and 58% were susceptible to streptomycin, sulphadiazine and tetracycline, respectively. They also found that the resistance to one or more drugs was detected in 85% while 72% were multiple-resistant strains. They showed 24 different resistance patterns, varying from resistance to one drug to resistance against seven drugs combined. This study also showed that, at least until then, trimethoprim-sulphamethoxazole was the best alternative drug for treating shigellosis, particularly in regions with multiple drug-resistant strains. Similarly, the study of Mogessie Ashenafi and Messele Gedebou (1985), again in Addis Ababa, also showed that almost all *Shigella* isolates were sensitive to cephalothin, gentamicin, kanamycin, polymyxin B and trimethoprim-sulphamethoxazole. About 175 were sensitive to the 11 drugs tested and

multiple-resistance was detected in 62% of their isolates while the most common was to six drugs (27%).

Afeworki Gebre-Yohannes and his groups were also able to indicate that *S. flexneri* and *S. dysenteriae*, the hyper-endemic strains together, comprised about 80% of the total *Shigella* isolates and that these serogroups had already given enough clinical and epidemiological problems with their multiple drug resistance properties. Although there were ample clinical indications of multiple drug resistance within *Shigella* species, there had not been much satisfactory laboratory reference works on this subject matter, except only for the single study by Messele Gedebou and Alebachew Tassew in 1979 on the multiple drug resistance and R-factor in 69 *Shigella* strains. Later after 1980, Afeworki Gebre-Yohannes with different group members (Afeworki Gebre-Yohannes and Yetnebersh Limenih, 1980; Afeworki Gebre-Yohannes, 1984; Afeworki Gebre-Yohannes and Eyassu Habte-Gabr, 1984b; Afeworki Gebre-Yohannes and Drasar, 1988a), conducted different studies with the aim of assessing the multiple drug resistance within *Shigella* species, in general, and its four serogroups, in particular, hoping that it would serve as base-line work for further identification of specific serotypes emerging as multiple drug resistant strains. For these purpose, they used a total of 165 *Shigella* isolates at one time (Afeworki Gebre-Yohannes and Yetnebersh Limenih, 1980) and around 360 at other times (Afeworki Gebre-Yohannes, 1984; Afeworki Gebre-Yohannes and Eyassu Habte-Gabr, 1984b; Afeworki Gebre-Yohannes and Drasar, 1988a) from among the 1974-1985 culture collections. Applying the standard agar-disc diffusion sensitivity testing of the Kirby-Bauer method, all the strains were tested against the commonly used drugs: cephalothin (Ce), tetracycline (T), chloramphenicol (C), ampicillin (A), carbenicillin (Cb), kanamycin (K), polymyxin-B (Po), streptomycin (S), trimethoprim-sulphamethoxazole (Sxt) and sulphadiazine (Su) antibiotics.

As indicated in Table 6 below the highest resistance level within *Shigella* species as a whole, was to sulphadiazine (63.6%) followed by tetracycline, streptomycin, chloramphenicol, carbenicillin, and ampicillin. The *Shigella* strains tested were always sensitive to trimethoprim-sulphamethoxazole, cephalothin, polymyxin-B and gentamycin, while only a single strain (0.6%) of the total 165 *Shigella* isolates was resistant to kanamycin. These results of the sensitivity tests were similar to the findings of many other authors elsewhere. However, unlike this study in Ethiopia, limited resistance to cephalothin, and susceptibility to Sxt was noted by Schlossberg in 1975

(Afeworki Gebre-Yohannes and Dekker, 1981). In some countries, like Austria it was observed that 100% susceptibility of *Shigella* species to kanamycin and 59% to Sxt were recorded.

Table 6 Drug resistance within *Shigella* species and serogroups A, B, C and D.

Drugs	All R-S. spp (n=165)		Group A (n = 48)						Group B (n = 81)		Group C (n = 19)		Group D (n = 17)	
			All		Ty2-10		Type 1							
	No	%	No	%	No	%	No	%	No	%	No	%	No	%
Su	105	63.6	40	83	8	50.0	32	100.0	51	63.0	2	11.1	13	76.5
T	87	52.1	33	69	5	31.3	29	90.6	43	53.1	3	16.7	8	47.1
S	76	46.1	38	81	8	50.0	30	94.0	29	35.8	3	16.7	11	64.7
C	41	24.8	28	58	2	12.3	26	81.3	10	12.3	1	5.6	2	11.8
A	37	22.4	26	54	1	6.3	25	78.1	10	12.3	0	0.0	1	5.9
Cb	38	23.0	26	54	1	6.3	25	78.1	11	13.6	0	0.0	1	5.9
K	1	0.6	1	2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
G	0	0.0	0	0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Sxt	0	0.0	0	0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Cep	0	0.0	0	0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Po	0	0.0	0	0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0

Su = Sulphadiazine; T = Tetracycline; S = Streptomycin; C = Chloramphenicol; A = Ampicillin; Cb = Carbinicillin; K = Kanamycin; G = Gentamycin; Sxt = Trimethroprim-sulphametoxazole; Cep = Cephalothin; Po = Polymyxin-B; F = Framycetine; N = Neomycin; Nx = Nalidixic acid.

Serogroup A was shown to have the highest overall resistance levels, followed by serogroups B, D and C in that order (Table 6). Within serogroup A, *S. dysenteriae,* serotype 1 showed unusually high resistance level to Su in 100%, Str in 93.8%, T in 90.6%, C in 81.3%, and both A and Cb in 78.1% each. It had also been noted that the non-shiga serotypes (that is, serotypes 2-10) of serogroup A were comparatively very sensitive to many drugs and had low rate of resistance against those like Su and S in 50% each, T in 31.3%, C in 12.3% and A and Cb in 6.3% each.

