IDENTIFICATION OF SEROTYPES AND ASSESSMENT OF MULTIPLE DRUG RESISTANCE IN 360 SHIGELLA ISOLATES

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by
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A C K N O W L E D G E M E N T S

This study has been made possible by the unreserved aid of many individuals. Notable among these include: Dr. M. Toucas (Chief of National Shigella Center, Paris) who identified our rare biochemotypes; Dr. Don J. Brenner (Chief of Enteric Section, Center for Disease Control, Atlanta, Georgia) who supplied us, free of charge, a complete set of Shigella control organisms; Dr. H. Rische (Chief of the Laboratory for Experimental Epidemiology, Wernigerode, DDR) who gave us, free of charge, large quantities of Shigella antisera; Dr. Hailemariam Kahsay (WHO National Co-ordinator for Ethiopia) who helped us acquire a high grade DIFCO antisera, through WHO sources.

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A B S T R A C T

Three hundred and sixty urban and rural Shigellae isolates were analysed in respect to serogrouping, serotyping, biochemotyping and drug resistance. S. flexneri (50.55%) was most common, followed by S. dysenteriae (32.78%), S. boydii (11.39%) and S. sonnei (5.28%). Isolation rates of S. dysenteriae, S. flexneri and S. sonnei were comparable in rural and urban areas, except for S. boydii which is more common in urban areas (significant at P=0.05).

Out of the thirty-two known Shigella serotypes, twenty-two were identified in this study (i.e. S. dysenteriae 1, 2, 3, 4, 6, 7; S. flexneri 1, 2, 3, 4, 6; S. boydii 1, 2, 3, 4, 5, 8, 9, 10, 12, 14; and S. sonnei). Urban isolates were represented by 22 serotypes compared to only 11 in rural areas. S. dysenteriae serotype 1 (Shiga's bacillus) was more common in rural areas (34.48%) than in urban areas (17.22%), and this difference is significant (P=0.05).

Ninteen patterns of drug resistance were observed, with TSu (21.11%), TCACbSSu (19.72%) and TSSu (12.50%) being comparatively more common. There were nine patterns of drug resistance in serogroup A, 12 patterns in B, 8 patterns in C, and 5 patterns in D. TCACbSSu pattern (53.39%) in serogroup A, TSu (41.76%) in B, and TSSu (26.31%) in D were observed. Within serogroup A, 81.82% of S. dysenteriae serotype 1 was associated with the TCACbSSu pattern. There were 17 patterns of resistance in urban areas compared to 10 in rural areas. The prominence of TCACbSSu pattern in rural areas was related to the high isolation rate of S. dysenteriae type 1 in

these places. In rural areas, 14.94% of Shigella isolates were classified as sensitive to all drugs tested, compared to 26.37% in urban areas and this difference is significant (P=0.05).

Identification of rare biochemotypes included: a) three strains of mannitol negative S. flexneri type 6, b) a strain of gas producing S. boydii type 14, and c) a strain of 'invasive' E. coli (O:164) serologically cross reacting in S. dysenteriae serotype 3 antiserum.

Based on the present study, it is recommended that: a) a further study of Shigella be persued, b) a Shigella reference center be established, c) an improvement of sanitation be stressed, d) chemotherapy be discouraged and e) a national policy for drug sale be enacted.

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INTRODUCTION AND LITERATURE REVIEW

DEFINITION OF TERMS AND ABBREVIATIONS

Definition of Terms

Shigella: non-motile bacteria that conform to the definition of the family Enterobacteriaceae and the tribe Escherichieae (1).

Shigella serogroups: the four divisions of genus Shigella; namely, S. dysenteriae (A), S. flexneri (B), S. boydii (C) and S. sonnei (D).

Shigella serotypes: these constitute subdivisions of the four serogroups. There are 10 serotypes within S. dysenteriae, 6 within S. flexneri, 15 within S. boydii and only 1 within S. Sonnei.

Strain: in this study, 'strain' signifies a Shigella isolate within a given serotype.

Isolate: an organism recovered by routine cultural investigation.

Drug: in this study 'drug' signifies any antimicrobial agent of natural or synthetic origin.

Drug resistance (in vitro): an organism is termed resistant to a given drug when it tolerates a concentration of antibiotic, significantly higher than that which inhibits the growth of susceptible organism of the same species (2).

Multiple drug resistance: a term used to denote simultaneous resistance to three or more drugs.

Dysentery: a disease marked by frequent watery stools, often with blood and mucous and characterized clinically by pain, tenesmus, fever, and dehydration (3).

Bacillary dysentery: dysentery caused by members of genus Shigella.

R-Factor: infectious, cytoplasmic DNA particle which multiplies independently of host chromosomes (4,5).

Abbreviations

For the sake of convenience, the following abbreviated names of drugs will be used, whenever reference is made to multiple drug resistance:

Cephalothin	Ce
Tetracycline	T
Chloramphenicol	C
Ampicillin	A
Carbenicillin	Cb
Kanamycin	K
Gentamicin	G
Polymyxin	Px
Streptomycin	S
Trimethoprim — Sulphamethoxazole	Sxt
Sulphadiazine	Su
Framycetine	F
Neomycin	N
Nalidixic acid	Nx

THE PROBLEM

The Magnitude of the Problem

In developing countries, diarrheal diseases are important causes of morbidity and mortality, especially in pre-school age children (6,7). Together with plague, cholera, influenza, bacillary dysentery has been one of the great scourges of the world. A survey carried out in 1957 showed that 150 of 189 countries of the world and 55 to 58 African countries listed bacillary dysentery as a major cause of morbidity and mortality (8). Considering the magnitude of the problem in Africa, availability of information on its incidence, prevalence and epidemiology is relatively scarce. In fact, Bokkenheuser (9) writing from South Africa remarks that "our ignorance of Shigella infection is almost complete". This is because facilities for Shigella study in Africa are still not adequate (7).

Shigellosis and the Younger Age-group

Shigellosis is quite common in the younger age group (10,11). In stool culture surveys of normal population groups in Guatemala, Beck et al. (12) reported a 2.7% incidence rate in those under 1 year of age and a peak of 11.8% in the 1 to 2 years age-group (the peak decreasing in each succeeding age-group). In Senegal, Baylet and Dauchy (13) found a 3% carrier rate of shigellosis in infants. In Central America, Gangarosa et al. (14) similarly reported the highest Shigella attack rates in the 6 months to 2 years age-group. Gordon et al. (15) found a 6% Shigella carrier rate in 1000 Guatemalan children. This incidence rate was again confirmed by Pierce et al. (16)

in single rectal cultures. Guatemalan children with chronic, recurrent shigellosis were revealed as dangerous sources of infection (17). The highest death rate (955.9/100,000) in Guatemalan children (1-4 years) was due to synergism of malnutrition and infection (18). In Yugoslavia (19), Mravunac and Weber reported a 2.3% death rate in children up to 2 years. The correlation with weaning practices and the attendant mal-nutrition was sufficient to characterize weaning diarrheae as an epidemiological entity (19,20,21). In addition, Weissman et al. (22) mentioned that shigellosis was becoming important in modern pre-school, day-care centers.

Pathogenicity of Shigella

Shigellosis is a self limiting disease, whose clinical manifestations range from mild to severe forms. Within Shigella serotypes, S. dysenteriae type 1 (Shiga's bacillus) is exceptional among enteric pathogens in possessing two potential modes of attack: a) by invading the cells of intestinal mucosa and b) by releasing exotoxins, acting on the mucosal cells and resulting in outpouring of fluids (23). Neurotoxin is also elaborated by this serotype (24). Two strains of S. flexneri and one of S. sonnei were reported to produce cell-free cytotoxin (25).

The disease is usually restricted to the alimentary canal, though extra-intestinal shigellosis is also reported in wound infections (26), skin lesions (27,28) as well as in haemolytic syndromes (29).

Lack of Protective Immunity (fallowing infection or vaccination)

The fact that relapses and reinfections are common in Shigellosis shows that active immunity is only of short duration. In addition, efforts to produce polyvalent vaccines have not yet materialized. Vaccines produced from streptomycin dependant S. flexneri 2a mutants were reported to provide type-specific immunity in experimental infections (30,31,32), while heat killed and irradiated vaccines showed no significant immunological properties (33).

FACTORS THAT AGGRAVATE THE PROBLEM

The Role of Contaminated Water and Food

In most developing countries, conditions that favour endemicity of shigellosis are always present, resulting in continuous transmission of the disease, with periodic occurrence of outbreaks or epidemics. Many studies have shown the correlation between Shigella prevalence and low socio-economic conditions (12,34,35,36). In tropical and subtropical regions, methods of faecal disposal may be quite primitive or entirely lacking. As such, the 'faecal peril' becomes the source of all bacterial, viral and parasitic diseases. Drinking water and food are readily contaminated (37). Even large bodies of water, like rivers, may be contaminated with enteric bacteria (38). The role of contaminated water (39,40,41,42,43,44, 45,46,47) and contaminated food (46,48) in the transmission of shigellosis has been amply documented in developed countries. In fact, Keusch (49) described shigellosis as "past plague" and

"modern menace", and Weissman et al. (40) characterized contaminated water as "significant problem of contemporary urban life".

In a well controlled study by Rajasekaran et al. (50) in India, it was found that personal hygiene appeared as important as readily available water supply. Surveys have shown that incidence of shigellosis was directly proportional to the distance of water supply from living quarters (51,52), and that readily available water was more important than its quality (51).

The Role of House Flies (M. domestica)

In many tropical and subtropical areas devoid of modern sanitation, flies can be as effective in spreading infection as do contaminated fingers, utensils, water and food (53). Studies have shown that effective fly control has resulted in reduction of : a) Shigella carrier rate, b) morbidity rates and c) mortality rates, in infants less than 2 years old (53). The abundance of M. domestica sorbens which breeds in human faeces and feeds on human secretions is so much linked with poor sanitation that, whenever health statistics are lacking, bacteriological investigation of flies may provide "essential epidemiological information" (54).

The Role of Minimal Infective Dose

The spread of shigellosis is also aided by the fact that the minimal infective dose of Shigella is unusually small. When at least 10^5 virulent S. flexneri 2a were fed to volunteers, 77% developed a variety of clinical symptoms (55). For S. dysenteriae

serotype 1, as few as 10 organisms produced disease in human volunteers (23). Considering that Shgella may attain a concentration of 10^6-10^{10} organisms/gram of stool in human colon (56) and a concentration of 10^5-10^8 organisms/gram of stool in children with clinical shigellosis (57), the 'faecal peril' under poor sanitary conditions is extremely dangerous. Active cases of shigellosis (newly infected or recurrent infection) are important in transmission, since asymptomatic carriers are uncommon (58) and the convalescent carrier rate is usually brief (17).

The Role of Multiple Drug Resistance

Another dimension to the problem of shigellosis is their tendency to develop multiple drug resistance, generally becoming unresponsive to most commonly used antibiotics. It is obvious, that the usual mechanism by which bacteria acquire resistance, i.e. by spontaneous mutation and selection, could not account for the rapid increase in the incidence of Shigella resistant to three, four or more drugs. It was observed that: a) the same patients often excreted sensitive and multiresistant Shigella of the same serological types, b) untreated patients excreting multiple drug resistant Shigella often excreted E. coli with the same pattern of drug resistance and c) patients formerly excreting sensitive Shigella often excreted multiresistant Shigella, after therapy with a single drug (6).

The hypothesis that Shigellae might have received their multiple drug resistance from E. coli already in patients' intestinal

tract was substanitiated by in-vitro demonstrations that the transfer of resistance could take place between E. coli and Shigella under conditions that allow cell contact (4,5,59,60,61,62,63,64). Similarly, in-vivo transfer of drug resistance using newly hatched chicken was shown by Walton (65).

It was later found that this type of multiple drug resistance was mediated by cytoplasmic DNA particle, which replicated autonomously and more rapidly that host chromosomes (4,5). Plasmids code for a variety of factors and are now by far the major source of acquired antibiotic resistance in Enterobacteriaceae (2).

Transferable drug resistance factors (R-factors) in Enterobacteriaceae are formed by a combination between two initially independent elements — transfer factors and resistance determinants (4.66). If strains carrying transfer factors gain access to strains carrying non-transferable resistant determinants to which they can become attached, transfer of drug resistance is initiated (67). Passage experiments have shown R-factors to be relatively stable; in general, R-factors are most stable in E. coli, least stable in Salmonella, with Shigella occupying an intermediate position (5). Artificial elimination of R-factors is possible by treatment with acriflavine (68). In addition, the spread of infective drug resistance is probably hastened by the use of antimicrobial drugs (2), which exerts a pressure effect towards conjugation and transfer of R-factors in the human gut (66).

Multiple drug resistance was first noted in Japan in the early 1950's (69,70), the majority being resistant to tetracycline, chloramphenicol, streptomycin and sulphonamides (TCSSu). As early as 1964, a strain of E. coli isolated in Germany was found by Watanabe et al. (61) to transfer resistance to tetracycline, chloramphenicol, streptomycin, sulphadiazine, kanamycin and neomycin (TCSSuKN).

Although multiple drug resistant strains were uncommon at the time of their discovery, they rapidly increased in prevalence (4). By the end of 1967, about 80% of Shigella strains isolated in Japan were resistant to two or more drugs (71). In the United States, Smith and Armour (72) demonstrated R-factors in enteric bacteria, causing infection of the genito-urinary tract. Similarly, R-factors of the intestinal flora were shown by Smith and Halls (73) in England and by many authors in the United States (6,74,75,76,77,78). Infectious resistance has also been demonstrated in many strains of Salmonella, E. coli and other gram negative bacilli in Japan, Europe and Israel (79). In Ethiopia, Mann and Messele (80) demonstrated R-factors in intestinal flora and later Messele and Alebachew demonstrated these factors in Salmonella and Shigella isolates (81).

