

Isolation, identification and sensitivity
testing of Microorganisms from
stool received from patients at Kenyatta
National Hospital

BY

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A dissertation submitted in partial
fulfilment for the award of the Bachelor
of Pharmacy Degree

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D E D I C A T I O N

My entire project is affectionately dedicated
to:-

My Parents

My sister Rashida

Mr Kassamali Ganiwalla

My fiancee Rehana

for their moral support and their utmost patience
during my entire Academic career.

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

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S U M M A R Y

The aim of this project was to isolate microorganisms from stools of patients from Kenyatta National Hospital, to identify them using various microbiological techniques and finally their sensitivity against commonly used therapeutic agents. The antibiotics used included:-

- (1) Ampicillin 25 microgramme
- (2) Tetracycline 25 microgramme
- (3) Trimethoprim/Sulphamethaxazole 25 microgramme
- (4) Streptomycin 10 microgramme
- (5) Kanamycin 30 microgramme
- (6) Gentamicin 10 microgramme
- (7) Polymixin B Sulphate 250 units
- (8) Neomycin 30 microgramme

Stool samples were plated on the Mac Conkey's Agar and colonies obtained were identified using Gram stain method, Motility and subculturing on TSI (Triple Sugar Iron).

Finally sensitivity testing was done with the antibiotic discs on Mac Conkey's.

From the results obtained, it was found that:-

- (1) The organisms isolated were E. coli, Salmonella and Shigella.
- (2) In majority of the patients (16 out of 24), the causative organism was E. coli.
- (3) All the microorganisms were most sensitive to Gentamicin. E. coli was least sensitive to Tetracycline while Salmonella and Shigella were not sensitive at all to Ampicillin, Tetracycline, Trimethoprim/Sulphamethoxazole and Streptomycin.

I N T R O D U C T I O N

The enteric group of bacteria includes a large number of species of gram negative non-sporulating rods whose natural habitat in most instances in the gastro-intestinal tract of man and other animals.

Some of these enteric bacteria are pathogenic for man and cause various types of gastro-intestinal diseases such as typhoid, enteric fevers, gastro-enteritis and dysentery.

Three of the common enteric bacteria pathogenic to man include:-

- (1) Shigella
- (2) Salmonella
- (3) E. coli (This is pathogenic mostly to children)

ESCHERICHIA COLI

History and Morphology - E. coli was isolated from faeces by Escherich in 1885. It was found universally in the intestinal tract of man and animals. It is also the predominant organism in the colon where it is commonly referred to as the "colon bacillus".

E. coli is a gram-negative bacillus, rod-shaped, 2 - 3 microns long and about 0.6 micro in breadth. The microorganisms are non-sporing and most strains are motile.

BIOCHEMICAL REACTIONS

E. coli ferments variety of carbohydrates, including dextrose, lactose, maltose, mannitol and Xylose with the production of acid and gas.

It produces Indole, does not utilize citrate and also does not liquefy gelatin. .

PATHOGENECITY

E. coli as a pathogen is associated with two main clinical symptoms:-

- (1) Acute gastra enteritis in infants upto 2 years of age and less
- (2) Infection of Urinary tract particularly in married women.

MODE OF TRANSMISSION

Epidemic diarrhoea usually predominant in infants under 2 years is usually acquired by infants as a secondary infections. Infact, situations have been reported that patients in hospitals for treatment of other conditions become infected with *E. coli*.

Before 1971, there were reports of water-borne outbreaks of *E. coli* in the United States. Also between 30th October, 1971 to 10th December, 1971, there were over 200 persons suffering from food poisoning due to *E. coli*.

CLINICAL FEATURES

Infants with epidemic diarrhoea can exhibit a variety of symptoms, including a bluish coloration of the skin, convulsions, dehydration, diarrhoea, joundice, nausea and vomiting.

LABORATORY DIAGNOSIS

The identification of enteropathogenic *E. coli* depends on its isolation from a freshly obtained stool specimen on a suitable and selective media such as Mac Conkey's, Eosin-Methylene Blue Agar.

Differentiation from other Gram negative organism invalues the use of biochemical tests which are specific to *E. coli*.

TREATMENT

The essential treatment of severely ill dehydrated infants is rehydration and restoration of the electrolyte balance.

Antibiotics although of little use, are sometimes given to prevent spread of other susceptible micro-organism.

CONTROL MEASURES

Generally, the prevention of infantile gastroenteritis demands that bottled milk feeds served to infants must be sterile and in hospitals this is best effected by terminal heat treatment of the fully prepared feed in the feeding bottle. In the home, mothers should be encouraged to observe the same standards but this is difficult to implement in conditions of poverty and poor environment. The most effective measure is breast feeding without supplements for the 1st 6 - 9 months of life.

SALMONELLA

History and Morphology

Salmonella enteritidis was first isolated in 1888 by Gaerlner from a patient who died following consumption of meat contaminated with this organism. Shortly after this Durham and Noeble isolated Salmonella typhimurium from patients suffering from gastroenteritis.

Salmonella are Gram-negative, motile, non-sporing rods. They average about 2 - 3 micron in length and about 0.6 micron in width but may vary in size under different environmental conditions.