Afeworki Gebre-Yohannes and Yetnebersh Limenih (1980) had also shown that multiple drug resistance pattern was observed among the *Shigella* serogroups as indicated in Table 7. Resistance to 6 drugs was shown by 31 (18.8%) of all the *Shigella* species. The corresponding proportions of strains consecutively resistant to a combination of less than six drugs were also shown to be 2 (1.2%) strains resistant to 5 drugs, 9 (5.4%) to 4 drugs, 25 (15.2%) to 3 drugs, 35 (21.2%) to 2 drugs and 13 (7.9%) to 1 drug. Within serogroup A, *S. dysenteriae* type 1 again showed multiple drug resistance to 6 drugs in 75% of the strains. On the other hand, only 30.3% of the whole *Shigella* species were sensitive to all tested drugs, while none of the *S. dysenteriae* serotype 1 isolates was uniformly sensitive to all drugs.

Therefore, this baseline study demonstrated the existence of multiple drug resistance within *Shigella* spp., in general, and within serogroups A and B, in particular. Among serogroup A, all the *S. dysenteriae* serotype 1 strains were responsible for drug resistance against 1-6 combined drugs (Table 7).

Table 7 Multiple drug resistance by *Shigella* species, serogroups A, B, C, D and Shiga's bacillus from the 1974-1978 sample collections.

No. of Drugs	All species (n = 165)		Group A(n = 48)						Group B (n = 81)		Group C (n = 19)		Group D (n = 17)	
			All As		Shiga's		Ty2-10							
	No	%	No	%	No	%	No	%	No.	%	No.	%	No.	%
1 drug	13	7.9	1	2.1	1	3.1	0	0.0	9	11.1	2	10.5	1	5.9
2 "	35	21.2	5	10.4	2	6.3	3	12.5	25	30.9	0	0.0	5	29.4
3 "	25	15.2	4	8.3	3	9.4	1	6.2	15	8.5	1	5.3	5	29.4
4 "	9	5.4	4	8.3	1	3.1	3	12.5	3	3.7	1	5.3	1	5.9
5 "	2	1.2	2	4.2	1	3.1	1	6.2	0	0.0	0	0.0	0	0.0
6 "	31	18.8	24	50.0	24	75.0	0	0.0	6	7.4	0	0.0	1	5.9
allSen	50	30.3	0	16.7	0	0.0	8	50.0	23	28.4	15	78.9	4	23.5

A = *S. dysenteriae*; B = *S. flexneri*; C = *S. boydii*; D = *S. sonnei*; Shiga's = *S. dysenteriae* type 1 (Shiga bacillus); Ty2-10 = Serotypes 2-10 of *S. dysenteriae*.

Later in 1984, Afeworki Gebre-Yohannes (1984), Afeworki Gebre-Yohannes and Eyassu Habte-Gabr (1984b), Afeworki Gebre-Yohannes and Drasar (1988a, 1989a) analyzed drug resistance not only at the genus and the four serogroup levels but also at the specific serotypes level. They conducted this work on 360 *Shigella* isolates taken from the culture collection stocks of January 1974 to February 1980 and 140 additional isolates from another collection stock of 1980-1985 at the Bacteriology Division of the former National Research Institute of Health (NRIH). From the results of these studies it was observed that a total of 14 resistance patterns, represented by 70.6% were obtained among the total of 500 *Shigella* isolates of the whole collections of 1974-1985 (Table 8). The isolates tested were resistant against 1 to 6 multiple drugs and no single pattern that included antibiotics like Cep, Pol, G and Sxt were encountered in the study. The most common pattern was TSSu (16.0%), followed by TSu (15.2%) and TCACbSSu (14.2%). The drug-resistance pattern to six drugs (TCACbSSu) was most common in *S. dysenteriae* (55.3%), while the TSu pattern was common in *S. flexneri* (43.9%), and the TSSu pattern in *S. sonnei* (38.4%), *S. flexneri* (17.9%) and *S. boydii* (11.4%).

According to the above results, there were nine resistance patterns within *S. dysenteriae*, only four of which were common showing the highest rate of multiple-drug resistance patterns in over 4% of strains, TCASSu and TCACbSSu being the most highly repeated patterns in 85.0% and 81.8% of the *S. dysenteriae* type 1 strains, respectively (Table 9). One strain of this serotype was resistant to 7 drugs, TCACbKSSu (data not shown). As shown in the same table of results, *S. dysenteriae* type 2 was commonly associated with the TCSSu pattern (29.9%). The prominence of TCACbSSu pattern in rural areas was directly related to the Shiga's bacillus prevalence (Table 10).

Table 8 Common patterns of drug resistance in 500 *Shigella* isolates and the major prevalent serogruops from the 1974-1985.

Resistance pattern	All *Shigella* spp.		*S. dysenteriae*		*S. flexneri*		*S. boydii*		*S. Sonnei*	
T	10	2.0	0	0.0	3	1.7	3	2.1	4	5.5
S	5	1.0	0	0.0	0	0.0	5	3.6	0	0.0
Su	23	4.6	2	1.8	16	9.2	3	2.1	2	2.7
TA	2	0.4	0	0.0	0	0.0	2	1.4	0	0.0
TS	9	1.8	0	0.0	0	0.0	5	3.6	4	5.5
TSu	76	15.2	0	0.0	76	43.9	0	0.0	0	0.0
SSu	40	8.0	8	7.0	8	4.6	18	12.9	6	8.2
TAS	2	0.4	0	0.0	0	0.0	2	1.4	0	0.0
TSSu	80	16.0	5	4.4	31	17.9	16	11.4	28	38.4
TACS	11	2.2	0	0.0	0	0.0	11	7.9	0	0.0
TASSu	3	0.6	0	0.0	0	0.0	3	2.1	0	0.0
TCSSu	18	3.6	15	13.2	1	0.6	1	0.7	1	1.4
TACSSu	3	0.6	0	0.0	0	0.0	2	1.4	1	1.4
TCACbSSu	71	14.2	63	55.3	7	4.0	0	0.0	1	1.4
All Sen.	147	29.4	21	18.4	31	17.9	69	49.3	26	35.6
TOTAL	500	100.0	114	100.0	173	100.0	140	100.0	73	100.0

Table 9 Patterns of drug resistance in *S. dysenteriae* types 1, 2 and 3 from the 1974-1985 collections.