In 1968-1970, an epidemic strain of S. dysenteriae serotype 1 showed multiple drug resistance to tetracycline, chloramphenicol, streptomycin and sulphonamides (TCSSu) in Central America (14); i.e. in El-Salvador (82), Honduras (82), Guatemala (35) and Mexico (83). Ampicillin resistance was later reported from Mexico City in

1972 (84) and from Bangladesh in 1974 (85). The strains from Mexico City and Bangladesh were resistant to tetracycline, chloramphenicol, ampicillin, streptomycin and sulphonamides (TCASSu), but were sensitive to kanamycin. During 1973-1976, Shigella strains isolated in Somalia showed multiple drug resistance to as many as 7 drugs, mainly within S. dysenteriae serotype 1(86). In Washington area, patterns of resistance to tetracycline, ampicillin, streptomycin and sulphonamides (TASSu) were common, and in the same area, resistance to ampicillin increased from 6% to 95% in only four years (87). In France, Szturm-Rubinsten et al (88) reported multiple drug resistance up to 5 drugs (TCASSu) in some strains of S. flexneri and S. sonnei. A similar study in Brazil (63) revealed multiple resistance up to 8 drugs (TCAKNFSSu) in S. flexneri types 1 and 2, as well as in S. sonnei. In Ethiopia, multiple drug resistance and R-factors in 69 Shigella isolates were reported by Messele and Alebachew (81), and comparative multiple drug resistance within Shigella serogroups was studied by Afeworki and Yetnebersh (89). In the latter study, multiple resistance to as many as 6 drugs was noted mainly within S. dysenteriae serotype 1 (identified biochemically).

THE NEW DIMENSION IN SHIGELLOSIS

A new development in technologically advanced countries is the venereal transmission of shigellosis (90,91,92). In this case, shigellosis has been most common in young people who have adopted an "alternative life-style". The mode of transmission is thought to be via faecal ingestion during analingus.

DISTRIBUTION OF SHIGELLA SEROGROUPS AND SEROTYPES

The world distribution of Shigella serogroups and their serotypes is highly variable, though some general trend could be observed in developed and developing countries (Table 1). By 1968, Christie (37) reported that S. flexneri has given way to S. sonnei as the dominant serogroup in England. Similarly, 1977, S. sonnei constituted 64.5% of all Shigella isolated in the United States (10). In this report (10), S. flexneri type 2a, type 3a, type 1b and type 1a, in order of decreasing incidence, were reported. In the U.S.A., S. dysenteriae and S. boydii accounted for less than 1% of all isolates in 1969 (11). In Poland (93), for the period 1965 to 1968, the predominant strain was reported to be S. sonnei with 19,181 isolates, followed by S. flexneri, S. boydii and S. dysenteriae (with 16,717, 340 and 25 isolates, respectively). In Malagasy (94), for the year 1979, incidence rates for Shigella serogroups were: 70.02% for S. flexneri, 18.25% for S. sonnei, 5.72% for S. dysenteriae and 3.51% for S. boydii. Common serotypes in Malagasy included S. flexneri type 2a, S. flexneri type 4, S. dysenteriae type 1 and S. dysenteriae type 2. For the period of 1966 to 1970, serogroups of Shigella in Tangier showed 70.9% for S. flexneri, 13.2% for S. sonnei, 9.9% for S. boydii and 3.7% for S. dysenteriae (95); within S. flexneri, serotype 2 was most common, followed by serotypes 1 and 3, respectively. In Peru (96), S. flexneri 2a was reported as most common. In 1977, the teaching hospital in Lagos (97) reported S. flexneri as most common (50.27%), with serotype 2 predominating, and followed by serotypes 4,6,1 and 3 respectively; S. boydii accounted 23.8% of all

isolates with serotype 4 predominating and S. dysenteriae accounted for 7.9% of all isolates with serotype 2 predominating. In this same study (97), S. sonnei accounted for 17.4% of all Shigella isolates. In France, for the years 1975 to 1977, S. sonnei was most commonly isolated (53.16%), followed by S. flexneri, S. boydii and S. dysenteriae (41.90%, 3.25% and 1.68%, respectively (98). In this same study, serotype 2 within S. dysenteriae, serotypes 2 and 6 within S. flexneri and serotype 2 within S. boydii were frequently isolated.

In Ethiopia, systematic followup of Shigella serogroup prevalence with time has not been adequately reported. For the period of 1974-1978, the dominant serogroup in Addis Abeba area was S. flexneri (49.1%), followed in order of prevalence by S. dysenteriae, S. boydii and S. sonnei (89). So far, identification of prevalent serotypes in Ethiopia has not been attempted. Lack of information and established methodology on Shigella serotypes, phage-types and colicin-types have hampered epidemiological investigation of Shigella infections.

DOMINANT SHIGELLA SEROGROUPS
DEVELOPED vs DEVELOPING COUNTRIES

A U T H O R	REF	COUNTRY	YEAR	Dominant serogroup	Dominant serotype(s)
Christie	(37)	England	1968	D -	-
Rosenberg et al.	(10)	U.S.A.	1977	D (64.50%)	-
Stypulkawska & Lachowicz	(93)	Poland	1965-1968	D (52.89%)	-
Piechaud & Toucas	(98)	France	1975-1977	D (53.16%)	-
Coulanges	(94)	Malagasy	1979	B (70.02%)	S. flexneri 2,4
Mailloux	(95)	Tunisia	1966-1970	B (70.90%)	S. flexneri 2
Balazar	(96)	Peru	1979	B -	S. flexneri 2
Wozuzu-Acholonu	(97)	Nigeria	1977	B (50.27%)	S. flexneri 2
Afeworki & Yetnebersh	(89)	Ethiopia	1974-1978	B (49.10%)	-

Table 1

THE PURPOSE OF THIS STUDY

Shigellosis is a world-wide problem with special prominence in developing countries (8), where many factors that promote its endemicity are always present (12,34,35,36). In addition, research activities in genus Shigella have been quite inadequate in developing countries as compared to those carried out in developed countries (2). Adequate and continuous information of prevalent Shigella serogroups, serotypes, phage-types and colicin-types, as well as effective surveillance of drug resistance are essential to take meaningful curative measures and epidemiological investigation.

In Ethiopia, published reports of studies on genus Shigella are very few in number (81,89), and more studies are required to ellucidate hitherto unknown aspects of this etiological agent. With this in mind, the purpose of the present study is to:

1. .... identify Shigella serotypes isolated from clinical cases of shigellosis,
2. .... assess all possible patterns of multiple drug resistance within specific Shigella serotypes,
3. ....develop a system of biochemotyping and serotyping at the Central Laboratory and Research Institute, which in turn, would lay a ground work for the establishment of Shigella reference center in Ethiopia, and
4. .... suggest guidelines for Shigella chemotherapy, based on current patterns of drug resistance.

CHAPTER 2

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

COLLECTION AND STORAGE OF SHIGELLA ISOLATES

The three-hundred and sixty Shigella isolates were collected from stool cultures of patients referred to the Central Laboratory and from field trip collections in rural areas. Each Shigella isolate was stocked in 0.5 ml Tryptic Soy Yeast (TSY) with 25% glycerol, and stored in deep freeze, -70°C. There were 273 Shigella isolates from Addis Abeba area and 87 from miscellaneous rural areas. The origin of the 87 rural Shigellae isolates was as follows: a) Field trip collections during epidemic calls (54 form Tatek, 2 from Metahara Sugar Estate, 7 from Amibara Agricultural Project, 2 from Gemu awraja, 4 from Dedessa, 2 from Humera, 5 from Jibat-Mecha awraja, 1 from Tole and 1 from Arba), and b) Routine collections from rural patients referred to the Central Laboratory (2 from Ogaden, and 1 each from Arba-Gugu awraja, Bahar Dar, Assela, Debrezeit and Arsi-Negelle).

The earliest collection date was on 19.1.1974, while the latest was on 18.2.1980. However, 84.2% of all isolates were collected between January 1978 and February 1980.

CONFIRMATION OF PURITY

Cultural Purity

Stocked organisms were sub-cultured on MacConkey (Oxoid) and Salmonella-Shigella (Oxoid) agars, and incubated at 35°C. for 18-24 hours. Cultural purity was visually ascertained.

Biochemical Purity

(a) Using a sterile, straight wire, a single colony from an isolate was picked up and used to inoculate about 4 ml of nutrient broth.

(b) The inoculated nutrient broth was incubated at 35°C. for 2-4 hours, or until culture was visually turbid.

(c) By means of a sterile Pasteur pipette, the broth culture of an isolate was used to inoculate the following set of biochemical tubes: Triple Sugar Iron (TSI), Lysine Iron Agar (LIA), Urea Slant, Simmon's Citrate, Motility Test Medium, 1% Mannitol Broth and 1 % Glucose Broth with inverted Durham tube.

These sets of tubes were used to determine the following biochemical characteristics of each isolate: a) fermentation of glucose, lactose and mannitol, b) production of hydrogen sulphide, c) hydrolysis of urea, d) utilization of citrate, e) observation of motility, f) production of indole, g) production of gas from glucose, h) decarboxylation of lysine and i) deamination of phenylalanine.

(d) To check the purity of broth inocula and thus the reliability of biochemical tests, each broth inoculum was sub-cultured on MacConkey (Oxoid) 'check plate' and incubated at 35°C. for 18 to 24 hours. Cultural purity of a broth inoculum was visually observed.

(e) For quality control of biochemical tubes: a) all biochemical sets were pre-incubated at 35°C. for 18-24 hours to ascertain sterility and b) standard quality control organisms (S. typhimurium, S. flexneri and P. mirabilis) were used to check the reactivity of representative biochemical tubes.

Serological Purity

Shigella isolates that met the minimal biochemical profile of genus Shigella were serogrouped using DIFCO commercial antisera.

BIOCHEMOTYPING

Serologically confirmed Shigella serogroups were biochemotyped, according to procedures recommended by Edwards and Ewing (1):

(a) Using a straight wire, a single colony from each Shigella isolate was inoculated separately into a 4 ml nutrient broth and incubated at 35°C. for 2 to 3 hours, or until visible turbidity was observed.

(b) A broth culture of each isolate was used to inoculate a set of 5 ml purple broth bases containing 1 % each of the following sugars: mannitol, dulcitol, xylose, raffinose, glycerol, lactose, arabinose and sorbitol. Part of the remaining broth inoculum was used to seed a tube of l-ornithine (Falkow) and the remaining portion was reserved for indole test (Kovacs). Betagalactosidase test was carried out by including a disc of O-nitrophenylgalacto-

pyranoxidase (ONPG)[1] in a 5 ml saline suspension of Blood agar cultural growth.

(c) All tests were incubated at 35°C. for 72 hours. Results were recorded as positive, weakly positive and negative.

(d) Quality control of prepared sugar fermentation tubes was carried out by: a) pre-incubating all tubes at 35°C. for 18-24 hours, to ascertain sterility and b) inoculating representative tubes with pairs of known positive and negative organisms, as recommended by CDC (99).

SEROTYPING

(a) Each Shigella isolate (already identified to serogroup level) was sub-cultured on Blood agar and incubated at 35°C. for 18-24 hours.

(b) The pattern of biochemical characterstic of each isolate was effectively used in selecting antisera for serotype identification.

(c) Serotypes within Shigella serogroups were identified by slide agglutination method, using DIFCO commercial antisera and counterchecked by antisera from the Institute of Experimental Epidemiology (German Democratic Republic). Agglutination reactions were observed with the naked eye over a source of fluorescent light.

(d) The specificity of each antisera was checked by control organisms representing all known Shigella serotypes. These control organisms were imported from the National Collection of Type Cultures (England) and from the Center for Disease Control (U.S.A.).

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[1] ONPG impregnated discs from Institute Pasteur (Paris).

AGAR DISC-DIFFUSION ANTIMICROBIAL SUSCEPTIBILITY TESTING

Susceptibility test of each isolate was carried out according to recognized procedures by Kirby and Bauer (100):

(a) Each Shigella isolate was sub-cultured onto a plate of MacConkey agar (Oxoid).

(b) From a pure culture of each isolate, 4 to 5 colonies were randomly selected and these were transferred (by touching the top of each colony successively with the same loop) to a tube containing about 4 ml of Tryptic Soy Yeast (TSY).

(c) The TSY broth was incubated at 35°C. until it produced cloudiness due to colonial growth. Such broth culture was diluted with sterile TSY broth to obtain a turbidity of 0.5 McFarland (0.5 ml of 1.175% $BaCl_2.2H_2$ plus 99.5 ml of 0.36N H_2SO_4).

(d) Using a sterile, non-tocic cotton swab dipped into the broth culture, the entire surface of a Mueller-Hinton (Oxoid) agar was swabbed evenly. The inoculated plate was left at room temperature for 3 to 5 minutes.

(e) With the aid of an automatic dispenser (BBL) a set of 11 sensitivity discs were placed on the surface of each Mueller-Hinton plate, and these discs were pressed down gently by sterile forceps to assure even contact.

(f) Inoculated Mueller-Hinton plate with sensitivity discs was then incubated at 35°C. for 18-24 hours.

(g) Finally, diameters of inhibition zones were measured by a metal caliper to the nearest millimeter. The resuts were recorded as Resistant (R), Intermediate (I) and Sensitive (S), according to standard interpretive charts (100).

(h) The sensitivity discs included the following drugs: cephalothin, tetracycline, chloramphenicol, ampicillin, carbenicillin, kanamycin, gentamicin, polymyxin-B, streptomycin and sulphadiazine.

(i) For quality control purposes, E. coli (ATCC-25922) was used to detect deficiencies in medium, inoculum and sensitivity discs.

INSTITUTIONS CONSULTED

We have freely contacted miscellaneous organizations, directly or indirectly connected with our study. These Institutes were: Center for Disease Control, Atlanta, Georgia (U.S.A.), National Collection of Type Cultures, Collindale Avenue, London (England); National Shigella Center, Institute Pasteur, Paris (France); Institute of Experimental Epidemiology, Burgstrasse, Wernigerode (German Democratic Republic); Institute Pasteur of Malagasy, Tanararive (Malagasy) and the World Health Organization, Brazzaville (African Region). We contacted these Institutes to provide us with Shigella antisera, control organisms, technical advice and reference material. In addition, national Shigella centers in Nigeria, Peru, Poland and Sweden were approached to provide us with information on Shigella serogroups and serotypes, as well as sensitivity patterns, in their respective countries.