Salmonella do not ordinarily form capsules when grown at 37°C but most species may give rise to mucoid colonies composed of Encapsulated bacilli.

BIOCHEMICAL REACTIONS

These bacteria do not ferment lactose, sucrose or salicin, while glucose, mannitol, maltose and dextrin are fermented with the production of acid and gas except in the S. typhosa which do not form gas. They all utilize citrate, while indole is not produced and also gelatin is not liquefied.

EFFECTS OF PHYSICAL AND CHEMICAL AGENTS

Most Salmonella are killed at a temperature of 60°C/15 minutes.

Brilliant green dye inhibit *E. coli* and *Shigella* while *Salmonella* species are resistant to its action.

Sodium deoxycholate and Selenium compounds also inhibit *E. coli* but not the *Salmonella*.

PATHOGENECITY

Salmonella species are the causative organisms for causing Thypoid and Parathypoid fever in man.

Salmonella fall into 3 groups with respect to their distribution and relationship to human diseases.

The first group are those which are primarily human pathogens and includes *S. typhosa*, *S. paratyphi* and *S. schattmuellere* and *S. hirschfeldic*.

The second group are those which are primarily pathogenic for animals including birds. This group contains majority of the *Salmonella*.

The third group are those which are known to be pathogenic only for animals or birds. *S. gallinarum-pullorum* is the most important organism in this group.

Salmonella also cause Gastroenteritis and Septicaemia.

MODE OF TRANSMISSION

Man acquires Salmonellosis through his consumption of contaminated water or food. A variety of foods have been implicated as sources of causative agents in outbreaks. These include cream containing bakery goods, ground meats, sausages and eggs.

Rodents infected with *Salmonellae* may become carriers and by means of these excreta contaminate certain foods for human consumption.

The possibility of food contamination by human carriers also exists.

CLINICAL FEATURES

The symptoms of gastroenteritis develop suddenly, within 48hrs after the ingestion of sufficient numbers of appropriate Salmonellae. Infected persons experience abdominal pain, diarrhoea, dizziness, fever, headache, nausea and vomiting. Poor appetite and difficulty in digesting solid foods also occur.

LABORATORY DIAGNOSIS

The diagnosis of Salmonella infection depends on isolation and identification of the causative agent from blood, faeces or urine.

Faeces or urine are plated on DCA medium or Triple Sugar Iron agar. On DCA media, pale, non-lactose fermenting colonies are obtained. On TSIA, production of Hydrogen sulphide gas resulting in black coloration is indicative of Salmonella species.

Biochemical tests specific for Salmonella are then done for confirmation. These include urease negative and positive motility.

TREATMENT

Formerly, treatment was mainly supportive and aimed at maintaining nutritional state and fluid balance of the patient.

However, more recently several specific therapeutic agents have been employed.

Chloramphenicol has been found to be the most effective antibiotic in the treatment of typhoid fever. Also very effective was Gentamicin.

Sulphonamide have also been tried but with disappointing results.

CONTROL MEASURES

These include:-

- (1) Proper cooking of foods obtained from animal sources such as ground meat and sausages.

- (2) Suitable refrigeration and covering of prepared foods.
- (3) Protecting food from contaminations by mice, rats or flies.
- (4) Periodic inspection of food handlers.
- (5) Suitable sanitation.

SHIGELLA

History and Morphology

Shigella dysenteriae which is the most common cause of Bacillary dysentery was first discovered by the Japanese bacteriologist Shiga in 1896. He isolated from the faeces and the intestinal walls of patients suffering from clinical dysentery.

The *Shigella* organisms are slender, gram-negative rods, approximately 1 micron by 0.5 micron. They are non-motile and non-sporulating. They are also non-capsulating.

Shigella bacilli are aerobes and facultative anaerobes and grow best at 37°C.

BIOCHEMICAL REACTIONS

These group of species do not liquefy gelatin nor do they produce Hydrogen sulphide. They also do not utilize citrate. Some of the species produce indole. They are also able to reduce nitrates to nitrites. They ferment a variable number of Carbohydrates with production of acid but no gas.

EFFECT OF PHYSICAL AND CHEMICAL AGENTS

They are killed at a temperature of 55°C/1hr. Also exposed to 1% phenol for 30min will kill them. However, they may survive in sea water for at least 3 days.

Under natural conditions in the stool, their survival appears to be short presumably due to bacteriophage action or because of their sensitivity to the acidity produced by growth of other organism.

PATHOGENECITY

Shigella are the causative organisms for causing bacillary dysentery. This is also sometimes called Shigellasis.

MODE OF TRANSMISSION

The human is the sole reservoir of infection. Although all age groups are susceptible to Shigellae infection, children and males between 20 - 30 years are more commonly infected.

Predisposing factors related to the occurrences of Shigellasis include lowered states of resistance, malnutrition, overcrowding and poor sanitation.

Bacillary dysentery is usually contacted through the ingestion of contaminated food or water. The causative agents can be transmitted by faeces, fingers, flies or food.