Resistance pattern	*S. dysenteriae* serotypes							
	Total		Type 1		Type 2		Type 3	
	No.	%	No.	%	No.	%	No.	%
S	30	13.9	0	0.0	30	52.7	0	0.0
Su	1	0.5	1	0.8	0	0.0	0	0.0
TSu	8	6.8	0	0.0	0	0.0	0	0.0
SSu	3	1.4	0	0.0	1	1.8	2	7.7
ACT	5	2.3	5	3.8	0	0.0	0	0.0
CSSu	1	0.5	1	0.8	0	0.0	0	0.0
TSSu	7	3.2	3	2.2	2	3.5	2	7.7
ACST	2	0.9	0	0.0	0	0.0	2	7.7
TCSSu	26	12.0	9	6.7	17	29.9	0	0.0
TACSSu	130	60.2	113	85.0	0	0.0	17	65.4
TACKSSu	1	0.5	1	0.8	0	0.0	0	0.0
TCACbSSu	63	83.4	63	81.8	0	0.0	0	0.0
All Sensitiv	10	4.6	0	0.0	7	12.3	3	11.5
Total	216	100.0	133	100.0	57	100.0	26	100.0

A multiple drug resistance pattern similar to that of *S. dysenteriae* types was exhibited by *S. flexneri* type 2a in Cape Town (Watson 1967). In the above study by Afeworki Gebre-Yohannes (1984) and others (Afeworki Gebre-Yohannes and Eyassu Habte-Gabr, 1984a,b), this TCACbSSu, type of resistance pattern was observed only in serotype 1 of *S. dysenteriae* at 81.8%, while it was observed in serotypes 1, 2, 3 and 4 at 3.9%, 6.3%, 15.0% and 4.5% of the *S. flexneri* tested isolates, respectively (Table 8, 9 and 11). Such multiple resistant pattern of *S. flexneri* serotypes, shown to three and more drugs, was also observed in various studies conducted at the same time in countries like Brazil, France and Sweden (Szturm-Rubinsten *et al.*, 1969; Piechaud *et al.*, 1974; Hansson *et al.*, 1981).

Table 10 Common patterns of drug resistance in the 360 total isolates and those from urban vs rural sites.

Resistance pattern	All *S.* spp. (n = 360)		*S.* spp. of Ur (n = 273)		*S.* spp. of Ru (n = 87)	
	No.	%	No.	%	No.	%
T	6	1.7	5	1.8	1	1.2
Su	23	6.4	21	7.7	2	2.3
TSu	76	21.4	57	20.9	19	21.8
SSu	20	5.6	19	7.0	1	1.2
TSSu	45	12.5	31	11.4	14	16.1
TCSSu	18	5.0	14	5.1	4	4.6
TCACbSSu	71	19.7	41	15.0	30	34.9
All Sensitive			72	26.4	13	14.9

Like in other developing countries, it was already confirmed that in Ethiopia also *S. flexneri* was the dominant serogroup associated with clinical shigellosis. Very early studies in Ethiopia had shown that resistance to less than or equal to three drugs (e.g., T, Su, Tsu and TSSu) was frequently encountered in *S. flexneri* isolates (Mesele Gedebu and Alebachew Tassew, 1979; Afeworki Gebre-Yohannes, 1980). The only multiple drug resistance pattern, which was shown in those earlier studies by a few strains of *S. flexneri*, was the TCACbSSu pattern. In 1982, there was a significant increase in the TCACbSSu pattern, and a sudden emergence of novel resistance patterns like, the TACbSSu, TACbS and TCACbS. As a result, it could be observed that a total of 59.7% of *S. flexneri* were associated with only the above mentioned four groups of multiple drug resistance patterns (Table 11). In addition, chloramphenicol sensitivity associated with the TACbSSu and TACbS resistance patterns was only at intermediate levels. Eight and six strains only were found to be resistant to Sxt and Kmn together with other drugs, respectively (Afeworki Gebre-Yohannes and Drasar, 1988a). It is interesting to note that, as of 1982, the role of Ampicillin in *Shigella* chemotherapy was greatly weakened, if not entirely lost. Another point of particular interest observed from the above mentioned Ethiopian studies was the fact that the TCACbSSu pattern was seen not only in sertoype 2, but also in serotypes 1, 3 and 4 but not in serotype 6 (Table 12). This situation is in contrast to the above-mentioned studies conducted elsewhere (Watson *et al.*, 1967; Szturm-Rubinsten *et al.*, 1969; Piechaud *et al.*, 1974), and to the situation in *S. dysenteriae* in Ethiopia (Messele Gedebu and Alebachew Tassew, 1979; Afeworki Gebre-Yohannes, 1980), where such resistance was shown to be restricted to a single serotype 1 (Shiga's bacillus). The fact remained, however, that the newer resistance patterns (TACbSSu, TACbS and TCACbS) in 1982 (Table 11) were exclusively associated with serotype 2 (Table 12), which predominated during the last two years of the study period (Table 11). The sudden increase of multiple drug resistance especially in 1982 was assumed to be a cause for

alarm, which was expected to be probably due to plasmid-mediated transfer of genetic information coding for multiple drug resistance (Afeworki Gebre-Yohannes, 1984). The continuous indiscriminate use of antimicrobial agents in Ethiopia was thus partially responsible for the above situation.

Table 11 Yearly occurrence of the common resistance pattern in *S. flexneri* from 1974 -1982.

Resistance pattern		1978		1979		1980		1981		1982	
		No.	%	No.	%	No.	%	No.	%	No.	%
Su	(29)	7	10.4	4	5.5	7	8.2	8	15.1	3	4.2
TSu	(116)	24	35.8	33	45.2	34	40.0	19	35.8	6	8.3
TSSu	(57)	12	17.9	22	30.1	12	14.1	7	13.2	4	5.6
TACbS	(7)	0	0.0	0	0.0	0	0.0	0	0.0	7	9.7
TCACbS	(7)	0	0.0	0	0.0	0	0.0	1	1.9	7	9.7
TACbSSu	(19)	0	0.0	0	0.0	0	0.0	3	5.7	16	22.2
TCACbSSu	(37)	4	6.0	1	1.4	13	15.3	6	11.3	13	18.1
Sensitive	(37)	14	20.9	7	9.6	10	11.8	7	13.2	9	12.5
Total		67	100	73	100	85	100	53	100	72	100

Table 12 Patterns of drug resistance in the various serotypes of *S. flexneri* isolates of 1974-1985 studies.