(Prominent Shigella experts consulted will be included in the acknowledgement list)

CHAPTER 3

R E S U L T S

SEROGROUP IDENTIFICATION

Serogrouping of the three-hundred and sixty Shigella isolates, included in this study, showed that S. flexneri is the most common, followed by S. dysenteriae, S. boydii and S. sonnei (50.55%, 32.78%, 11.39%, 5.28%, respectively) (Fig. 1).

Serogroup distribution (Fig. 2) in urban and rural areas is similar for S. dysenteriae, S. flexneri and S. sonnei, except for S. boydii which is more common in urban areas and this difference is significant (P=0.05).

The three-hundred and sixty isolates included in this study are arranged by monthly isolation (Fig. 3). The fact that shigellosis is a year round disease is clearly observed. The prevalence of shigellosis during the months of June and September, as hinted in Fig. 3, would need more controlled observation.

SEROTYPE IDENTIFICATION

Incidence rate of total Shigella serotypes is shown in Table 2. Out of the 32 known Shigella aserotypes (10 within S. dysenteriae, 6 within S. flexneri, 15 within S. boydii and 1 within S. sonnei), 22 of them (6 within S. dysenteriae, 5 within S. flexneri, 10 within S. boydii and 1 within S. sonnei) were identified.

Serotypes of S. dysenteriae are shown graphically in Fig. 4. Within this serogroup, S. dysenteriae serotype 1 was most commonly

SEROGROUPS OF 360 SHIGELLA ISOLATES

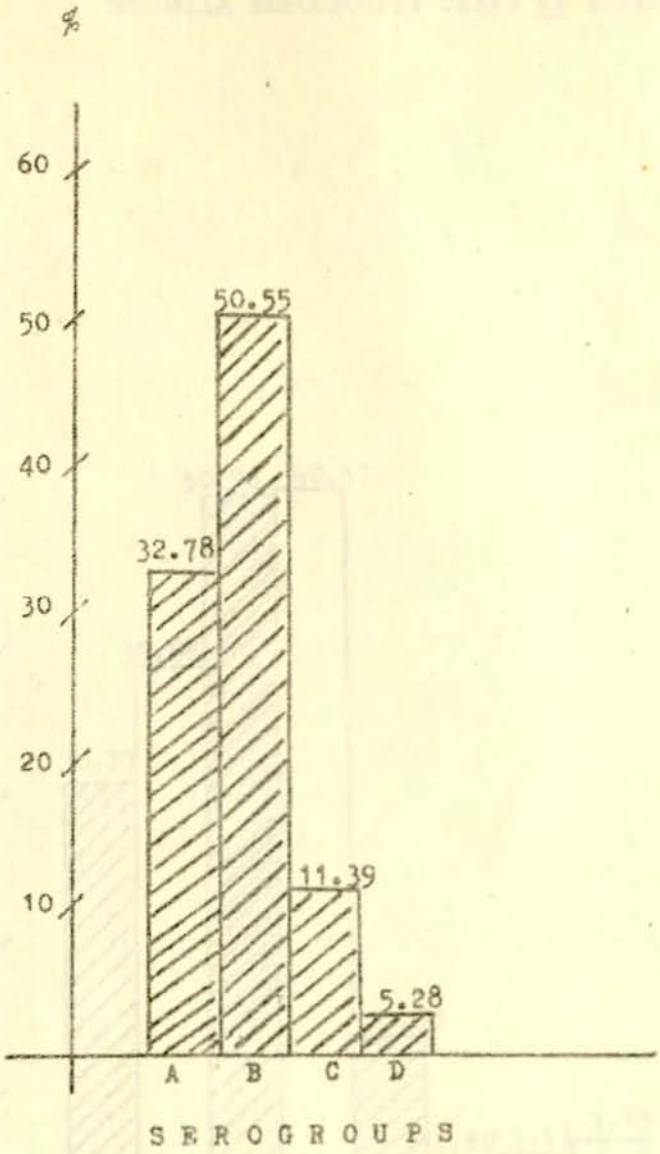

FIG. 1

SHIGELLA SEROGROUPS: URBAN vs RURAL

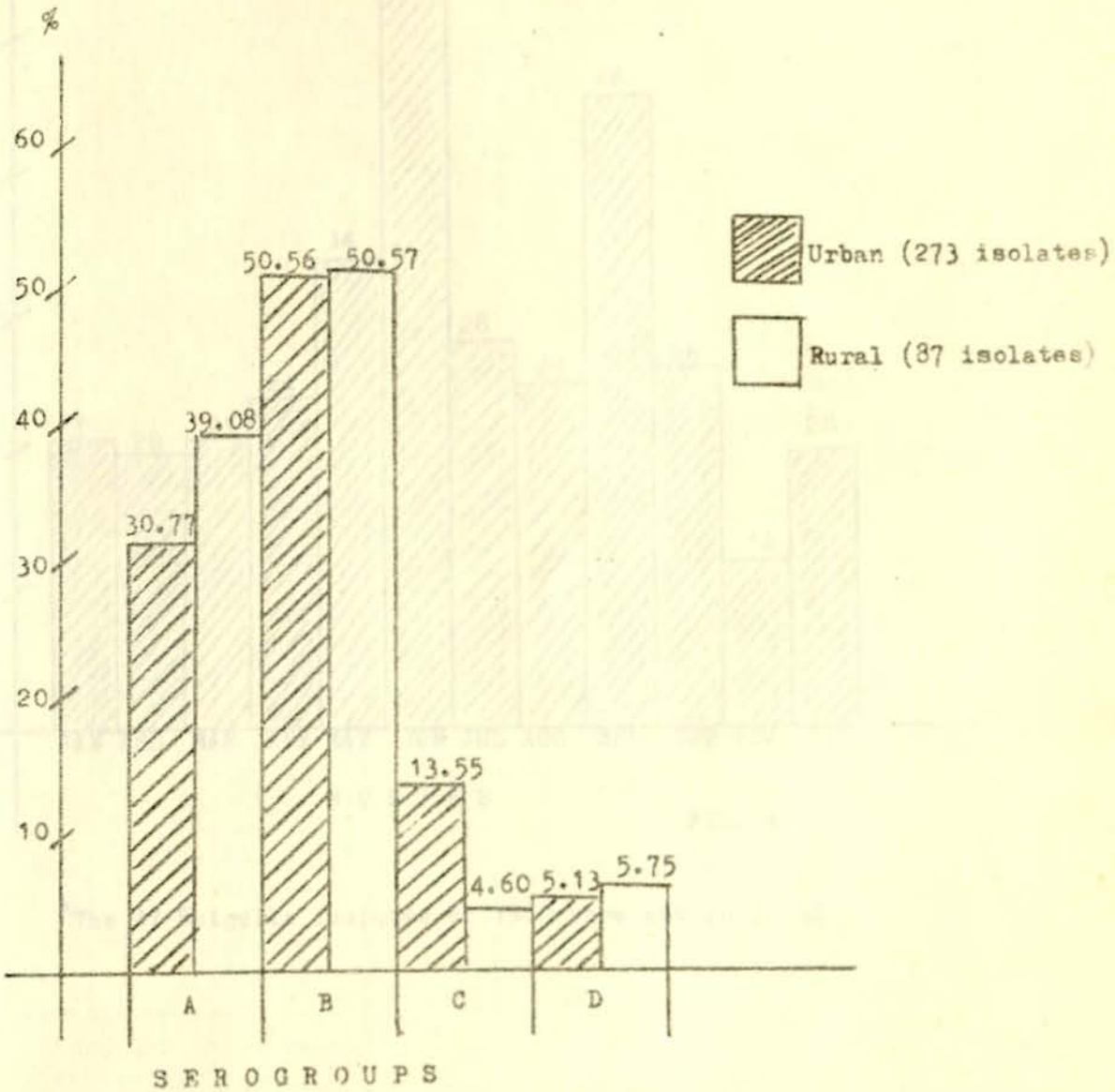

FIG. 2

MONTHLY SHIGELLA ISOLATES
(1974-1979)[a]

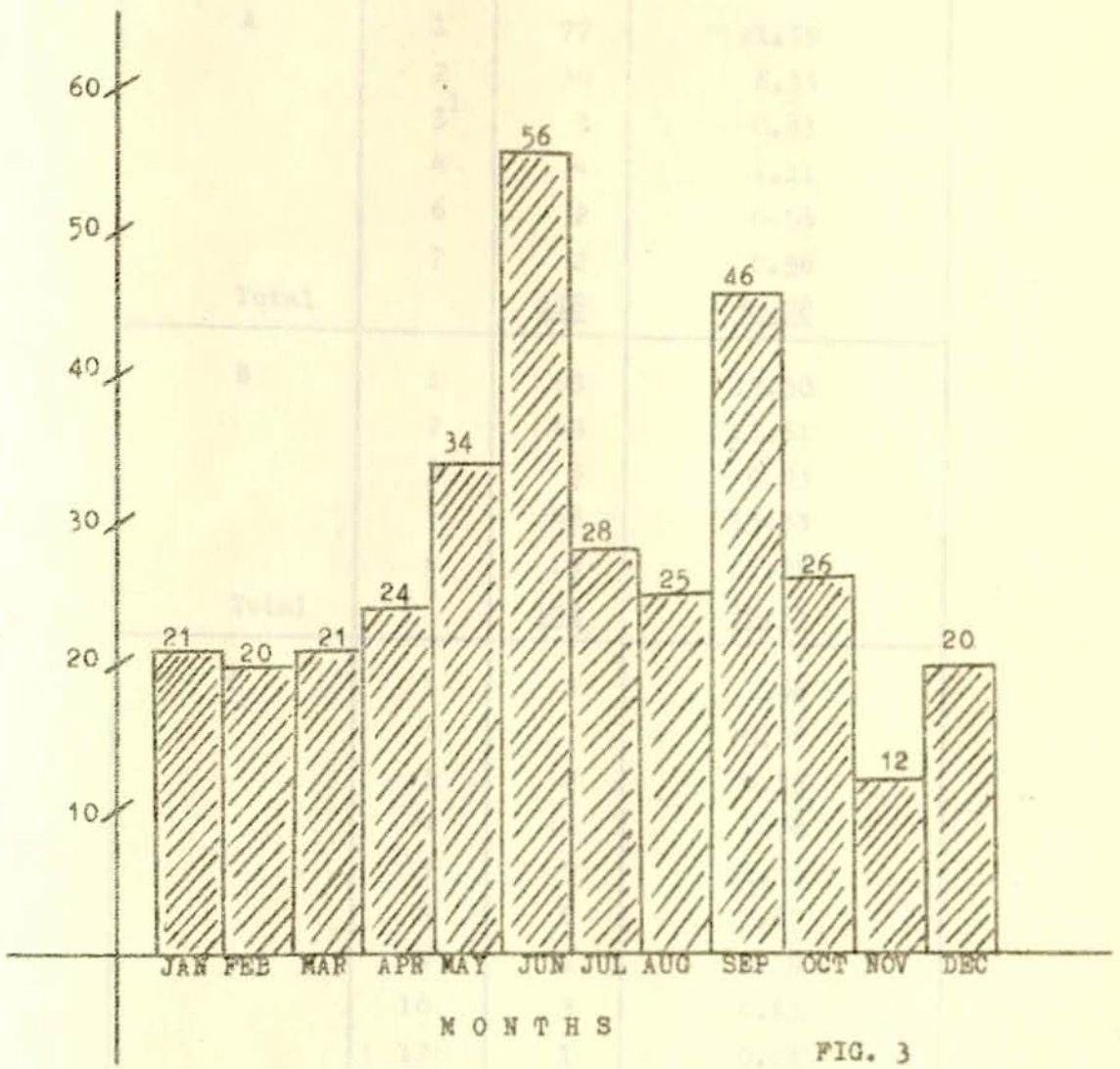

FIG. 3

[a]The 27 Shigella isolates of 1980 were not included

INCIDENCE-RATE OF SHIGELLA SEROTYPES

Serogroup	Type	No.	Percent of total isolates
A	1	77	21.39
	2	30	8.33
	3[1]	3	0.83
	4	4	1.11
	6	2	0.56
	7	2	0.56
Total		118	32.78
B	1	63	17.50
	2	49	13.61
	3	3	0.83
	4	48	13.33
	6	19	5.28
Total		182	50.55
C	1	7	1.94
	2	1	0.28
	3	2	0.55
	4	10	2.78
	5	9	2.50
	8	6	1.67
	9	1	0.28
	10	3	0.83
	12	1	0.28
	14	1	0.28
Total		41	11.39
D	1	19	5.27

[1] Includes one strain of invasive E. coli which showed strong agglutination in specific S. dysenteriae antiserum.