CLINICAL FEATURES

The incubation period for Shigellasis ranges from 1 - 14 days. The onset of symptom is usually abrupt. An infected individual generally experiences abdominal pain, diarrhoea, sharp fever (103 - 104°F) and malaise. In severe form of the disease, stools are primarily composed of blood, mucus and pus giving a "red current jelly" appearance.

A burning rectal sensation, dehydration, electrolyte imbalance, straining and vomiting may accompany defaecation. The average number of stools passed per day varies from 6 - 10.

LABORATORY DIAGNOSIS

An initial microscopic examination of the stool is done to eliminate the presence of protozoa or their cysts and also to note the character of the *colonies*

The material is plated on DCA media or Mac Conkey's and incubated at 37°C for 18 - 24hrs.

We obtain pale non-lactose fermenting colonies from DCA. These colonies are tested for biochemical reaction. These include urease negative, negative motility, no production of Hydrogen Sulphide on TSI agar.

TREATMENT

In mild dysentery it is advisable to give a non-specific agent e.g. Kaolin because in most cases such therapy gives rapid symptomatic relief with cessation of diarrhoea.

Specific antibiotic therapy should not be given unless the illness is severe and the laboratory should confirm that the drug selected is active against patients strain in vitro.

Gentamicin and Polymixin B sulphate were proved to be very effective invitro.

Chloramphenicol has also been found to be effective.

CONTROL MEASURES

Since human beings serve as the sole source of infectious agents, preventive measures must be directed towards infected persons, carriers and items which may have been contaminated by them.

The elimination of flies, the proper sanitary disposal of excreta and the protection of food and water are of great importance.

CHEMOTHERAPY

GENERAL MECHANISMS OF ACTION OF ANTIMICROBIAL AGENT

In order to exert its growth inhibiting action, the primary need of the agent is the ability to gain access to the target site.

Antibiotics gain access to the target site by two methods:-

- (1) Simple diffusion across the cell wall and cytoplasmic membrane. The rate of diffusion being determined by the external concentration of the drug.
- (2) Mediated transport against a concentration gradient.

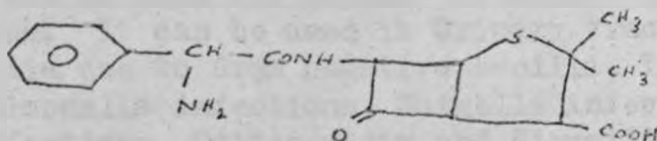
Once the target site is reached, the overall effect on bacteria can be bacteriostatic or bactericidal.

The bacteriostatic agents stops bacterial growth, allowing host defence mechanism additional time to remove the invading microorganism.

The bactericidal agents on the other hand, kill the microorganism.

AMPICILLIN

It is a semi-synthetic penicillin derived from the penicillin nucleus (6 - amino penicillanic acid). Ampicillin has the following structure:-



ANTIBIOTIC ACTIVITY

Ampicillin is active against most the Gram positive bacteria, in addition it is active against some Gram negative bacilli, which are penicillin G resistant.

MODE OF ACTION

This antibiotic act by selectively inhibiting the synthesis of mucopeptide in the bacterial cell of multiplying bacteria.

The antibacterial spectrum of ampicillin is wider compared to Penicillin G probably due to the ability of ampicillin to penetrate the outer membrane of the cell wall of some gram negative bacilli.

Ampicillin is also less susceptible to inactivation by some of the beta-lactomases produced by these organism.

RESISTANCE

- (1) E. coli - Many strains of E. coli are resistant. This is always due to beta-lactomase production. This may be either R plasmid mediated or Chromosomally mediated.
- (2) Salmonella - Resistance of Salmonellae to ampicillin is usually due to transferable drug resistance.
- (3) Shigella - Resistance is due to the production of a number of different Beta lactomases. This is mediated either by Chromosomal mutation or by R plasmid.

CLINICAL USE

It can be used for variety of microorganism mediated infections. It can be used in Urinary tract infection, Septicaemia due to Gram negative bacilli, Typhoid fever and other Salmonella infections, Shigella infections, Respiratory tract infections, Otites media and Sinusitis, Pertussis, Bacterial meningetis, Biliary infections.

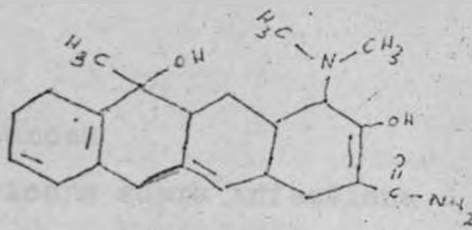
TOXICITY

This includes:-

- (1) Rashes and allergic reactions
- (2) GIT side effects
- (3) Neuropathy
- (4) Aggronulo cytosis
- (5) Encephalopathy
- (6) Drug Interactions. Ampicillin can impair the absorption of oral contraceptives.

TETRACYCLINE

Structure



CHEMISTRY

Tetracyclines are crystallins, amphoteric substances of low solubility. They are formulated as hydrochlorides which are more soluble.

ANTIBIOTIC ACTIVITY

Tetracycline is a broad spectrum antibiotic. It is effective against many Gram positive and Gram negative bacteria. In addition it is also effective against some protozoa, Rickettsia and some viruses.