Resistance pattern	*S. flexneri* serotypes											
	Type 1		Type 2		Type 3		Type 4		Type 6		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
T	1	0.5	0	0.0	0	0.0	2	1.8	0	0.0	3	0.4
Su	11	6.0	2	0.7	3	7.5	1	0.9	13	18.6	30	4.2
TS	0	0.0	0	0.0	0	0.0	0	0.0	2	2.9	2	0.3
TSu	68	37.4	1	0.3	2	5.0	43	38.4	3	4.3	117	16.5
SSu	1	0.5	5	1.6	2	5.0	1	0.9	21	30.0	30	4.2
TAC	1	0.5	4	1.3	0	0.0	3	2.7	0	0.0	8	1.2
TSSu	75	41.2	45	14.8	9	22.5	44	39.3	8	11.4	181	25.6
TAS	0	0.0	2	0.7	0	0.0	0	0.0	0	0.0	2	0.3
TACS	1	0.5	61	20.1	0	0.0	1	0.9	0	0.0	63	8.9
TASSu	1	0.5	2	0.7	0	0.0	0	0.0	0	0.0	3	0.4
TACbS	0	0.0	7	2.3	0	0.0	0	0.0	0	0.0	7	1.0
TACCbS	0	0.0	7	2.3	0	0.0	0	0.0	0	0.0	7	1.0
TACSSu	8	4.4	95	31.3	6	15.0	6	5.4	3	4.3	118	16.7
TACbSSu	0	0.0	19	6.3	0	0.0	0	0.0	0	0.0	19	2.7
TACKSSu	2	1.1	2	0.7	0	0.0	1	0.9	0	0.0	5	0.7
TACSSuSx	2	1.1	4	1.3	0	0.0	1	0.9	0	0.0	7	1.0
TACCbSSu	7	3.8	19	6.3	6	15.0	5	4.5	0	0.0	37	5.2
TACKSSuSx	0	0.0	1	0.3	0	0.0	0	0.0	0	0.0	1	0.1
Sens. to all	4	2.2	28	9.2	12	30.0	4	3.6	20	28.6	68	9.6
Total	182	100	304	100	40	100	112	100	70	100	708	100

According to results on Table 13 (column 7), all the six serotypes of the 350 *S. flexneri* isolates were studied but only serotypes 1,2, and 4 combined comprised 83.4% of the total isolates. The yearly incidence of *S. flexneri* serotypes is also shown in this Table (columns 2-6) in which one can observe that, as of 1981, *S. flexneri* serotype 1 seemed to have given way of its dominance to the serotype 2 strains in Ethiopia. On the basis of the multi-resistant *S. flexneri* isolates reported from other countries and earlier studies from Ethiopia, Afeworki Gebre-Yohannes (1984) analyzed the yearly difference in patterns of all their serotypes identified during the 1978-1982 collection of *Shigella* spp. (Afeworki Gebre-Yohannes and Drasar, 1988a,c).

Table 13 The incidence rate of *S. flexneri* serotypes collected during the years of 1978 to 1982.

S. flexneri	1978		1979		1980		1981		1982		Total	
serotypes	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Type 1	27	40.3	26	35.6	33	38.8	13	24.5	8	11.1	107	30.6
Type 2	16	23.9	21	28.8	12	14.1	20	37.7	49	68.1	118	33.7
Type 3	1	1.5	0	0.0	11	12.9	5	9.4	4	5.6	21	6.0
Type 4	12	17.9	25	34.2	17	20.0	9	17.0	4	5.6	67	19.1
Type 5	0	0.0	0	0.0	0	0.0	1	1.9	0	0.0	1	0.3
Type 6	11	16.4	1	1.4	12	14.1	5	9.4	7	9.7	36	10.3
Total	67	100	73	100	85	100	53	100	72	100	350	100

From the patterns of drug resistance in *S. flexneri* shown in Table 12, one can see that there were more than 15 patterns of drug resistance in the isolates of the 1978 to 1985 study period. However, only five patterns (Su, TSu, TSSu, TACSSu and TCACbSSu) comprised 78.2% of the total patterns and a sudden increase of multiple resistant strains was observed from 1982 onwards. The common patterns obtained in 1982 (Table 8c) included TACbSSu (22.2%), TCACbSSu (18.1%), TACbS (9.7%) and TCACbS (9.7%) (Table 11).

The main purpose of this analysis was to demonstrate the worsening situation of drug resistance problem, particularly in relation to *S. flexneri* and to relate such resistance to specific serotypes. The analysis of the common resistance patterns of the common drugs as shown in Table 12 indicates that serotypes 1, 2, 3, 4, and 6 have exhibited different rates of patterns for similar or varying drug combinations.

Shigella boydii is unique among all *Shigella* isolates in that multi-drug resistance is comparatively limited (Nowortyta 1972, cited in Afeworki Gebre-Yohannes and Drasar, 1988a). This could be attributed to its low isolation rate and, therefore, lower contact with other resistant bacteria. The most common drug resistance pattern within *S. boydii* serotypes was TSSu (11.4%). The rate of resistance patterns to SSu and TSSu together by *S. boydii* was 12.12% as observed in the 1984 study and was later increased to 24.3% in the 1988 study (Table 8) indicating significant problem in the clinical and epidemiological status of even the supposedly rare serogroup members. Over 50% of *S. boydii* isolates were sensitive to all drugs tested and among the remaining 50% significant resistance was shown only to streptomycin, sulphadiazine and tetracycline. In Ethiopia, this situation is in sharp contrast to *S. dysenteriae* and *S. flexneri*, where multiple drug resistance is still very high.