Table 2

SEROTYPES OF S. DYSENTERIAE

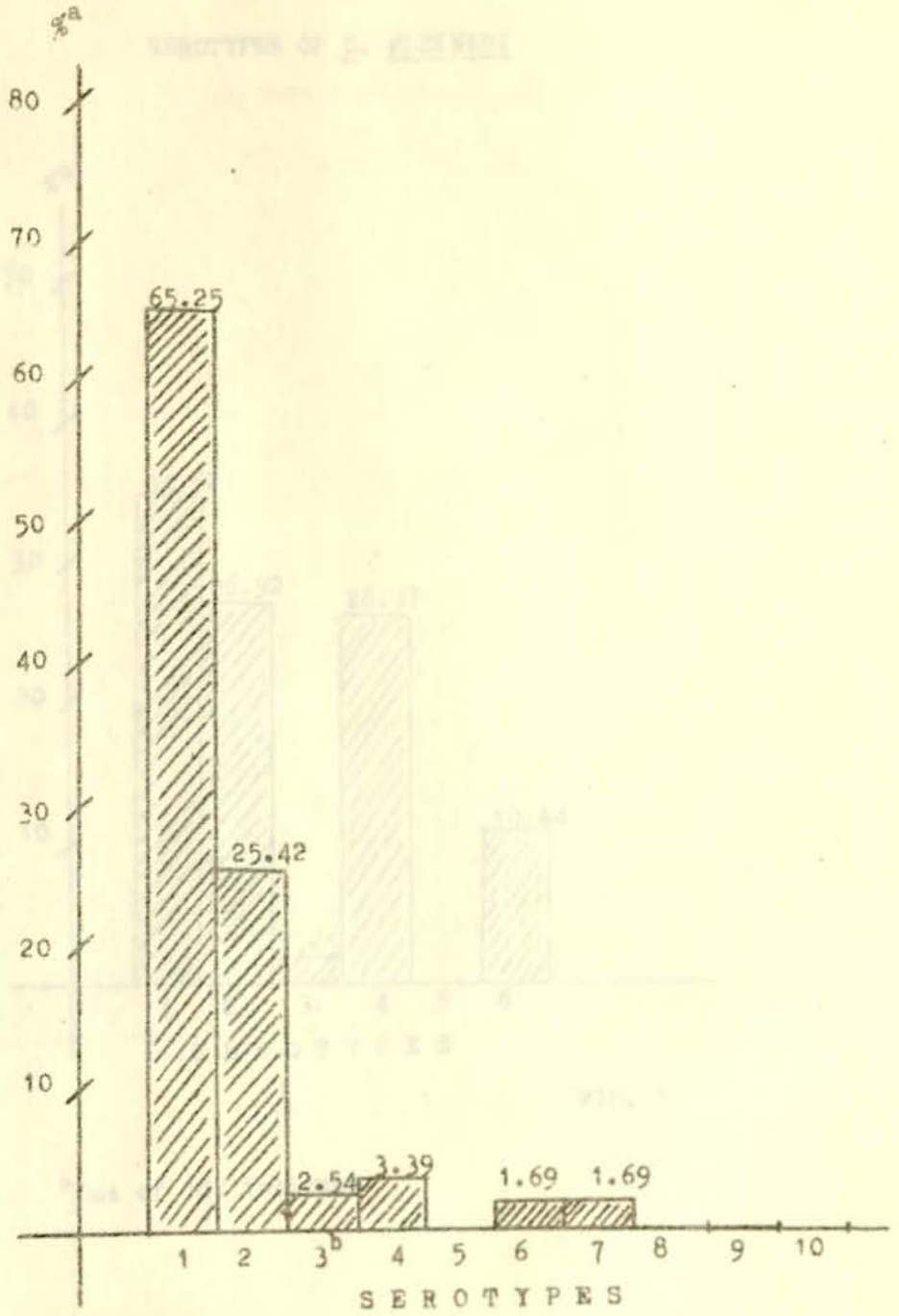

FIG. 4

[a] Out of 118 isolates

[b] Includes 1 strain of E.coli, cross reacting serologically with S. dysenteriae serotype 3.

SEROTYPES OF S. FLEXNERI

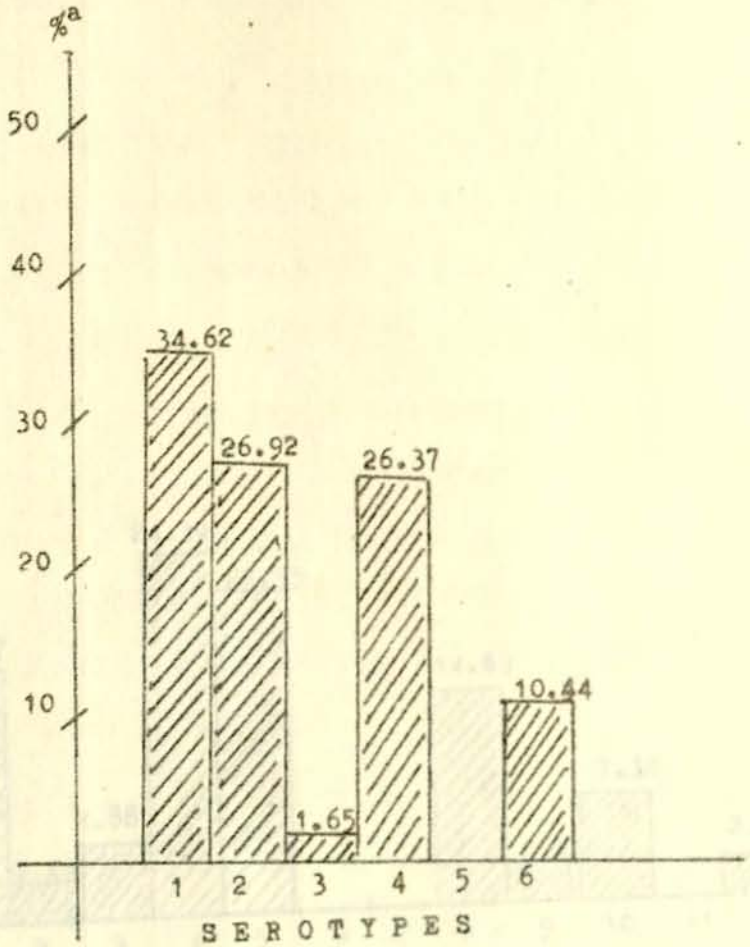

FIG. 5

[a]Out of 182 isolates.

SEROTYPES OF S. BOYDII

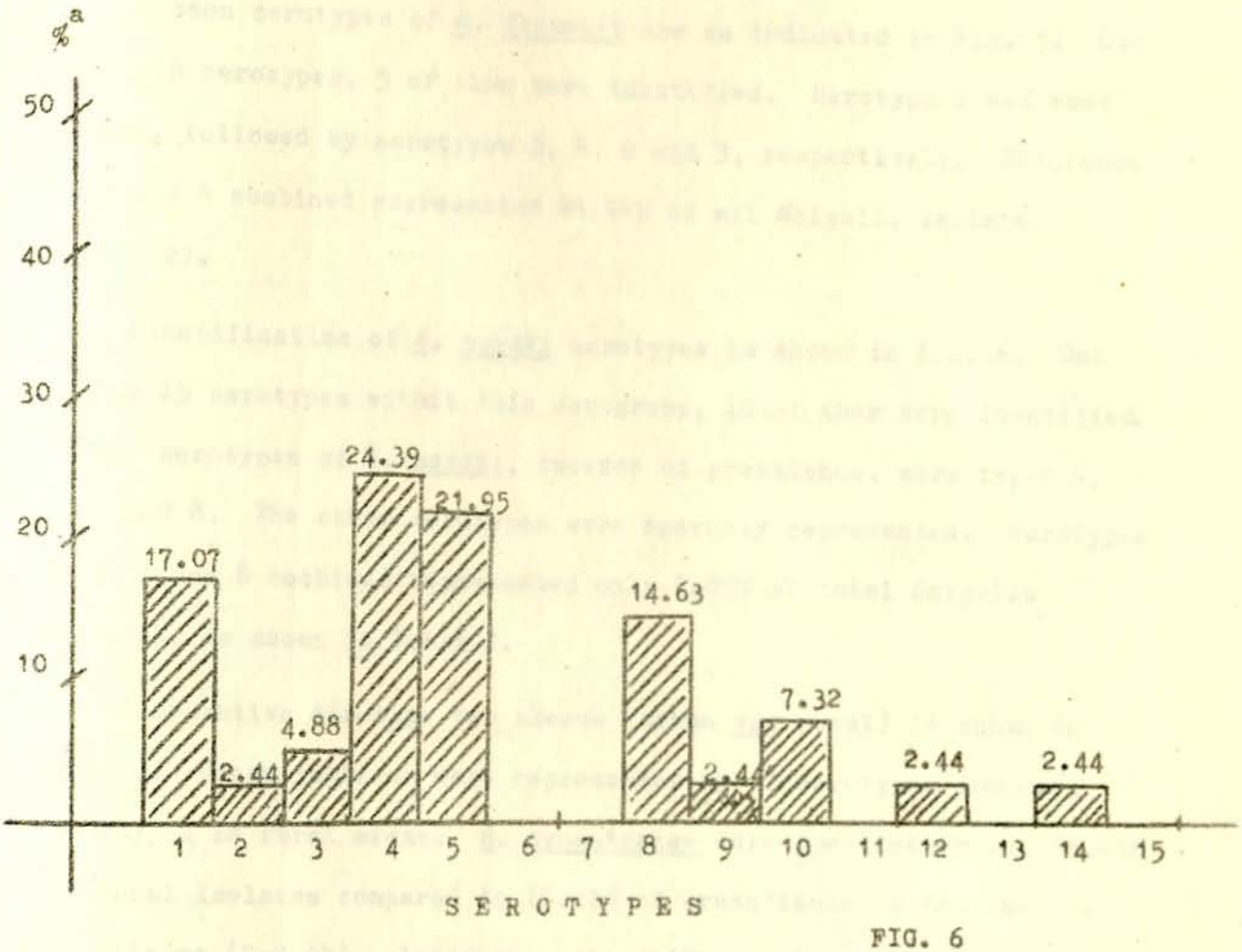

FIG. 6

[a]Out of 41 isolates

isolated. This strain comprised 21.39% of all Shigella isolates (Table 2). Other serotypes, inorder of prevalence, were types 2, 4 and 3. Serotypes 6 and 7 came next. Serotypes 1 and 2 combined represented 29.72% of total Shigella isolates (Table 2).

Common serotypes of S. flexneri are as indicated in Fig. 5. Out of the 6 serotypes, 5 of them were identified. Serotype 1 was most common, followed by serotypes 2, 4, 6 and 3, respectively. Serotypes 1, 2 and 4 combined represented 44.44% of all Shigella isolates (Table 2).

Identification of S. boydii serotypes is shown in Fig. 6. Out of the 15 serotypes within this serogroup, 10 of them were identified. Common serotypes of S. boydii, inorder of prevalence, were types 4, 5, 1 and 8. The other serotypes were sparcely represented. Serotypes 4, 5, 1 and 8 combined represented only 8.89% of total Shigella isolates, as shown in Table 2.

Comparative serotype prevalence (urban vs. rural) is shown in Table 3. Urban isolates were represented by 22 serotypes compared to only 11 in rural areas. S. dysenteriae serotype 1 comprized 34.48% all rural isolates compared to 17.22% of urban isolates and this is significant (P=0.05). Incidence rate differences for the other serotypes are not significant (P=0.05).

BIOCHEMOTYPING

Results of biochemical tests have greatly facilitated intelligent and economical use of antisera, by narrowing down possibilities in agglutination trials. Rare biochemotypes identified include:

(a) Three strains of mannitol negative S. flexneri type 6,

(d) [§] An invasive E. coli (O:164) which showed strong agglutination in specific S. dysenteriae serotype 3 antiserum, as shown in Table 4, and

(c) A rare gas producing variant of S. boydii serotype 14 (Table 4).

PATTERNS OF DRUG RESISTANCE

There were nineteen patterns of drug resistance within the 360 Shigella isolates (Table 5). However, six patterns represented 70.28% of total patterns. The Shigella isolates tested showed drug resistance ranging from 1 to 7. The most common pattern was TSu (21.11%), followed by TCACbSSu (19.72%) and TSSu (12.50%).

As shown in Table 5, the six drug multiple resistance (TCACbSSu) was most common in serogroup A (53.39%). Similarly, TSu pattern was common in serogroup B (41.76%) and TSSu in serogroup C (9.76%). The SSu and TSSu patterns were both common in serogroup D (15.79% and 26.32%, respectively). Non resistance (sensitive or intermediate) was shown by 17.80% of serogroup A, 17.03% of B, 63.41% of C and 36.48% of D.

There were nine patterns of resistance within serogroup A as shown in Table 7. The most common resistance pattern, TCACbSSu, was shown by 81.82% of S. dysenteriae serotype 1 (Plate 1). One strain

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[§] This isolate was initially identified as mannitol positive S. dysenteriae serotype 3. Further biochemical tests, however, revealed that it is an E. coli, sharing common antigen with S. dysenteriae type 3.

of this serotype was resistant to 7 drugs (TCACbKSSu). It is interesting to note that serotypes 2, 3, 4, 6, and 7 did not develop resistance to more than 4 drugs. S. dysenteriae serotype 2 commonly showed the TCSSu pattern (20%) (Plate 2).

There were twelve types of presistance pattern within serogroup B as shown in Table 8. Common pattern of drug resistance by serotypes 1 and 4 was TSu (68.25%, and 68.75%, respectively) (Plate 3). S. flexneri type 2 commonly showed the TSSu pattern (49%) (Plate 4). Of the 19 members of serotype 6, 11 (57.89%) showed unit resistance to sulphadiazine (Plate 5).

There were only eight types of resistance pattern within serogroup C, as shown in Table 9. Members of S. boydii were commonly sensitive to all drugs (63.31%), and there was no commonly associated resistance pattern for any of its serotypes.

There were five types of resistance pattern within serogroup D, as shown in Table 5. The TSSu pattern was comparatively more common (26.32%) (Plate 6), followed by SSu pattern (15.79%).

Comparative drug resistance in rural and urban areas is shown in Table 6. There were 17 patterns in urban areas, compared to 10 in rural areas. The TCACbSSu pattern was more common in rural areas (34.48%) than in urban areas (15.02%). This difference reflects the prevalence of S. dysenteriae serotype 1 in rural areas, as mentioned earlier. The incidence rate (urban vs. rural) of TCACbSSu showed significant difference (P=0.05). Urban isolated showed 26.37% non-resistance (sensitive or intermediate) to all drugs tested, compared to 14.94% in rural isolates and this difference is significant (P=0.05).