MODE OF ACTION

The most important mechanism of action is the ability of tetracycline to block the binding of the transfer RNA - amino acid complex to the ribosome. Thus no amino acid is available to messenger RNA and no polypeptides can be produced, thereby preventing protein synthesis at this level.

RESISTANCE

Resistance usually develop due to R plasmid transmission in *E. coli*, *Shigella* and *Salmonella*.

CLINICAL USE

Tetracycline can be used in Respiratory tract infections, Pertussis, *Mycoplasma pneumonia*, Staphylococcal infections, Surgical infections, Biliary infection, Urinary tract infection, Cholera, *Rechttsial* infections, Gonorrhoea, Syphillis, *Shigella* dysentery, Traveller's diarrhoea, Acne, Amoebic dysentery, etc.

TOXICITY

These include:-

- (1) GIT disturbances
- (2) *Candide albicans* supra infections
- (3) Hypersensitivity reactions
- (4) Photosensitivity
- (5) Teeth pigmentation
- (6) Terratogenecity
- (7) Hipato toxicity
- (8) Nephro toxicity
- (9) Vitamin depletion
- (10) Vastabular disturbance

TRIMETHOPRIM/SULPHAMETHOXOZOLE (SULPHANOMIDE)

The combination of these two drugs has synergestic effect against certain bacteria. Sulphamethoxazole which is a medium acting sulphanomide was selected because its rate of absorption and excretion closely parallels that of trimethoprim. Trimethoprim is a pyrimidine derivative and has both antibacterial and antimalarial activity.

MODE OF ACTION

Trimethaprim interferes with the action of dihydrofolic acid reductase, an enzyme which converts dihydrofolic to tetrahydrofolic acid, an essential stage in bacterial purine and ultimately, DNA synthesis. This enzyme acts at a stage which immediately follows the enzyme conversion of para-amino benzoic acid to dihydrofolic acid, which can be competitively blocked by Sulphamethoxazole. This sequential action explains the synergistic action of this combination against sensitive bacteria.

RESISTANCE

Animal cells are unable to synthesize folic acid but depend on exogenous sources and for this reason are not susceptible to the sulphonamide action.

Some bacteria produce large excess of PABA which thus compete with Sulphonamide and are thus resistant. Others may actually destroy Sulphonamide and acquire resistance.

CLINICAL USE

Co-trimoxazole can be used in Urinary tract infections, Septicaemia due to Gram negative bacilli, Plague, Meningitis, Bronchitis and Pneumonia, Staphylococcal infections, Typhoid fever, Gonorrhoea and other venereal diseases, Cholera, Malaria.

TOXICITY

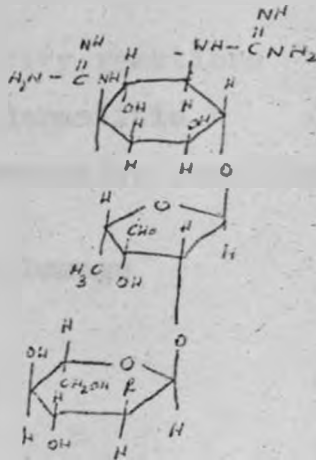
- (1) GIT disturbances
- (2) Haemolytic anaemia
- (3) Immuno suppressive effects
- (4) Nephrotoxicity
- (5) Hypersensitivity reactions

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STREPTOMYCIN

Chemistry

Streptomycin is a highly polar organic base with large number of hydrophilic and functional groups. The drug is made up of three components - streptidine, streptose and N-methyl-L-glucosamine



MODE OF ACTION

Streptomycin and other aminoglycosides act directly on the ribosomes, where they inhibit protein biosynthesis and decrease the fidelity of translation of the genetic code.

The site of action of streptomycin is the 30 S Ribosomal subunit.

RESISTANCE

This may be acquired by a single mutational step, and there is selection for such microorganisms in the presence of the antibiotic.

Resistant forms may also develop by virtue of their inability to transport streptomycin to an intracellular site or by the induction of enzymes that metabolise the drug (streptomycin phospho transferase and adenylate synthetase). Such resistance is carried by R plasmids.

CLINICAL USES

Streptomycin can be used in Bacterial endo carditis, Tularemia, Plague, Brucellosis, Respiratory tract infection, Peritonitis, Urinary tract infections, Bacterial meningitis and Tuberculosis.

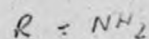
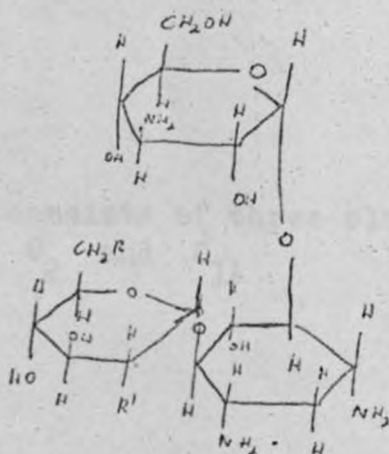
TOXICITY

- (1) Hypersensitivity reactions
- (2) Exfoliative dermatitis
- (3) Toxic and irritative reactions at the site of injection
- (4) Labyrinthine Damage
- (5) Deafness
- (6) Renal damage

KANAMYCIN

Chemistry

Kanamycin is a polybasic, water soluble substance. It contains two amino sugars in glycosidic linkage with 2-deoxystreptomine.