Strains of *S. sonnei* were found to be resistant in both studies of 1984 and 1988 by Afeworki Gebre-Yohannes and Eyassu Habte-Gabr (1984b) and

Afeworki Gebre-Yohannes and Drasar (1988a), always to sulphadiazine, streptomycin and tetracycline in the patterns of TSSu and SSu in 38.4% and 8.2% or more, respectively (Table 8). These were comparable to the results of multiple drug resistance patterns obtained elsewhere. However, in the developed countries significant resistance to useful drugs like Sxt was observed as in the study in Spain, where resistant pattern of TACSSuKSxt was reported (Lopoz-Brea *et al.*, 1983). From these studies it could be observed that the less endemic *Shigella* species, like *S. boydii* and *S. sonnei* in Ethiopia were comparatively sensitive to many of the commonly used drugs, which was assumed to be due to their low isolation rates, and thus less contact with R-factor bearing bacteria (Afeworki Gebre-Yohannes and Drasar, 1988a).

Plasmid Mediated Drug Resistance Analysis of *Shigella* species

Bacterial plasmids are replicons stably inherited as extra-chromosomal state of circular, double stranded DNA accessory elements that have been found to encode in more than 50 bacterial genera (Kopecko *et al.*, 1980, 1990). These genetic units are among the most important tools used to detect *Shigella* strains in faeces using DNA amplification methods (Frankel *et al.*, 1990). They can also mediate, among other bacterial properties, resistance to many antibiotics (Afeworki Gebre-Yohannes and Drasar, 1989b). In fact, plasmid encoded drug resistance has become the single most important mechanism by which bacteria exchange genetic information and become resistant to most useful drugs (Guiney, 1984). Studies by Moller *et al.* (1976) have shown that most naturally occurring strains of Enterobacteriaceae contain plasmids of different molecular weights.

The phenomenon of R-plasmid mediated multiple antibiotic resistance was first noted in Japan during the 1950's in bacterial strains that were resistant simultaneously to chloramphenicol, streptomycin, sulphonamides and tetracycline (Afeworki Gebre-Yohannes and Drasar, 1988b, c). It was then proposed in 1960 that the factor governing such multi-resistance could be transmitted by cellular contact (Akiba *et al.*, 1960). Almost at the same time, Ochial *et al.* (1959) were able to transmit resistance *in vitro* among strains of the genus *Shigella* and *Escherichia coli*.

Within the genus *Shigella*, R-plasmid mediated antibiotic multi-resistance has been observed to be especially common in *S. dysenteriae* type1, Shiga bacillus (Afeworki Gebre-Yohannes and Drasar, 1988c). Some of the examples reported as the first incidences of this property included the Central American populous epidemic in 1969-1971, where Shiga bacillus

with plasmid-mediated antibiotic multi-resistance to CSSuT appeared with devastating effects (Mata *et al.,* 1970). The second example is that followed by a severe but limited outbreak in Mexico City in 1970 (Olarle *et al.,* 1976), where the strain contained a transmissible plasmid (R-type CSSu) and a mobilisable plasmid encoding ampicillin resistance. R-plasmid mediated drug resistance types CSSuT and ACSSuT occurred in India (Macaden *et al.,* 1980) and Bangladish (Crosa *et al.,* 1977). The other example could be that reported by Frost *et al.* (1981), where isolates of Shiga bacillus with R-type ACSSuT from Somalia carried conjugative group-X plasmids coding for ACT, which mobilized the SSu-determinant. On the other hand, isolates of Shiga bacillus with the above R-type from Central African epidemic had conjugative ACT plasmids that failed to mobilize the SSu-determinant (Frost *et al.,* 1981; Ebright *et al.,* 1984). As it is well known, shigellosis is a major health problem in developing countries, where conditions favour the existence of endemic diseases. The emergence of plasmid-bearing antibiotic multi-resistant *Shigella* strains had been widely recognized, and has already remained a serious problem for chemotherapy with continuous transmission and sporadic outbreaks or epidemics of serious proportions in these countries. Since then, the R-factor-mediated drug resistance in Shiga bacillus had been reported repeatedly from developing countries including those from Africa (Afeworki Gebre-Yohannes and Drasar, 1988b).

The level of plasmid-mediated drug resistance in *S. flexneri,* which is the dominant species in developing countries, has not been amply studied, as most laboratory studies were centered on *S. dysenteriae* type 1 (Shiga bacillus) from outbreaks and epidemics of bacillary dysentery (Afeworki Gebre-Yohannes and Drasar, 1988b). In a study on transferable drug resistance in *S. flexneri* conducted by Frost and Rowe (1983), it was reported that over 60% of these strains with ACSSuT resistance type could not transfer it to an *E. coli* recipient. Analysis on serotype level indicated that this characteristic is associated with *S. flexneri* type 2 and not with 1, 3 and 4 (Afeworki Gebre-Yohannes and Drasar, 1988b). Resistance types ACST and AC_iST in *S. flexneri* type 2 were also non-conjugative. Transferable ACST resistance was reported in 2 of 8 *S. flexneri* isolated from Korea (Chun, 1984) and 65 of 86 isolates from India (Agarwal *et al.,* 1984).

Within the last few years, some of the previously arcane methods of molecular biology have been simplified (Afeworki Gebre-Yohannes and Drasar, 1990a). According to Howe (1982), the appearance of conjugally

transferable resistance among bacteria had been recognized as consequence of the use of antibiotics for some 25 years. It is possible to use relatively simple, quick and inexpensive methods to prepare plasmid DNA from bacterial isolates and to visualize it after agarose gel electrophoresis. Accordingly, the separation of these plasmids by this method shows strain-specific patterns (finger-prints) and such molecular analysis of plasmids has been applied to the investigation of epidemiological problems of bacterial infections (Afeworki Gebre-Yohannes and Drasar, 1990a). Prado *et al.* (1987) evaluated the usefulness of plasmid analysis for typing *S. sonnei* isolates. Similarly, the plasmid profile analysis of *S. dysenteriae* type 1 of African and Asian strains had been described elsewhere by many investigators (Ebright *et al.*, 1984; Frost *et al.*, 1985; Haider *et al.*1988).