SHIGELLA SEROTYPES:

URBAN vs RURAL

Serogroup	Type	Uraban No.	Uraban %	Rural No.	Rural %	Z
A	1	47	17.22	30	34.48	-3.42
	2	27	9.89	3	3.45	+1.89
	3	3	1.10	0	0.00	
	4	3	1.10	1	1.15	-0.27
	6	2	0.73	0	0.00	
	7	2	0.73	0	0.00	
Total		84	30.77	34	39.08	
B	1	47	17.22	16	18.39	-0.25
	2	35	12.82	14	16.39	-0.77
	3	1	0.37	2	2.30	-1.72
	4	38	13.92	10	11.49	+0.60
	6	17	6.23	2	2.30	+1.42
Total		138	50.56	44	50.57	
C	1	5	1.83	2	2.30	-0.28
	2	1	0.37	0	0.00	
	3	2	0.73	0	0.00	
	4	8	2.92	2	2.30	+0.31
	5	9	3.30	0	0.00	
	8	6	2.20	0	0.00	
	9	1	0.37	0	0.00	
	10	3	1.10	0	0.00	
	12	1	0.37	0	0.00	
	14	1	0.37	0	0.00	
Total		37	13.57	4	4.60	
D	1	14	5.13	5	5.75	

NB Urban isolates 273

Rural isolates 87

Table 3

BIOCHEMICAL PROFILES OF RARE ISOLATES

(a) An invasive E. coli (O:164) agglutinating in specific S. dysenteriae type 3 antiserum, and

(b) A rare gas producing variant of S. boydii serotype 14.

Characterstic	(a)	(b)
Indole	+	-
Urease	-	-
Citrate (Simmon's)	-	-
Citrate (Christensen's)	+ 7 days	-
Motility	-	-
Methyl Red	+	+
Voges Proskauer	-	-
Hydrogen sulphide	-	-
Betagalactosidase	+ weak	-
Phenylalanine desminase	-	-
Lysine decarboxylase	-	-
Arginine dehydrolase	-	+
Ornithine decarboxylase	-	-
Glucose	+	+
Gas (Glucose)	-	+
Mannitol	+	-
Lactose	-	-
Sucrose	-	-
Dulcitol	-	-
Xylose	+	-
Rhamnose	+	-
Raffinose	-	-
Glycerol	+ weak	+ weak
Arabinose	+	+
Sorbitol	+	-
Malonate	-	-
Inositol	-	-
Adonitol	-	-
Salicin	+ 7 days	-

NB: Incubation was at 35°C. for 3 days, unless indicated.

Table 4

PATTERNS OF DRUG RESISTANCE
IN 360 SHIGELLA ISOLATES

Resistance Patterns	All Shigella (360)a		Group A (118)		Group B (182)		Group C (41)		Group D (19)	
	No.	%	No.	%	No.	%	No.	%	No.	%
1. T	6	1.67	0	0.00	3	1.65	3	7.32	0	0.00
2. S	2	0.55	0	0.00	2	1.10	0	0.00	0	0.00
3. Su	23	6.39	2	1.69	16	8.79	3	7.32	2	10.53
4. TSu	76	21.11	0	0.00	76	41.76	0	0.00	0	0.00
5. SSu	20	5.55	8	6.78	8	4.39	1	2.44	3	15.79
6. TCSu	1	0.28	0	0.00	1	0.55	0	0.00	0	0.00
7. TACb	2	0.56	0	0.00	2	1.10	0	0.00	0	0.00
8. TSSu	45	12.50	5	4.24	31	17.03	4	9.76	5	26.31
9. ACbK	1	0.28	0	0.00	0	0.00	1	2.44	0	0.00
10. TCACb	3	0.83	0	0.00	3	1.65	0	0.00	0	0.00
11. TCSSu	18	5.00	15	12.71	1	0.55	1	2.44	1	5.26
12. TACbS	1	0.28	0	0.00	0	0.00	1	2.44	0	0.00
13. TACbSu	1	0.28	0	0.00	1	0.55	0	0.00	0	0.00
14. TKSSu	1	0.28	1	0.85	0	0.00	0	0.00	0	0.00
15. TACbSSu	1	0.28	0	0.00	0	0.00	1	2.44	0	0.00
16. CACbSSu	1	0.28	1	0.85	0	0.00	0	0.00	0	0.00
17. TCACbSSu	71	19.72	63	53.39	7	3.85	0	0.00	1	5.26
18. CACbKSSu	1	0.28	1	0.85	0	0.00	0	0.00	0	0.00
19. TCACbKSSu	1	0.28	1	0.85	0	0.00	0	0.00	0	0.00
20. Sensitive	85	23.61	21	17.80	31	17.03	26	63.41	7	36.84

[a]Figures in parenthesis refer to number of isolates.

Table 5

PATTERNS OF DRUG RESISTANCE:
URBAN vs RURAL

Resistance patterns	All Shigella (360)a		Urban (273)		Rural (87)		Z-value
	No.	%	No.	%	No.	%	
1. T	6	1.67	5	1.83	1	1.15	+0.43
2. S	2	0.55	2	0.73	0	0.00	
3. Su	23	6.39	21	7.69	2	2.30	+1.79
4. TSu	76	21.11	57	20.88	19	21.84	-0.19
5. SSu	20	5.55	19	6.96	1	1.15	+2.07
6. TCSu	1	0.28	1	0.37	0	0.00	
7. TACb	2	0.56	2	0.73	0	0.00	
8. TSSu	45	12.50	31	11.35	14	16.09	-1.16
9. ACbK	1	0.28	1	0.37	0	0.00	
10. TCACb	3	0.83	2	0.73	1	1.15	-0.36
11. TCSSu	18	5.00	14	5.13	4	4.60	+0.20
12. TACbS	1	0.28	1	0.37	0	0.00	
13. TACbSu	1	0.28	1	0.37	0	0.00	
14. TKSSu	1	0.28	1	0.37	0	0.00	
15. TACbSSu	1	0.28	1	0.37	0	0.00	
16. CACbSSu	1	0.28	1	0.37	0	0.00	
17. TCACbSSu	71	19.72	41	15.02	30	34.48	-3.97
18. CACbKSSu	1	0.28	0	0.00	1	1.15	
19. TCACbKSSu	1	0.28	0	0.00	1	1.15	
20. Sensitive	85	23.61	72	26.37	13	14.94	+2.18

[a]Figures in parenthesis refer to number of isolates.

Table 6

PATTERNS OF DRUG RESISTANCE:

S. DYSENTERIAE

Resistance pattern	Serogroup A (118)a		SEROTYPES											
			1(77)		2(30)		3(3)		4(4)		6(2)		7(2)	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1. Su	2	1.69	0	0.00	2	6.67	0	0.00	0	0.00	0	0.00	0	0.00
2. SSu	8	6.78	0	0.00	1	3.33	1	33.33	3	75.00	2	100.00	1	50.00
3. TSSu	5	4.24	2	2.60	1	3.33	1	33.33	1	25.00	0	0.00	0	0.00
4. TCSSu	15	12.71	9	11.69	6	20.00	0	0.00	0	0.00	0	0.00	0	0.00
5. TKSSu	1	0.85	0	0.00	1	3.33	0	0.00	0	0.00	0	0.00	0	0.00
6. CACbSSu	1	0.85	1	1.30	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
7. TCACbSSu	63	53.39	63	81.82	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
8. CACbKSSu	1	0.85	1	1.30	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
9. TCACbKSSu	1	0.85	1	1.30	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
10. Sensitive	21	17.80	0	0.00	19	63.33	1	33.33	0	0.00	0	0.00	1	50.00

[a]Figures in parenthesis refer to number of isolates.

Table 7

PATTERNS OF DRUG RESISTANCE:

S. FLEXNERI

Resistance patterns	Serogroup B (182)a		SEROTYPES									
			1(63)		2(49)		3(3)		4(48)		6(19)	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1. T	3	1.65	1	1.59	0	0.00	0	0.00	2	4.17	0	0.00
2. S	2	1.10	1	1.59	1	2.04	0	0.00	0	0.00	0	0.00
3. Su	16	8.79	4	6.35	0	0.00	0	0.00	1	2.08	11	57.89
4. TSu	76	41.76	43	68.25	0	0.00	0	0.00	33	68.75	0	0.00
5. SSu	8	4.39	1	1.59	3	6.12	0	0.00	1	2.08	3	15.79
6. TCSu	1	0.55	1	1.59	0	0.00	0	0.00	0	0.00	0	0.00
7. TACb	2	1.10	0	0.00	1	2.04	0	0.00	1	2.08	0	0.00
8. TSSu	31	17.03	4	6.35	24	49.00	1	33.33	1	2.08	1	5.26
9. TCACb	3	1.65	1	1.59	1	2.04	0	0.00	1	2.08	0	0.00
10. TCSSu	1	0.55	0	0.00	1	2.04	0	0.00	0	0.00	0	0.00
11. TACbSu	1	0.55	1	1.59	0	0.00	0	0.00	0	0.00	0	0.00
12. TCACbSSu	7	3.85	2	3.17	1	2.04	0	0.00	4	8.33	0	0.00
13. Sensitive	31	17.03	4	6.35	17	34.69	2	66.66	4	8.33	4	21.05

[a]Figures in parenthesis refer to number of isolates.

Table 8

PATTERNS OF DRUG RESISTANCE

S. boydii

Resistance patterns	Serogroup C (41)a	S E R O T Y P E S													
		1(7)		2(1)		3(2)		4(10)		5(9)		8(6)		9(1)		10(3)		12(1)		14(1)	
	No. %	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1. T	3 7.32	0	0.00	0	0.00	0	0.00	2	20.00	0	0.00	1	16.67	0	0.00	0	0.00	0	0.00	0	0.00
2. Su	3 7.32	0	0.00	0	0.00	0	0.00	1	10.00	1	11.11	0	0.00	0	0.00	0	0.00	1	100.00	0	0.00
3. SSu	1 2.44	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	1	100.00
4. TSSu	4 9.76	0	0.00	1	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	3	100.00	0	0.00	0	0.00
5. ACbK	1 2.44	0	0.00	0	0.00	0	0.00	1	10.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
6. TCSSu	1 2.44	0	0.00	0	0.00	0	0.00	0	0.00	1	11.11	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
7. TACbS	1 2.44	1	14.29	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
8. TACbSSu	1 2.44	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	1	16.67	0	0.00	0	0.00	0	0.00	0	0.00
9. Sensitive	26 63.41	6	85.71	0	0.00	2	100.00	6	60.00	7	77.77	4	66.67	1	100.00	0	0.00	0	0.00	0	0.00

[a]Figures in parenthesis refer to number of isolates.

Table 9

CHAPTER 4

DISCUSSION AND CONCLUSION

SEROGROUPS AND SEROTYPES

The predominance of S. flexneri in developing countries is well known (94,95,97,101). Similarly, in Ethiopia, the prominence of S. flexneri has been confirmed in this study and in previous reports (81,89). In developed countries, like U.S.A. (10), France (98), England (37), Australia (102), Poland (93), S. flexneri has given way to S. sonnei as the dominant serogroup in these countries (Table 1). Slopek (103) mentions that during the years 1900 to 1924, S. dysenteriae was common in Europe, but later this serogroup was dominated by S. flexneri (1926-1949), which was in turn overwhelmed by S. sonnei since the 1950's. According to Christie (37), the factor responsible for this change of serogroup prevalence with time is difficult to account. In Ethiopia, as in other developing countries (97,94,95), all four serogroups coexist, albeit in different proportions.

In this study, twenty-two of the thirty-two known Shigella serotypes were identified. The fact that S. dysenteriae serotype 1 represented 21.39% of all Shigella isolates included in this study indicates the prevalence of this widely reported (14,35,82,84,86, 104) serotype in the Ethiopian environment. Overall isolation rate of S. dysenteriae type 1 in urban areas (17.22%) was about half that in rural areas, and this is significant (P=0.05). The predominance

of S. dysenteriae type 1 in rural Shigella outbreaks was demonstrated during our field trips to Amibara, Metahara, Gamo Awraja and Jiba-Mecha Awraja. During these outbreaks S. dysenteriae type 1 constituted 13/16 (81.25%) of total Shigella isolates from these areas.

Of the twenty-two serotypes identified in this study, only eleven of them were represented in rural areas. The larger number of urban Shigella serotypes could probably be due to importation of foreign serotypes by the international community residing in urban centers.

After a long absence from the world, S. dysenteriae serotype 1 (Shiga's bacillus) reappeared in populous Central America, during the years 1969 to 1972 (14,35,82,84). The disease was unfortunately misdiagnosed as amoebiasis (2), and as a result, an estimated 122,000 cases of dysentery with 3,800 deaths occurred during the first ten months of the 1969 Guatemalen epidemic (14). In recent years, S. dysenteriae type 1 was reported to have caused outbreaks on a coral island in the bay of Bengal, affecting 33% of its entire population in three months (105). Similar outbreaks occurred in Bangladesh (104), in Sri-Lanka (106) and in rural Somalia (38).

Among the other S. dysenteriae serotypes, type 2 was reported to be more common in Poland (93) and was second to S. dysenteriae type 1 in North Africa, Equatorial Africa and Indochina (101).

Shigella flexneri serotype 1 (34,62%), followed by serotype 2 (26,92%) were commonly isolated in this study. In Nigeria (97),

Malagasy (94), Australia (107), Peru (96) and Vietnam (108), S. flexneri type 2 was reported as most common. Similarly, a mammouth study by Piechaud et al. (101) indicated the prevalence of S. flexneri type 2 in North Africa, Equatorial Africa, Indochina and Turkey.

Among Shigella boydii serotypes, we found that S. boydii type 4 was most common (24.39%), closely followed by serotype 5(21.95%). In Nigeria (97), similar prevalence of S. boydii type 4 was reported for the years 1976 and 1977.