MODE OF ACTION

Same as Streptomycin.

RESISTANCE.

Bacteria may become insensitive to Kanamycin by acquisition of a R-factor during conjugation. This resistance is associated with the presence of enzymes that can phosphorylate, acetylate or adenylylate the drug.

CLINICAL USES

Kanamycin is used most often for the therapy of infections due to Gram-negative microorganisms, especially Klebsiella, Acrobacter, Proteus and E. coli. It is also used in treatment of human tuberculosis, gastroenteritis. It is also used prophylactically to suppress the intestinal flora prior to surgery. Here it is given orally.

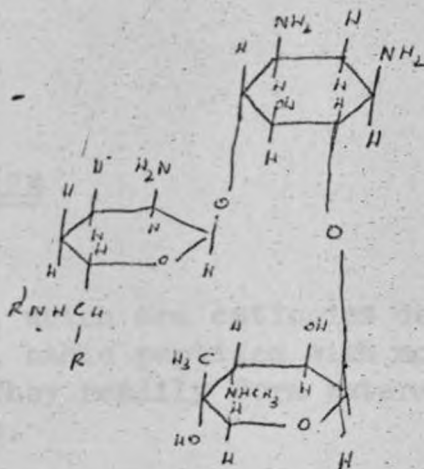
TOXICITY

- (1) Hypersensitivity reactions
- (2) Ototoxicity and nephrotoxicity
- (3) Restlessness, nervousness, headache, etc.

GENTAMICIN

Chemistry

Gentamicin consists of three closely related components -
gentamicins C, C₂ and C_{1A}



MODE OF ACTION

Same as Streptomycin.

RESISTANCE

Same as Streptomycin.

CLINICAL USES

The antibiotic is very useful in serious gram-negative microbial infections. Among these are Urinary tract infections, bacteremia, meningitis, tuleramia, infected burns, osteomyelitis, pneumonia, peritonitis, gonorrhoea and infections of ear, nose and throat.

TOXICITY

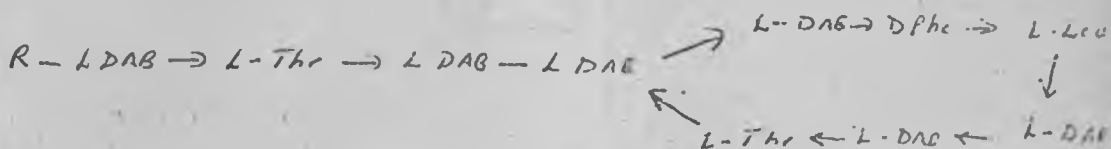
These icnlude:-

nausea, vomitting, headache, transient proteinuria, clevation of blood urea nitrogen, increase in serum transominases, overgrowth of Candida after oral administration and ototoxicity.

POLYMYXIN B SULPHATE

Chemistry

The polymyxin, which are cationics detergents, are relatively simple, basic peptides with molecular weights of about 1,000. They readily form water-soluble salts with mineral acids.



MODE OF ACTION

Polymyxin B is a surface active agent, containing lipophilic and lipophobic groups separated within the molecule. The ability of these to become oriented between lipid and protein films is thought to produce a disorientation of the lipoprotein membrane of bacteria, so that it no longer functions as an effective osmotic barrier and thereby allows cell contents to escape. Permeability changes in the membrane begin immediately on contact with the drug.

CLINICAL USES

Primary use of Polymyxin B is for treatment of infections caused by Gram-negative bacteria especially Pseudomonas. It is also used in Urinary tract infections, Meningeal infections, pulmonary infections, infections of the skin, eye and ear,

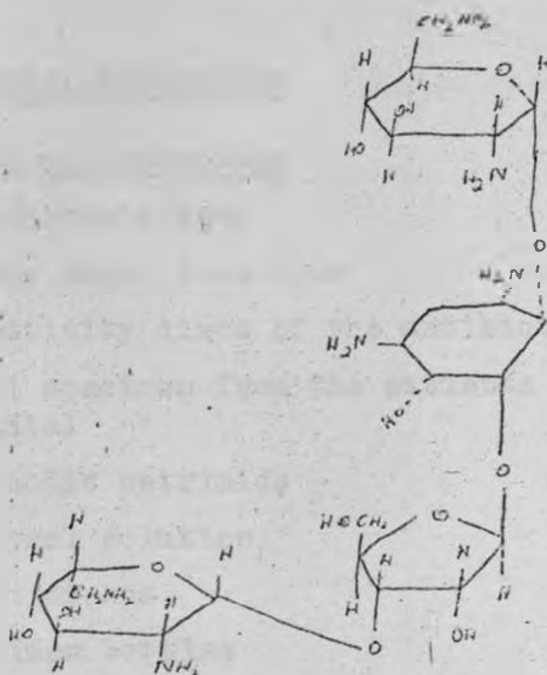
TOXICITY

- (1) Hypersensitivity reactions
- (2) Facial flushing, dizziness, atoxia, paresthesias
- (3) generalized weakness, slurred speech, acute tubular necrosis
- (4) Proteinuria, Haematuria, etc.