From repeated results of many studies in many developing countries including Ethiopia, it had been confirmed that *S. dysenteriae* and *S. flexneri* were the dominant serogroups causing both clinical and epidemiological shigellosis problems. In Ethiopia, we have tried to show above that the prevalence of various *Shigella* serotypes has been described and their resistance patterns determined by various workers (Afeworki Gebre-Yohannes and Yetnebersh Limenih, 1980; Afeworki Gebre-Yohannes and Dekker, 1981; Afeworki Gebre-Yohannes and Eyassu Habte-Gabr, 1984a, b; Afeworki Gebre-Yohannes and Drasar, 1987, 1988a, 1989a) at different times. *S. flexneri* and *S. dysenteriae* were then found to comprise over 80% of the total *Shigella* isolates. However, reports on R-plasmids first appeared only in few studies of Messele Gedebu and Alebachew Tassew (1979) and similarly studies on plasmid profile and R-plasmid characterization had never been attempted until the late 1980's, when Afeworki Gebre-Yohannes and his group (Afeworki Gebre-Yohannes and Drasar, 1987, 1988b, c, 1989b, 1990a, b, 1991) started to design different studies of such genotypic characterization of the major *S. dysenteriae* and *S. flexneri* serotypes as indicated below.

In order to determine the prevalence of R-plasmids in specific serotypes of *S. dysenteriae* and *S. flexneri*, Afeworki Gebre-Yohannes and Drasar (1988b, c), designed a study by using standardized methods, which included determination of transfer frequency and mobilization of non-conjugative plasmids. For this study, they used a total of 199 *S. dysenteriae* and 412 *S. flexneri* isolates resistant to one or more antibiotics and belonging to serotypes 1, 2, 3, 4, 6 and 7 of *S. dysenteriae* as well as serotypes 1 - 6 of *S. flexneri*, to examine, by an overnight one-step broth-mating with *Escherichia coli* K-12 and, if non-conjugative, additionally by triparental

crosses with the conjugative plasmids X and Δ. Direct transfer of plasmids was examined by the Anderson and Threlfall (1974) methods, whereby broth cultures of *Shigella* strains and the recipient strains (*E.coli* K-12, F-, Lac+, Nal prototrophic) were grown to exponential phase with continuous agitation at 37°C. Equal volumes (0.5 ml) of the cultures were mixed and incubated overnight at 28 and 37°C. Then 0.01 ml of serial ten-fold dilution preparations in PBS were spread with a calibrated loop on MacConkey agar containing the respective antimicrobial agent. After an overnight incubation period of these selective plates, the ex-conjugant colonies were counted and 5-10 colonies from each selective plate were resistance-typed by agar dilution method (Steers *et al.*, 1959). Transfer frequency was expressed as the proportion of resistance progeny per recipient cell. For the non-conjugative plasmids mobilization by triparental crosses with Fi+, group F_{II} plasmid X and the F', group I_1 plasmid Δ, was assessed using the same procedure as for the direct transfer except that 0.5 ml of donor and 0.5 ml of intermediate host (containing X or Δ) were incubated for 18 hr at 37°C before the addition of 1 ml of the final recipient (*E. coli* K12).

In Table 14a the comparative transfer frequencies of conjugative plasmids in the Shiga bacillus with R-type ACSSuT isolated during the years 1974-1979 and 1980-1985 are indicated. Table 14b shows the spectrum of donor R-type encountered, the number with conjugative plasmids, the R-phenotype transferred to *E. coli* K-12 with the estimated transfer frequencies (for *S. dysenteriae* type 1). Of these isolates 96% harboured conjugative plasmids and most of these transferred at high frequencies. Similarly, Table 15 shows those for *S. flexneri* isolates. Conjugative plasmids coding for ACSSuT resistance transferred at low frequencies from strains isolated during 1974-1979, while after 1980, 50% of the conjugative plasmids transferred at high frequencies and expressed only the ACT determinant in recipients. Transferable or mobilisable antibiotic resistance detected in *S. dysenteriae* 2, 3, 4, 6 and 7 is shown in Table 14b, too. Conjugative plasmids coding for SSuT resistance were detected in *S. dysenteriae* types 2, 3 and 4. Non-conjugative SSu determinants in type 3 were mobilized by transfer factors X and Δ. The CSSuT phenotype in types 2 and 7 and the ACST phenotype in type 3 were neither transferable nor mobilisable. It is possible that some of these isolates of the Ethiopian Shiga bacillus are related to the earlier indicated African strains. The above cited preliminary plasmid finger-print analysis of *S. dysenteriae* type-1 isolates and their R-plasmid bearing *E. coli* counterparts had shown that the strain, which first appeared in Ethiopia in 1980, was identical with the Zairian strain (Frost *et*

al., 1985), whereas the second strain was endemic to Ethiopia and may have an independent R-plasmid evolution (Afeworki Gebre-Yohannes and Drasar, 1988c).

Table 14a Transfer kinetics of conjugative plasmids in *S. dysenteriae* type 1 with R-type ACSSuT isolated during the years 1974-79and 1980-85.

Transfer[a] frequency	Number (%) of transfer plasmids in isolates from		
	1974-1979	1980-1985	
		Plasmid	R-Type ACT
10^{0}			1 (1.9)
10^{-1}		5 (9.3)	13 (24.1)
10^{-2}			12 (22.2)
10^{-3}			
10^{-4}	2 (3.8)		
10^{-5}	10 (18.9)	4 (7.4)	
10^{-6}	24 (45.3)	10 (18.5)	
10^{-7}	16 (30.2)	9 (16.7)	
10^{-8}	1 (1.9)		
Total	53 (100.1)	28 (51.9)	26 (48.1)

[a] Overnight crosses in broth mating at 37°C with selection on media containing chloramphenicol, ampicillin or tetracycline.