Prevalence of Shigella serotypes in selected countries is shown in Table 10. The analysis refers to culture collections over a period of one year or more. The studies were conducted between 1964 and 1980 and specimen numbers vary considerably. With these limitation in mind, the table offers some idea of comparative serotype prevalence in these areas. S. dysenteriae type 1 is prevalent in Ethiopia, Equatorial Africa and Indochina. S. flexneri type 2 is most common in all these countries, with the exception of Ethiopia, where it takes a second place within S. flexneri serotypes. Shigella sonnei is prevalent in France, a developed country. Rare Shigella serotypes include: S. dysenteriae types 5 and 10, as well as S. boydii types 6, 7, 12, 13, and 15.

SHIGELLA SEROTYPES:

COMPARATIVE PREVALENCE

Group	Type	No. of Shigella isolates				
		Ethiopia	Malagasy (94)	France (98)	Eq. Africa (101)	Indochina (101)
A	1	77	7	1	96	33
	2	30	7	4	85	23
	3	3	4	2	19	2
	4	4	0	1	9	0
	5	0	0	0	1	2
	6	2	1	0	17	1
	7	2	1	2	10	4
	8	0	0	0	0	0
	9	0	1	1	0	0
	10	0	0	0	0	0
B	1	63	12	26	109	55
	2	49	117	123	444	231
	3	3	29	40	148	74
	4	48	54	35	104	44
	5	0	0	1	36	47
	6	19	30	37	72	15
C	1	7	1	0	28	3
	2	1	2	6	11	19
	3	2	0	0	14	6
	4	10	0	1	18	16
	5	9	1	1	47	9
	6	0	1	0	0	0
	7	0	0	0	0	0
	8	6	0	1	6	0
	9	1	0	0	12	0
	10	3	0	3	8	0
	11	0	1	1	16	11
	12	1	0	0	0	0
	13	0	0	0	0	0
	14	1	0	2	0	0
	15	0	1	0	1	0
D		19	66	332	162	64
No. Isolates		360	367	620	1474	659

Table 10

MULTIPLE DRUG RESISTANCE

Ninteen patterns of drug resistance were exhibited by the 360 Shigella isolates, included in this study. In Poland, Noworyta (110) found 26 different patterns. This problem of multiple drug resistance is world-wide and is the result of indiscriminate use of antibiotics by man, and also use of antibiotics in animals (2). After analysis of the "trends and consequences" of antibiotic use in the U.S.A., Simmons and Stolley (115) concluded that much of the increasing use of these drugs was not justifiable. This misuse of antibiotics is especially dangerous in Shigella chemotherapy, where treatment with a single drug is known to result in the excretion of multiple resistant strains (6).

Though no correlation study has been attempted to-date, S. dysenteriae serotype 1 has consistently been associated with unusually large multiple drug resistance. The Central American epidemic due to S. dysenteriae type 1 (14,35,82) had a TCSSu pattern, while that of Bangladesh (104) had a TCASSu pattern. A similar pattern was demonstrated in Mexico City (84). In Somalia, resistance to 5, 6 or 7 drugs was noted (86). In our study, out of the 77 serologically confirmed S. dysenteriae type 1, 63(81.82%) were resistant to 6 drugs (TCACbSSu) and one strain was resistant to 7 drugs (TCACbKSSu). In this connection, it is interesting to note that, though the isolation rate of S. dysenteriae type 2 was reasonably frequent (8.33% of total Shigella isolates), not a single strain within this serotype was associated with the TCACbSSu pattern. On the contrary,

S. dysenteriae serotypes, other than serotype 1, were reasonably sensitive to most drugs (89). Another attribute of S. dysenteriae type 1, in this study, was the fact that not a single strain within the 77 isolates was uniformly sensitive to all drugs.

Multiple drug resistance pattern similar to that of S. dysenteriae type 1 was exhibited by S. flexneri type 2 in Cape Town (64). In our study, this type of resistance was shown, not only by S. flexneri type 2, but also by serotypes 1 and 4. Among the 182 S. flexneri isolates, 7 showed the TCACbSSu pattern. The findings of Watson (64) differs from our finding in that patterns like TCKSSu (which included kanmycin) were not encountered. Lewis (78) in 1967 reported S. flexneri type 2a with TCASSuNx from an outbreak in a mental hospital. In Brazil (63), Piechaud et al. reported a TCAKFNSSu pattern in 2 out of 16 strains of S. flexneri type 2, and TCASSu pattern was observed in strains of S. flexneri types 2 and 6 in France (109).

Shigella boydii is unique among Shigella serogroups in that multiple drug resistance is comparatively limited (89,110). Whether or not this is due to its low isolation rate and, therefore, less contact with other resistant bacteria remains to be seen. In our study, the most common pattern of drug resistance within S. boydii serotypes was TSSu (9.76%), while Noworyta (110) reported TSu pattern in Pland.

In a study of 590 S. sonnei of 21 countries in the 5 continents, Szturm-Rubinsten et al. (111) mentioned that SSu pattern was the

most common (60.0%); further they concluded, that S. sonnei has in the same period, in the same area, the same pattern of resistance. Our results show that TSSu pattern (26.32%) is the most common, followed by SSu (15.79%) and Su (10.53%) patterns. In Australia, S. sonnei with TCASu pattern was reported (112), and in South Africa, the TCSSu pattern was noted (64). In addition, Davis et al. (113) in England reported that 70% of S. sonnei were resistant to 3 or more drugs. Comparative sensitivity patterns of Shigella serogroups in Ethiopia showed that resistance to 2 and 3 drugs were exhibited by 58.8% of 17 S. sonnei isolates (89).

While studying the state of drug resistance within Shigella isolates, our attention was drawn by the various patterns of drug resistance and the possibility of differentiating serotypes (within serogroups) on the basis of their commonly associated resistance pattern. At present, however, the large number of these patterns (19 in this study) has more or less limited the usefulness of this method for identification of Shigella serotypes. Inspite of their large number, however, certain patterns are more commonly associated with specific serotypes (Plates 1-6); a) within S. dysenteriae serogroup, the TCACbSSu pattern was commonly demonstrated in S. dysenteriae serotype 1 (81.82%), while S. dysenteriae serotype 2 was often associated with TCSSu (20.00%); b) within S. flexneri serotypes, TSu was most frequently seen in serotypes 1 and 4 (68.25% and 68.75%, respectively), while TSSu is comparatively more common in serotype 2 (49.00%); c) for S. sonnei TSSu is

reasonably frequent (26.32%). At present, the usefulness of multiple drug resistance for serotype identification is limited, but such studies should be routinely carried out to detect the stability of such patterns with place and time.

Another use of surveys of multiple drug resistance is to guide treatment of shigellosis in areas where laboratory facilites are not available. In such cases, information of drug resistance patterns, preferably linked to specific geographic area, could be helpful in selecting appropriate chemotherapy (110), i.e. by minimizing indiscriminate chemotherapy and the risks inherent in such procedures. Inspite of the fact that resistance to antibiotics is not usually predictable (114), Noworyta (110) in Poland was able to delineate the "western resistant part" of Poland from the "eastern sensitive" one.

BIOCHEMOTYPING

Serological identification of Shigella is greatly facilitated if it is preceded by complete biochemical study, as recommended by Edwards and Ewing (1). Accordingly, we have followed biochemotyping recommended by these authors. Our results were as expected. However, the following report on identification of rare biochemotypes is presented:

(a) Mannitol negative strains of S. flexneri serotype 6. Our study of 182 S. flexneri isolates has revealed 3 strains of mannitol negative S. flexneri serotype 6. These strains, though comparatively rare, are well documented (1). There is a tendency in routine

laboratories to try serological agglutination of mannitol negative strains with S. dysenteriae antiserum only. Accordingly, some positive cultures can easily be missed. If clinical, cultural and biochemical tests are typical for genus Shigella, mannitol negative strains that fail to agglutinate in S. dysenteriae antiserum should not be discarded without checking them with S. flexneri and S. boydii antisera.

(b) A gas producing variant of S. boydii serotype 14. Gas production has been commonly reported in the Manchester and Newcastle strains of S. flexneri type 6, but not in S. boydii (1). The mannitol negative variant of S. boydii type 14 in this study showed large quantities of gas in Triple Sugar Iron and Lysine Iron Agar slants. In routine laboratories, gas producing strains are discarded as non-Shigella, after ruling out the Manchester-Newcastle strains. In this particular case, we were forced to try with all Shigella antisera because: a) the strain was isolated from a typical case of shigellosis, b) colonial characteristics were typical of Shigella and c) biochemical characteristics, except for gas formation, were also typical of Shigella. To counter-check our result, this strain was referred to the 'Centre National des Shigella' in Paris, which confirmed our identification of the strain as gas producing S. boydii serotype 14. Although this is the first report of such an isolate in Ethiopia, its incidence has been reportedly increasing in Europe, Asia and some African countries (116).

(c) An invasive E. coli (O:164) which showed strong agglutination in specific S. dysenteriae serotype 3 antiserum. According to Edwards and Ewing (1):

> The entire S. dysenteriae subgroup is mannitol negative. The only known exception to this is a mannitol positive variant of S. dysenteriae serotype 3, described by Dr. K.P. Carpenter/ Personnal communication, 1956/ and mentioned by Ewing et al., 1958.

The strain isolated in our study is different from the mannitol positive variant of S. dysenteriae type 3 (mentioned above) in that: a) it was unusually carbohydrate active, fermenting mannitol, xylose, rhamnose, glycerol, arabinose and sorbitol, b) it was weakly betagalactosidase positive and c) it gave delayed positive results in Christensen's citrate and salicin. Fermentation of xylose and rhamnose by S. dysenteriae has not been reported, according to Edwards and Ewing (1). We concluded, therefore, that this isolate was an E. coli sharing common antigens with S. dysenteriae serotype 3. To check our result, we also referred this strain to 'Centre National des Shigella' in Paris. On the basis of exhaustive biochemical and serological tests, Toucas (116) tentatively classified the organism as "invasive E. coli O:164". E. coli type O:164 is an invasive strain, which has caused outbreaks of diarrheae in countries like Australia and England (116). Further study will be made to see if this organism is common in Ethiopia, and if it is always associated with diarrheal outbreaks.

SHIGELLA CHEMOTHERAPY: PAST HISTORY AND PRESENT OPTION

The Discouraging History of Shigella Chemotherapy

Almost all common antibiotics had had their ups and downs in Shigella chemotherapy.. The golden age of sulpha therapy started to wane in the 1950's (117). In 1966, Haltalin and Nelson (118) reported that 87% of S. sonnei and 59% of S. flexneri were resistant to sulphadiazine. In Australia, 95% of combined S. sonnei and S. flexneri were resistant to sulphadiazine (102). Chloramphenicol was reported effective in the 1950's (119,120). Clinical trial using gentamicin sulphate in Shigella was termed "useful new drug", though 11% failure rate was noted in the same study (121). The tetracyclines fared no better (87,113). However, the single dose tetracycline therapy in adults was reported as effective, irrespective of susceptibility test results (122). The use of Ampicillin in shigellosis gave impressive results compared to sulpha drugs (123). Compared to the non-absorbable drugs, like neomycin, ampicillin was found more effective (124), though oral ampicillin treatment often resulted in superinfection with Candida and Pseudomonas species (125). Comparative study of oral and intramuscular ampicillin showed that the latter method exhibited faster clearing of Shigella from stools and was less likely to cause superinfection (126). The rise of multiple drug resistance (69,70,71,84,85,86,87,88,89,127) in Shigella nullified the effectiveness of most common drugs including tetracycline chloramphenicol and ampicillin (113). The present drug of choice, especially where the problem of multiple drug resistance

prevails, is trimethoprim ___ sulphamethoxazole (128,129,130), though resistance to this drug is also being reported (86,102,106).

The Present Option for Shigella Chemotherapy in Ethiopia

In Ethiopia, a recent study has shown that 63.6% of 165 Shigella isolates were resistant to sulphadiazine and 52.7% resistant to tetracycline (89). Similarly in this study, TSu, TSSu and TCACbSSu patterns combined constituted 53.55% of all patterns. As such, treatment of shigellosis with tetracyclines or sulpha drugs is to be discouraged. Ampicillin resistance was mostly demonstrated by members of S. dysenteriae, in this study, as well in the previous study (89). However, since the other serogroups, including the dominant S. flexneri, are generally sensitive to ampicillin, this drug is to be considered useful in present Shigella chemotherapy. In cases of ampicillin failure, one can assume with a fair degree of accuracy, that the etiological agent in question is a multiresistant (TCACbSSu) S. dysenteriae type 1, which in this study and in the previous study (89) was found to respond to trimethoprim-sulphamethoxazole.

RECOMMENDATIONS

Based on the present study, the following recommendations are made:

(a) Present level of Shigella study should be intensified. Shigellosis is highly endemic in Ethiopia. Published reports on genus Shigella are, however, quite scanty. The few field trips we made to rural areas have amply demonstrated that shigellosis is still rampant in many parts of Ethiopia. As such, a survey of shigellosis, with special emphasis on rural areas, is deemed essential.

(b) Methods of Shigella phage-and colicin-typing should be instituted. For accurate epidemiological studies, present diagnostic capabilities should be supplemented with finer methods of typing.

(c) Genetic studies of multiple drug resistance should be continued. The present level of genetic study should be intensified to monitor the mechanism of the acquisition of drug resistance in Ethiopia. The reason why certain serotypes, like S. dysenteriae type 1 are usually multiresistant should be investigated.

(d) A national Shigella reference center should be established. A national surveillance programme, in addition to early detection of the appearance of multiresistant strains, may guide clinicians in choosing the most suitable antimicrobial agents, when laboratory support is not available. It may

also provide partial contribution for the development of national and international policies concerning the manufacture and importation of antibiotics.

(e) The role of improved personal hygiene and environmental sanitation should be stressed. The somewhat discouraging history of Shigella chemotherapy underlines the fact that shigellosis can only be effectively tackled by improving hygienic conditions. This effort should be coupled with: i) effective fly-control, ii) safe sewage-disposal system and iii) easily available potable water. In this connection, the need for appropriate health education cannot be overemphasized.