NEOMYCIN

Chemistry

Neomycin is a polybasic, water soluble substance that readily forms salts with a variety of acids. It is composed of Neamine and Neo biosomine.



MODE OF ACTION

Same as Streptomycin.

RESISTANCE

Same as Streptomycin.

CLINICAL USES

Neomycin has been widely used for topical application in a variety of infections of the skin and mucus membranes. These include burns, wounds, ulcers, and infected dermatoses.

Oral administration of neomycin has been employed primarily for "preparation" of the bowel for surgery. It is also used in the therapy of intestinal infections, primarily in children due to pathogenic strains of *E. coli*

TOXICITY

- (1) Hypersensitivity reactions
- (2) Renal damage and nerve deafness
- (3) Intestinal malabsorption and suprainfections
- (4) Overgrowth of yeasts in the intestine.

EXPERIMENTAL PROCEDURES

Apparatus and materials

1. Mac Conkey's Agar
2. Triple Sugar Iron Agar
3. Sensitivity discs of the antibiotics
4. Stool specimen from the patients at Kenyatta National Hospital
5. Alcoholic cetrimide
6. 2% Lysol solution
7. Petri dishes
8. Specimen bottles
9. Swabs
10. Refrigerator
11. Oven
12. Wire loops
13. Microscopic and cover slides
14. Microscope
15. Autoclave

PREPARATION OF MAC CONKEY'S AGAR

52.0 gms of Mac Conkey's agar was dissolved in 1 litre of water. The solution was heated to boiling to complete dissolution. The solution was then transferred to ten 100ml bottles which were then sterilized by autoclaving at 121°C/15 minutes.

20mls of the sterile hot solution was then transferred aseptically to each petri dish which had been previously autoclaved.

The plates were then left to settle to solidify and then stored in the refrigerator until required.

PREPARATION OF TRIPLE SUGAR IRON AGAR

The media was made up of the following:-

| | |
|---|-----------|
| Beef extract | 3.0g |
| Yeast extract | 3.0g |
| Peptone | 20.0g |
| Glucose | 1.0g |
| Lactose | 10.0g |
| Sucrose | 10.0g |
| Ferrous Sulphate ($\text{Fe SO}_4 7\text{H}_2\text{O}$) | 0.2g |
| Sodium Chloride | 5.0g |
| Sodium thiosulphate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$) | 0.3g |
| Agar | 20.0g |
| Distilled water | 1000.00ml |
| 0.2% Phenol red solution | 12.0ml |

The powders are dissolved in water with aid of heat. After complete dissolution, phenol red solution was added and mixed completely. The solution was then sterilized at $115^\circ\text{C}/20$ minutes.

About 25mls of the solution was then transferred aseptically to each test tube, which were placed in a slant position so as to form a slope with a deep butt.

PREPARATION OF COTTON SWABS

These were prepared from cotton wool and applicator sticks. These were then double wrapped in sets of fives and autoclaved at 115°C/30 minutes. They were then stored until required.

CLEANSING AND STERILIZATION OF PETRI-DISHES AND SPECIMEN BOTTLES

The glass ware were washed with a detergent, rinsed with tap water and finally further rinsed with distilled water. They were then dried in a hot air oven.

These were then wrapped in autoclaving paper packets and sterilized in an autoclave at 121°C/15 minutes.

PREPARATION OF GRAM STAINING REAGENTS

METHYL VIOLET - 0.5gms of Methyl violet was dissolved in 100mls of distilled water to give a 0.5% Methyl violet solution.

IODINE SOLUTION - 1.0g of Iodine was dissolved in 100mls of 2% potassium Iodine solution.

ACETONE - ALCOHOL - Equal volumes of 50mls of acetone and alcohol were mixed together.

NEUTRAL RED - To 0.1% aqueous solution was added to 1 drop of 5% acetic acid per 100ml to get the final solution.

PREPARATION OF ALCOHOLIC CETRIMIDE

0.5gms of Cetrinide was dissolved in 100mls of 70% alcohol.

METHODOLOGY

The whole experimental procedure can be categorised into 3 sections:-

1. Isolation of the microorganisms i.e. E. coli, Shigella and Salmonella
2. Identification
3. Sensitivity testing

ISOLATION OF THE MICROORGANISMS

Stools from the patient at Kenyatta National Hospital were used for the isolation of the microorganism.

Using sterile cotton swabs, stool samples were plated on the Mac Conkey's Agar. Streaking was then done using a heated wire loop.

The plates were then incubated at 37°C for 24 hours.

IDENTIFICATION

This was done under the following sub headings:-

- (a) Fermentation of lactose on Mac Conkey's agar
- (b) Gram-staining and Microscopy
- (c) Motility test
- (d) Reaction on the Triple Sugar Iron Agar

(a) FERMENTATION OF LACTOSE ON MAC CONKEY'S AGAR

This was by observing the colonies obtained on the Mac Conkey's agar. Pink colonies showed the presence of a lactose fermenter while whitish mucoidal colonies showed the presence of a non-lactose fermenter.