In the case of *S. flexneri*, results in Table 15 show that conjugative plasmids of type 1 were detected in most of its resistance types (R-types), even though the overall drug transfer rate was 24 of 124 isolates (19.4%). For *S. flexneri* type 2, 13 of the 180 strains (17.2%) transferred their resistance partially or in total to the recipient strain. Most of the strains with R-types ACSSuT and ACiSSuT among those of type 2 were unable o transfer these resistances to an *E. coli* recipient strain which, thus, confirms the similar findings obtained elsewhere by Frost and Rowe (1983). The changing pattern of drug resistance in this *S. flexneri* type 2, compared between two ranges of years (1974-79 and 1980-85), was also shown in Table 16 of the work by Afeworki Gebre-Yohannes and Drasar (1988b). During the 1974-79 work the only significant R-type was SSuT. After 1980, R-types like ACSSuT, ACST, ACiST and ACT became common. But resistance types ACST and ACiST in these *S. flexneri* type 2 were non-conjugative. Significant plasmids coding for multi-drug resistance were noted in *S. flexneri* type 4, while only a few conjugative plasmids were detected in types 3 and 6 as shown in Table 15, although these serotypes were comparatively rare in Ethiopia (Afeworki Gebre-Yohannes and Drasar, 1988b). Armed with this array of multiple drug resistance, *S. flexneri* type 2 replaced type 1 as the dominant serotype in Ethiopia (Afeworki Gebre-Yohannes and Drasar, 1987). No comparable increase in multiple-drug resistance was observed in the other serotypes of these *S. flexneri* strains (Afeworki Gebre-Yohannes and Drasar, 1988b). In this Ethiopian study, it

was also found that conjugative plasmids coding for SSuT were present in types 1,2,3,4 and 6. In agreement with the other previous studies elsewhere (Frost and Rowe, 1983), the SSu-determinant in all the Ethiopian *S. flexneri* serotypes with R-type SSu were non-conjugative.

Table 14b Transferable and mobilizable antibiotic resistance in strains of *S. dysenteriae* types1, 2, 3, 4, 6 and 7 isolated in Addis Ababa, Ethiopia (1974-1985).

Donor N. R-type*	Serotype	Direct transfer[a]		Mobilization[c]		Transfer frequency[d]		
		No tested	No. with R-plasmid (R-type)[b]	No. tested	No. mobilized (R-type)	High	Medium	Low
1. Su	1	1	1(Su)	-	-	+	-	-
2. S	2	12	0	4	0	-	-	-
3. SSu	3	2	0	2	2(Δ,SSu)[e] (X,SSu)	-	-	-
4. SSu	4	5	0	3	0	-	-	-
5. SSu	6	3	0	2	0	-	-	-
6. SSu	7	2	0	2	0	-	-	-
7. ACT	1	5	5(ACT)	-	-	+	-	-
8. CSSu	1	1	1(CSSu)	-	-	+	-	-
9. SSuT	1	3	3(T)	-	-	-	+	-
10.SSuT	2	2	2(SSuT) +(T)	-	-	-	-	-
11.SSuT	3	2	2(SSuT)+ (T)	-	-	-	-	-
12.SSuT	4	1	1(SSuT)	-	-	-	-	-
13.CSSuT	1	9	8(CSSuT)	-	-	+	-	-
14.CSSuT	2	17	0	3	0	-	-	-
15.CSSuT	7	2	0	1	0	-	-	-
16.ACST	3	2	0	2	0	-	-	-
17.ACSSuT	1	112	107(ACT	-	-	+57%	+43%	-
18.ACSSuT	3	17	0	-	3(Δ,SSu) (X,SSu)	-	-	-
19.ACKSSuT	1	1	1(AC)(AK (ACKSiSu (ACSiSuT	-	-	+	-	-
20.ACKSSuTTp	1	1	1(AC)(ACSiSuTTp) (TTp)	-	-	+	-	-
Total		199	132	22	5			

* Resistance type

[a] Overnight broth culture at 37°C and 28°C

[b] *E. coli* K 12,F⁻, Lac⁺, Nal^r, prototrophic; Enteric Reference Laboratory (ERL no. 14R525)

[c] Triparental crosses with plasmids X (ERL no. 48R626) and Δ (ERL no.RT641);

[d] High: $10^{0} - 1^{-2}$, Medium: $10^{-3} - 10^{-5}$, Low:$10^{-6} - 10^{-8}$;

[e] Higher transfer in Δ-mediated transfer ($10^{-3} - 10^{-4}$).

A, ampicillin; C, chloramphenicol; K, kanamycin; S, streptomycin; Su, sulphadiaxine; T, tetracycline; Tp, trimethoprim; Nx, nalidixic acid; i, intermediate resistance.

Table 15 Plasmid-mediated drug resistance *S. flexneri* types 1, 2, 3, 4, 6 and 7 isolated in Addis Ababa (1974-1985).