(f) Chemotherapy should be discouraged. Generally speaking, chemotherapy in shigellosis is not advisable. However, in cases where chemotherapy is indicated, it should be guided by i) individual case analysis, ii) likelihood of secondary spread and iii) susceptibility of the organism to safe effective drug. The use of rehydration procedure, oral or systemic, should be given priority in Shigella therapy.

(g) A national policy for drug sale should be enacted. The present indiscriminate use of antibiotics is partially due to the fact that the public has an easy access to all drugs. To control present drug abuse, antibiotics should be sold only upon presentation of valid medical prescription.

(h) Finally, efforts to produce a Shigella vaccine should be intensified. To-date, the antigenic diversity of Shigella

has not lent itself to the production of multipotent vaccines. Such effort, however, should be supplemented with research in intestinal immunity and disease pathology, both of which may open new frontiers in vaccine production.

APPENDIX AND REFERENCE MATERIAL

COMMON PATTERNS OF DRUG RESISTANCE:

S. dysenteriae serotype 1

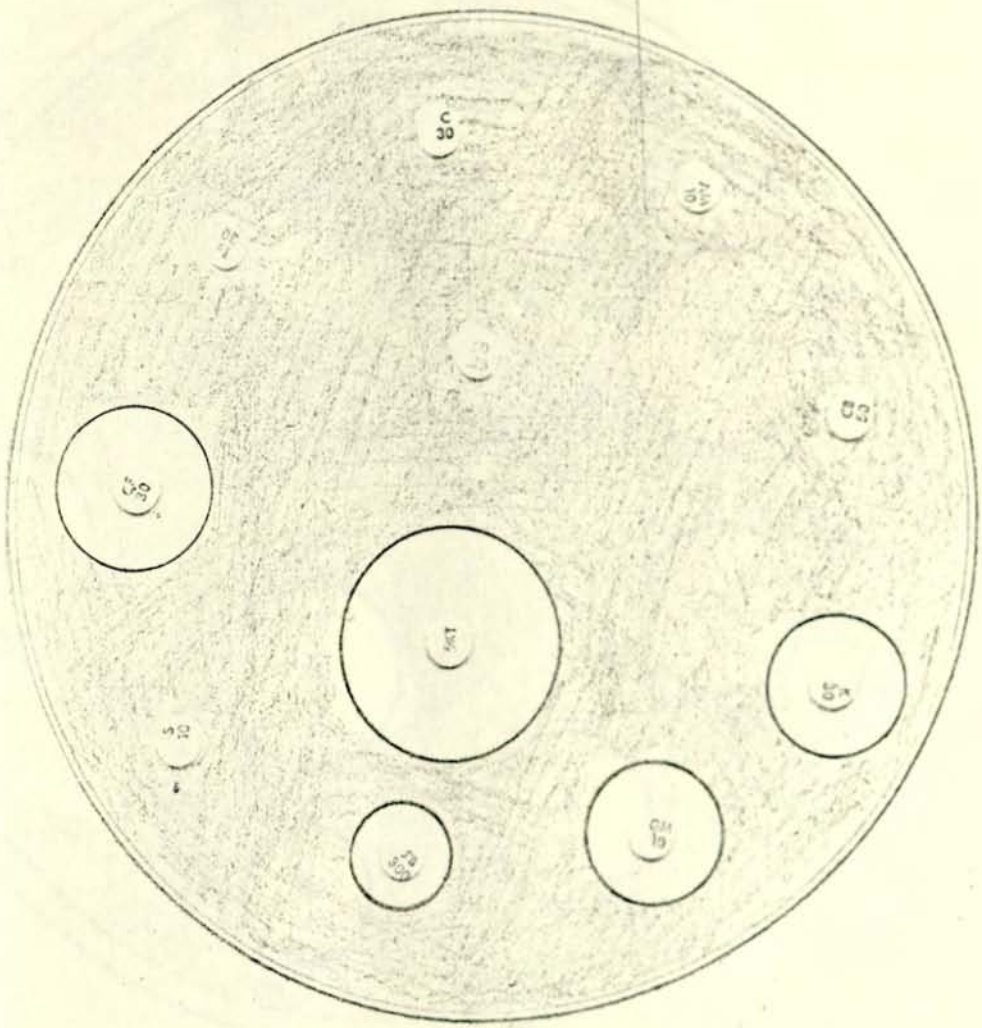

PLATE No. 1

SENSITIVE/ CF, K, Cm, Px, Sxt

RESISTANT/ T, C, A, Cb, S, Su (81.82%)

COMMON PATTERNS OF DRUG RESISTANCE:

S. dysenteriae serotype 2

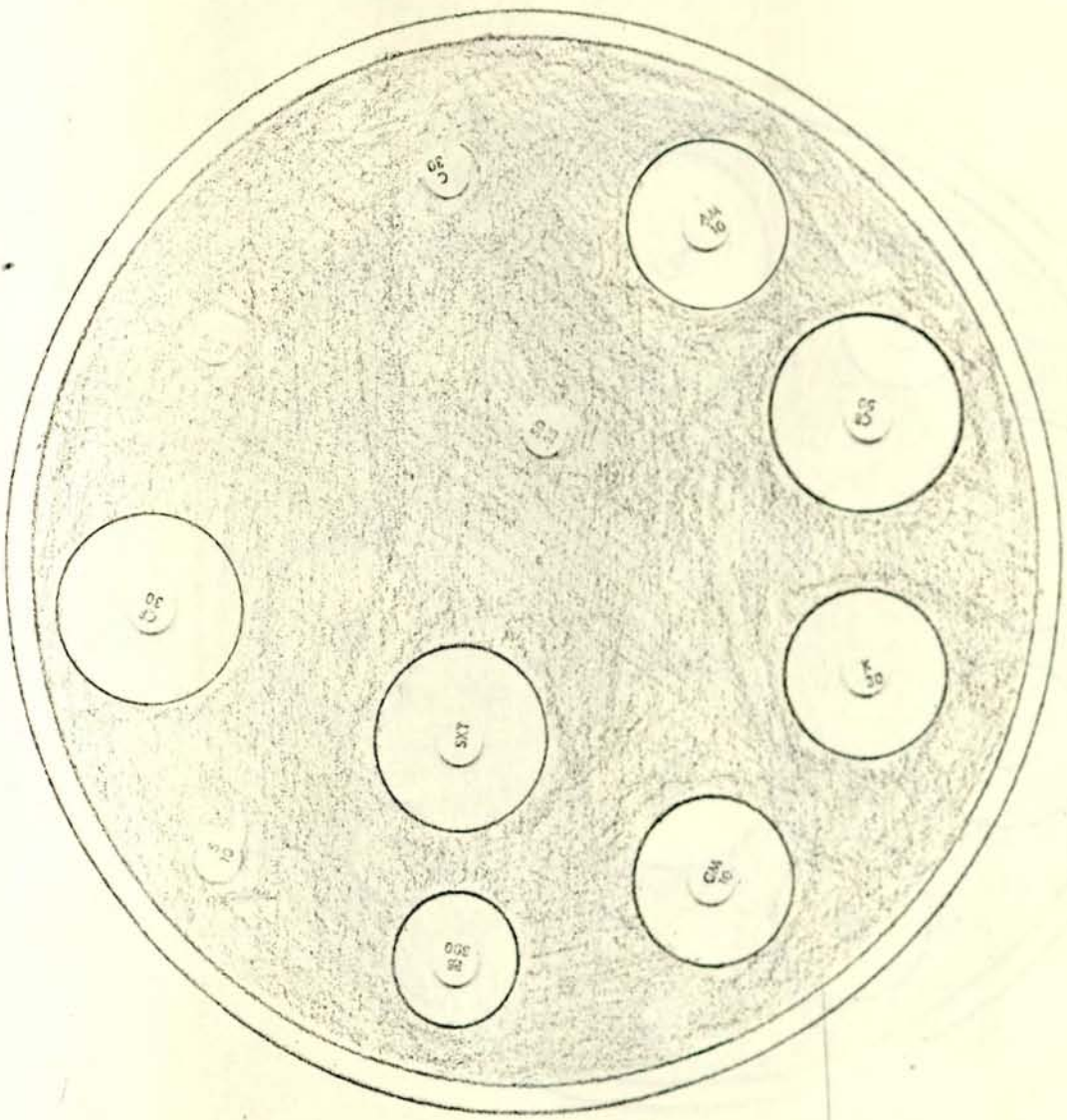

PLATE No. 2

SENSITIVE: CF, A, Cb, K, Gm, Px, Sxt

RESISTANT: T, C, S, Su (20.00%)

COMMON PATTERNS OF DRUG RESISTANCE:

. S. flexneri types 1 and 4

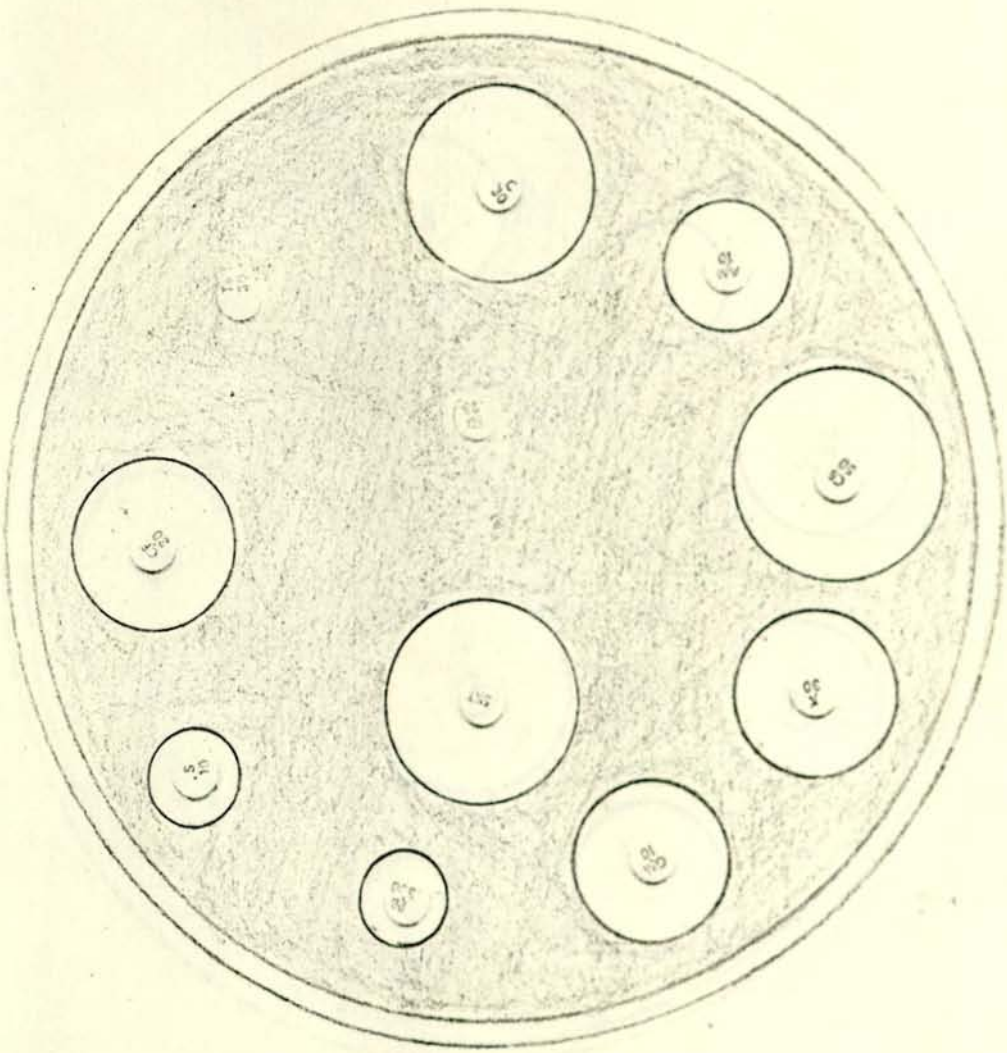

PLATE No. 3

SENSITIVE: CF, C, A, Cb, K, Gm, Px, S, Sxt

RESISTANT: T, Su (68.25% and 68.75%)

COMMON PATTERNS OF DRUG RESISTANCE:

S. flexneri serotype 2

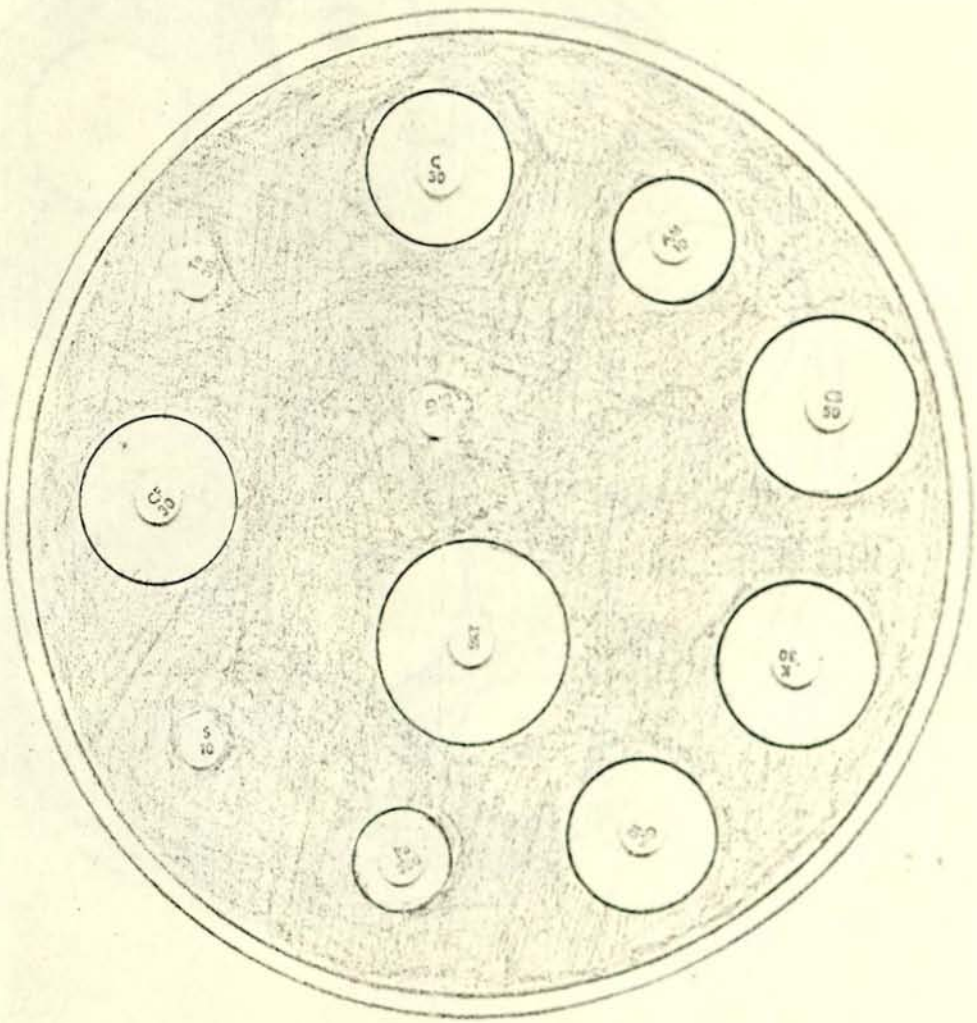

PLATE No. 4

SENSITIVE: CF, C, A, Cb, K, Cm, Px, Sxt

RESISTANT: T, S, Su (49.00%)