(b) GRAM-STAINING AND MICROSCOPY

Smear of the colony from the Mac Conkey's agar was made onto a microscope slide. This was allowed to dry and then fixed by passing through a bunsen burner 3 - 5 times.

The slide was then cooled and stained with Methyl violet for 15 - 30 seconds. The stain was drained and slide washed off with Iodine. The slide was flooded with more Iodine and allowed to stand for 30 seconds.

The Iodine was then drained off and holding the slide at an angle, the acetone-alcohol was added drop by drop until the Methyl violet ceases to stream out of the smear. Immediately then the slide was rinsed with water and stained with neutral red for 1 - 2 minutes.

Finally, the slide was washed with water, dried with a clean filter paper and then examined with the oil immersion lens of the microscope.

(c) MOTILITY TEST

A colony of the microorganism was looped from the Mac Conkey's agar into the nutrient broth. The broth was then incubated at 37°C for 24 hours.

A drop of the nutrient broth culture was then placed in the centre of a cover glass with the aid of a small inoculating loop. A ring of vaseline was made round the drop by squeezing the vaseline through a syringe (without a needle). A microscopic slide was then placed over the cover glass. The slide was then inverted and examined under the microscope at high power dry objective and reduced illumination for motility.

(d) REACTION ON THE TRIPLE SUGAR IRON AGAR (TSIA)

A colony of the microorganism was looped from the Mac Conkey's agar on to the TSIA. The media was then incubated at 37°C for 48 - 72 hours. Changes in the media was then noted.

(e) SENSITIVITY TESTING

Standardised commercial antibiotic polydiscs. KGL 2/2 were used. These contained known amounts of antimicrobial agents.

On to the sterile Mac Conkey's agar seeded with microorganisms (using a sterile cotton swab) were placed the polydiscs.

The plates were then incubated at 37°C for 24 hours. Clear zone round the disc gave the zones of Inhibition. The diameter of these zones were measured in millimeter and recorded.

R E S U L T S

SUMMARY OF THE RESULTS

| | |
|---|------|
| Total number of patients | = 24 |
| Number of patients with E. coli infections | = 16 |
| Number of patients with Shigella infections | = 6 |
| Number of patients with Salmonella infections | = 2 |

Number of E. coli sensitive to:-

| | |
|---|------|
| (1) Ampicillin 25mcg | = 3 |
| (2) Tetracycline 25mcg | = 1 |
| (3) Trimethoprim / Sulphomethoxazole 25mcg | = 2 |
| (4) Streptomycin 10mcg | = 2 |
| (5) Kanamycin 30mcg | = 11 |
| (6) Gentamicin 10mcg | = 16 |
| (7) Polymyxin B Sulphate 250 units | = 16 |
| (8) Neomycin 30 mcg | = 14 |

Most effective antibiotic = Gentamicin

Least effective antibiotic = Tetracycline

Number of Salmonella sensitive to:-

| | |
|-----|-----|
| (1) | = 0 |
| (2) | = 0 |
| (3) | = 0 |
| (4) | = 0 |
| (5) | = 1 |
| (6) | = 2 |
| (7) | = 1 |
| (8) | = 1 |

Most effective antibiotic = Gentamicin

Number of Shigella sensitive to:-

- (1) = 0
- (2) = 0
- (3) = 0
- (4) = 0
- (5) = 3
- (6) = 6
- (7) = 6
- (8) = 3

Most effective antibiotic = Gentamicin

| Number | Sex | Age | Gram stain | Lactose Fermenter | Reaction on TSIA | Motility | Micro-organism | SE | NS | IT | IV | I | TY | | |
|--------|-----|------------|------------|-------------------|-------------------|----------|----------------|----|----|----|----|----|----|----|----|
| | | | | | | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| 684 | F | 1½ Years | - | + | No acid No gas | + | E. coli | 6 | R | R | R | R | 7 | 5 | 7 |
| 685 | M | 1 Year | - | + | No acid No gas | + | E. coli | R | R | R | R | 12 | 10 | 6 | 12 |
| 687 | M | 58 Years | - | + | No acid No gas | + | E. coli | 7 | R | R | R | 14 | 8 | 8 | 10 |
| 335 | F | 17 Years | - | + | No acid No gas | + | E. coli | R | R | R | R | R | 18 | 8 | 10 |
| 336 | F | Paediatric | - | + | No acid No gas | + | E. coli | R | R | R | R | 16 | 14 | 14 | 8 |
| 337 | M | 23 Years | - | + | No acid No gas | + | E. coli | R | R | R | R | 24 | 15 | 5 | 13 |