	Serotypes 1, 2, and 3			Serotypes 4, 5 and 6			
No. donor R-type[a](No.)	Serotypes	No. tested	No.with conjugative R-plasmid[b] (R-type)[a]	Donor R-type[a](No)	Serotypes	No. of tests	No. with conjugative R-plasmid[b] (R-type)[a]
1. Su(4)	1	4	0 –	Su(1)	4	1	0 –
Su(1)	3	1	0 –	Su(1)	6	1	0 –
				S(1)	6	1	0 –
2. SiSu(8)	1	8	0 –	SiSu(1)	4	1	0 –
SiSu(2)	2	1	0 –	SiSu(16)	6	5	0 –
SiSu(2)	3	1	0 –	SiT(1)	5	1	1(T)
SSu(7)	2	3	0 –	Su(1)	4	1	0 –
-	-	-	-	SSu(5)	6	3	0 –
3. AT(1)	1	1	1 (AT)	-		-	-
4. SuT(14)	1	1	1 (T)	SuT(23)	4	6	0 –
SuT(2)	3	1	0 –	SuT(2)	6	1	1 (T)
-	-	-	-	SiT(2)	4	1	1 (T)
-	-	-	-	SiT(2)	6	1	1 (T)
5. SiSuT(59)	1	2	2(SSuT)(T)	SiSuT(34)	4	8	0 –
SSuT(14)	1	5	5 (SSuT)	SSuT(10)	4	4	1 (SSuT)
SSuT(44)	2	12	6 (SSuT)	SSuT(5)	6	4	1 (SSuT)
SSuT(9)	3	3	1 (SSuT)	ASiT(1)	4	1	1 (AT)
-	-	-	-	ACT(2)	4	1	1 (ACT)
6. ACSiT(2)	1	1	1 (ACT)	ACSiT	4	1	1 (ACT)
ACSiT(6)	2	3	1 (ACSiT)	-		-	-
ACSuT(1)	1	1	ACSuT,ACT	-		-	-
ACSiSu(1)	2	1	0 –	-		-	-
ACST(27)	2	27	0 –	-		-	-
ACiST(29)	2	27	0 –	-		-	-
ASiSuT(1)	1	1	1 (AT)	-		-	-
ASSuT(2)	2	1	0 –	-		-	-
CSSuT(1)	2	1	1 (CSSuT)	-		-	-
KSSuT(1)	2	1	1 (KSSuT)	-		-	-
7. AiCSSuT(3)	1	3	2(ACSi,SuT),1 (ACST)3(ACT	ACSSuT(7)	4	3	3(ACS,SUTi) 1(ACT)
ACSSuT(6)	1	5	5(ACSiSuT)1 ACT,1(ACSuT	ACSSuT(3)	6	3	1 (Su)
ACSSuT(53)	2	53	(2)ACSiSuT	-		-	-
ACiSSuT(40)	2	40	(8)ACSSuT,1A	-		-	-
ACSSuT(6)	3	2	(2)ACSSu,2AT	-		-	-
ASSuTTp(1)	2	1	(3)CSSuT(2)T (7)AT(1)SSuT (2)AC(1)SSu	-		-	-
8. ACKSSuT(2)	1	2	2(ACKSSuT), 1(ACSSuT)	ACKSSuT	4	1	1(ACKT), 1(CT)
ACSSuTTp(2)	1	2	2(ACSSuTTp)	-		-	-
ACSSuTTp(3)	2	3	2(ACSSuTTp) 1(ACSSuTp) 1(CSSuTTp) 1(CTTp)1(Tp)	-		-	-
ACiSSuTTp(1)	2	1	1(ASSuTTp)	ACiSSuTTp	4	1	1(ACKT) 1(CT)
9.ACKSSuTTp(1	2	1	1(ACKSSuTTp	-		-	-
10. Sensitive(4)	1	-	-	Sensitive(3)	4	-	-
Sensitive(28)	2	-	-	Sen(20)	6	-	-
Sensitive(12)	3	-	-	-		-	-
Total = 2716	1, 2 and 3	213	37	147	4,5 and 6	50	14

[a] Resistance type.

[b] Recipient *E. coli* K12, F⁻, Lac⁺, Nx^r, prototrophic , Enteric Reference Laboratory number (ERL no. 14R525). Drug abbreviation is as given in Table 14b.

Table 16 Changing patterns of drug resistance in *S. flexneri* type 2 (1974-79 and 1980-85).

R-type[a] (No.)	1974-79 Number (year)[b]	1980-85 Number (year)
SSu (7)	4 (1976-1979)	3 (1980-1982)
SSiu (2)	0	2 (1980-1982)
ACS (1)	0	1 (1984)
ACT (4)	0	4 (1985)
ASiT (2)	1 (1978)	1 (1982)
SSuT (44)	24 (1976-1979)	20 (1980-1985)
ACiSSu (1)	0	1 (1984)
ACST (27)	0	27 (1981-1985)
ACiST (24)	0	24 (1982-1985)
ACiSiT (5)	0	5 (1982-1983)
ACSiT (7)	1 (1977)	6 (1980-1985)
ASSuT (2)	0	2 (1981-1982)
CSSuT (1)	1 (1979)	0
KSSuT (1)	0	1 (1981)
ACSSuT (53)+ACiSSuT(40)	1 (1977) + 0 –	52(1980-85)+40(1981-85)
ASSuTTp(1)	0	1 (1983)
ACSSuTTp(4)+AciSSuTTp(1)	0 + 0	4 (1982-1983) + 1 (1983)
ACKSSuTTp (1)	0	1 (1984)
Sensitive (28)	18 (1974-1979)	10 (1980-1985)
TOTAL = 256	50 (1974-1979)	206 (1980-1985)

[a] Resistance type.

[b] Year of strains isolation and collection. Drug abbreviation, Table 14.

CONCLUSION AND RECOMMENDATIONS

In conclusion, it could be deduced, from the results of this baseline and other follow-up studies on the genus *Shigella* conducted by Afeworki Gebre-Yohannes and many other groups that the prevalent *Shigella* serogroups and serotypes, at least up to 1985, were recorded. Their data could also be used to map out the yearly serotype changes and seasonal distribution of the two most commonly encountered *Shigella* species in Ethiopia, *S. dysenteriae* and *S. flexneri.* The analysis of the recent surveys, that is constitutively conducted also shows the same pattern of distribution of the serotypes of these *Shigella* serogroups. Similarly, the antimicrobial resistance related studies conducted so far have demonstrated that R-plasmid mediated antibiotic resistance did occur more among the different serotypes of the *S. flexneri* and *S. dysenteriae* isolates than the other serogroups and that these organisms formed a reservoir of plasmids which can transfer antibiotic resistance to other members of the Enterobacteriaceae in the Ethiopian population.

Therefore, the recommendations that one can make following these conclusions of the reviewed issues could be: -

1. A continuous surveillance of the *Shigella* serogroups and serotypes should be performed in order to: -

i. Observe the periodic change of serogroup and serotype prevalence with time,

ii. Detect the introduction of new serotypes for each serogroup from outside the country,

iii. Establish methods for phage- and colicin-typing, and,

iv. Enhance the capability of studying *Shigella* epidemiology.

2. Continous surveillance of drug resistant *Shigella* should be conducted for the practical use of multiple drug resistance surveys, which can be used as a guideline for shigellosis treatment in areas where laboratory facilities are unavailable.

3. Antimicrobial therapy does not seem to be a solution to the control of shigellosis as there are strong resistance patterns within the different isolates of shigellae. Thus, it should be noted that sanitation must be given more emphasis for the control of shigellosis rather than to concentrate more on drug therapy.

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