COMMON PATTERNS OF DRUG RESISTANCE:

S. flexneri serotype 6

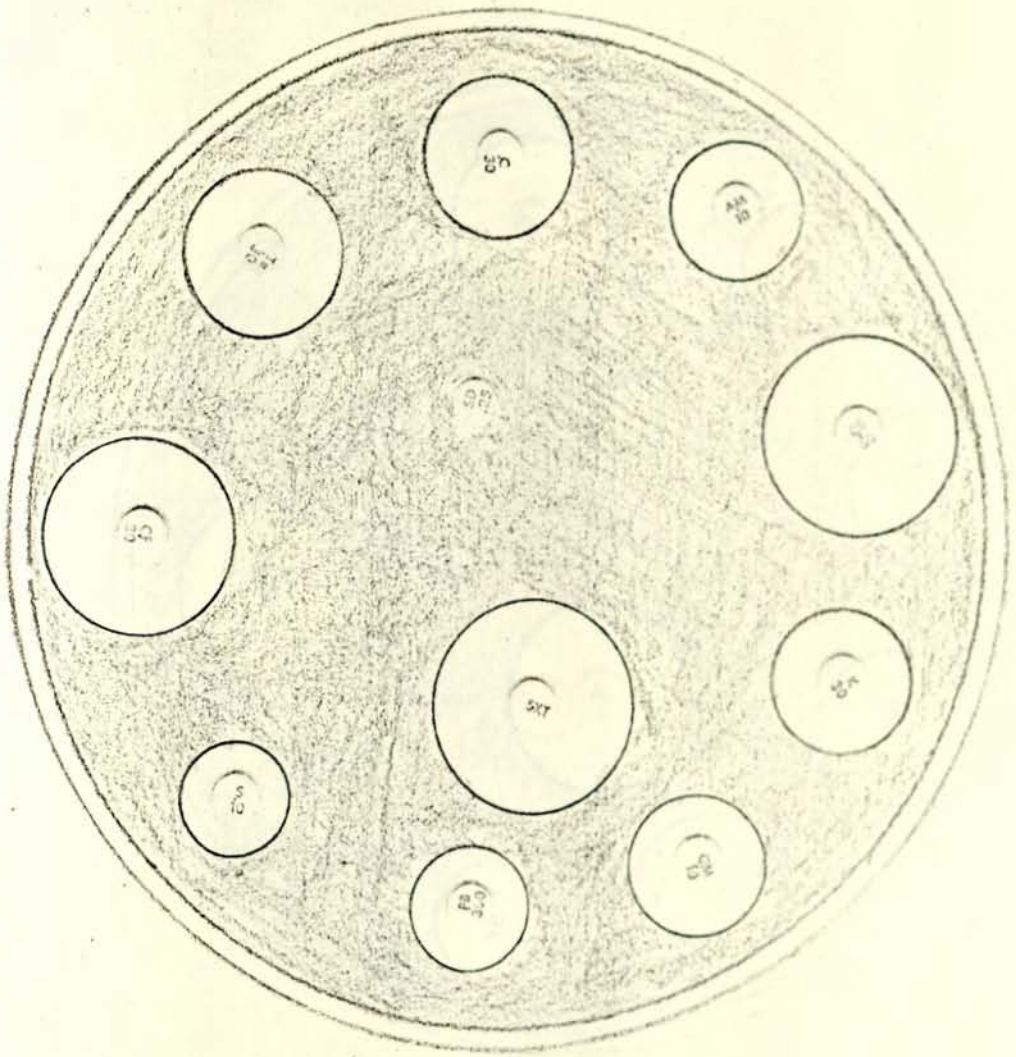

PLATE No. 5

SENSITIVE: CF, T, C, A, Cb, K, Gm, Px, Sxt

RESISTANT: Su (57.89%)

COMMON PATTERNS OF DRUG RESISTANCE:

S. sonnei

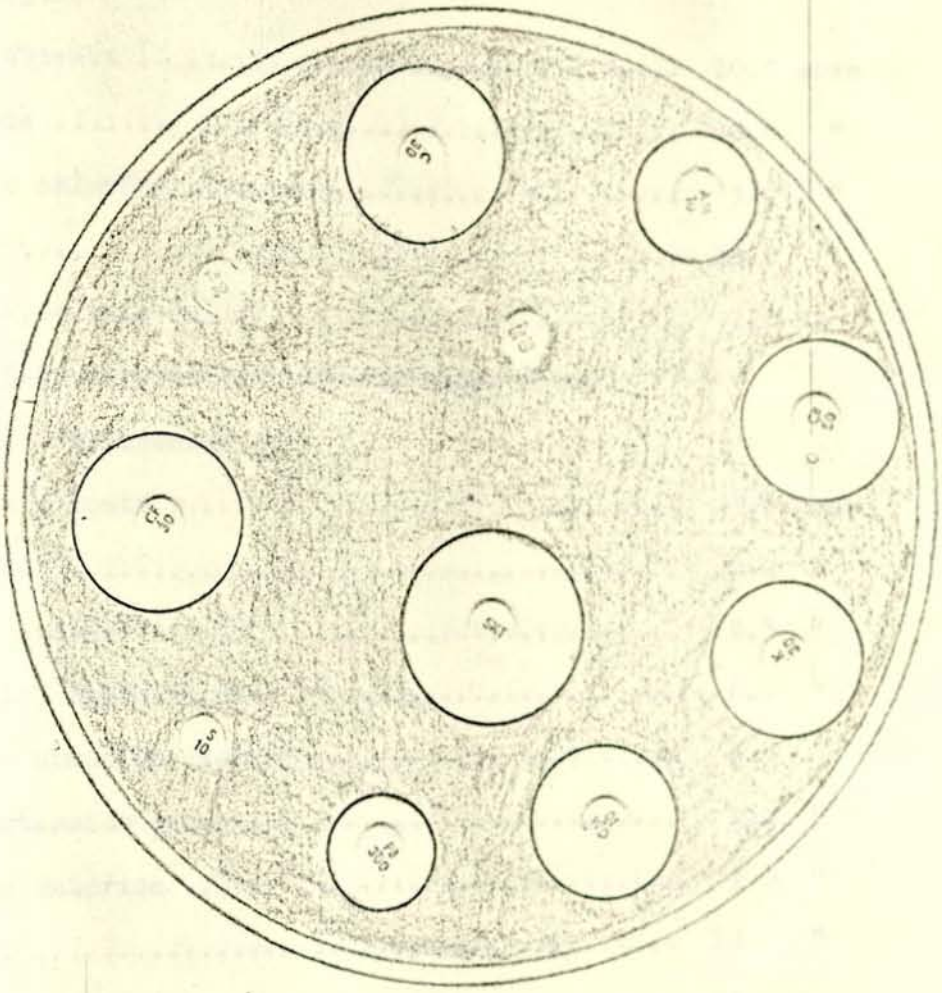

PLATE No. 6

SENSITIVE: CF, C, A, Cb, K, Gm, Px, Sxt

RESISTANT: T, S, Su (26.32%)

FORMULAE OF CULTURE MEDIA AND REAGENTS

1. Andrade's Indicator:

Acid fuchsin	0.5 gram
Sodium hydroxide (1.ON)	16.0 ml
Distilled water	100.0 ml

2. Blood Agar:

Beef extract	10.0 gram
Peptone	10.0 "
Sodium chloride	5.0 "
Agar	15.0 "
Distilled water	1.0 liter

Add: 5-10 percent defibrinated sheep blood.

3. Citrate (Christensen's):

Sodium citrate	3.0 gram
Glucose	0.2 "
Yeast extract	0.5 "
Cysteine hydrochloride	0.1 "
Ferric ammonium citrate	0.4 "
Monopotassium phosphate	1.0 "
Sodium chloride	5.0 "
Agar	15.0 "
Sodium thiosulphate	80.0 mg
Phenol red	12.0 "
Distilled water	1.0 liter

4. Citrate Agar (Simmon's):

Magnesium sulphate	0.2 gram

Dipotassium phosphate	1.0 gram
Monoammonium phosphate	1.0 "
Sodium citrate	2.0 "
Sodium chloride	5.0 "
Agar	15.0 "
Bromothymol blue	80.0 mg
Distilled water	1.0 liter

5. Glucose or mannitol peptone water (with Andrade's indicator):

Peptone water...	Proteose peptone	10.0 gram
	Sodium chloride	5.0 "
	Beef extract	3.0 "
	Distilled water	890.0 ml
Andrade's indicator		10.0 "
Sugar solution (10% glucose/mannitol=)		100.0 "

6. Kovac's reagent:

Amyl or iso-amyl alcohol	150.0 ml
Concentrated hydrochloric acid	50.0 "
p-dimethylaminobenzaldehyde	10.0 gram

7. Lysine Iron Agar:

Peptone	5.0 gram
Yeast extract	3.0 "
Dextrose	1.0 "
L-lysine	10.0 "
Ferric ammonium citrate	0.5 "
Sodium thiosulphate	40.0 mg

Bromocresol purple	20.0 mg
Agar	15.0 gram
Distilled water	1.0 liter

8. Malonate broth (Leifson):

Ammonium sulphate	2.0 gram
K_2HPO_4	0.6 "
KH_2PO_4	0.4 "
Yeast extract	1.0 "
Sodium chloride	2.0 "
Sodium malonate	3.0 "
Bromothymol blue	25.0 mg
Glucose	250.0 "
Distilled water	1.0 liter

9. MacConkey Agar:

Peptone	20.0 gram
Lactose	10.0 "
Bile salt No. 3	1.5 "
Sodium chloride	5.0 gram
Neutral red	30.0 mg
Crystal violet	1.0 "
Agar	13.5 gram
Distilled water	1.0 liter

10. Motility test medium (BBL):

Beef extract	3.0 gram
Peptone	10.0 "

Sodium chloride	5.0 gram
Agar	4.0 "
Distilled water	1.0 liter

11. Motility test medium (Modified):

BBL motility test medium	22.0 gram
Distilled water	1.0 liter
Triphenyl-tetrazolium (0.5%)	5.5 ml

12. MR-VP:

Polypeptone (buffered)	7.0 gram
Dextrose	5.0 "
Dipotassium phosphate	5.0 "
Distilled water	1.0 liter

13. Mueller-Hinton Agar:

Beef infusion	300.0 gram
Acid hydrolysate of casein	17.5 "
Starch	1.5 "
Agar	17.0 "
Distilled water	1.0 liter

14. Nutrient Broth:

Beef extract	3.0 gram
Peptone	5.0 "
Distilled water	1.0 liter

15. Ornithin decarboxylase both (Falkow):

Peptone	5.0 grams
Yeast extract	3.0 "
Dextrose	1.0 "

L-ornithine dihydrochloride	1.0 gram
Bromocresol purple .....(1.6%)	1.0 ml
Distilled water	1.0 liter

16. Purple broth base:

Peptone	10.0 gram
Beef extract	1.0 "
Sodium chloride	5.0 "
Bromocresol purple	15.0 mg
Distilled water	1.0 liter

17. Shigella-Salmonella Agar:

Beef extract	5.0 gram
Peptone	5.0 "
Lactose	10.0 "
Bile salts	8.5 "
Sodium thiosulphate	8.5 "
Agar	13.5 "
Brilliant green	0.33 mg
Neutral red	25.0 mg
Distilled water	1.0 liter

18. Triple Sugar Iron:

Beef extract	3.0 gram
Peptone.....	20.0 "
Lactose	10.0 "
Sucrose	10.0 "
Glucose	1.0 "

Ferrous sulphate	0.2 gram
Sodium thiosulphate	0.3 "
Sodium chloride	5.0 "
Agar	13.0 "
Phenol red	24.0 mg
Distilled water	1.0 liter

19. Tryptic Soy Yeast (TSY):

Trypticase soy yeast	30.0 gram
Yeast extract	10.0 "
Distilled water	1.0 liter

NB: for stock organisms, 25% glycerol is added.

20. Urea slant agar:

a) Urea concentrate

Peptone	1.0 gram
Sodium chloride	5.0 "
Monopotassium phosphate	2.0 "
Dextrose	1.0 "
Urea	20.0 "
Phenol red	12.0 mg
Distilled water	100.0 ml

b) Solidifying agent

Agar	15.0 gram
Distilled water	900.0 ml

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D E C L A R A T I O N

I, the undersigned, declare that this thesis is my work and that all sources of material used for the thesis have been duly acknowledged.

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