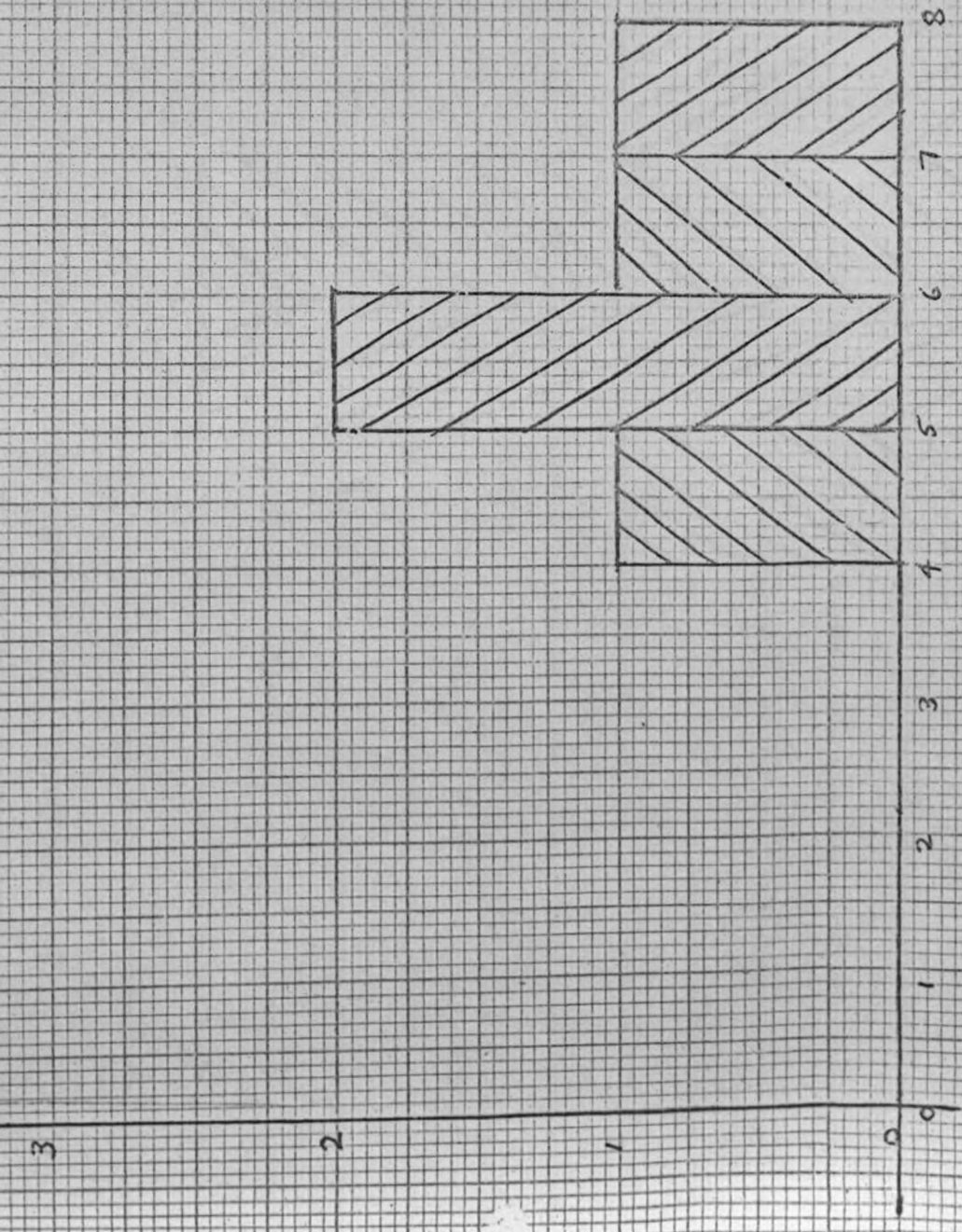
| Number | Sex | Age | Gram stain | Lactose Fermenter | Reaction on TSIA |
|--------|-----|----------|------------|-------------------|-------------------|
| 355 | M | 2 Years | - | + | No acid No gas |
| 843 | F | 30 Years | - | - | Acid No gas |
| 839 | M | 4½ Years | - | + | No acid No gas |
| 831 | M | 42 Years | - | + | No acid No gas |
| 829 | F | 2½ Years | - | + | No acid No gas |
| 548 | M | 9 Months | - | - | Acid No gas |

| Motility | Micro-organism | S | EN | SI | TI | VI | TY | | |
|----------|----------------|---|----|----|----|----|----|----|----|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| + | E. coli | R | R | R | R | 18 | 13 | 8 | 12 |
| - | Shigella | R | R | R | R | R | 7 | 10 | R |
| + | E. coli | R | R | R | R | 20 | 16 | 10 | 10 |
| + | E. coli | R | R | R | R | R | 15 | 11 | R |
| + | E. coli | R | R | R | R | R | 12 | 8 | R |
| - | Shigella | R | R | R | R | 17 | 14 | 8 | 12 |

| Number | Sex | Age | Gram stain | Lactose Fermenter | Reaction on TSIA | Motility | Micro-organism | S E N S I T I V I T Y | | | | | | | |
|--------|-----|----------|------------|-------------------|-----------------------------------|----------|----------------|-----------------------|---|---|---|----|----|----|----|
| | | | | | | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| 909 | M | 8 Months | - | + | No acid No gas | + | E. coli | R | R | R | R | 15 | 7 | 7 | 8 |
| 913 | F | 4 Months | - | + | No acid No gas | + | E. coli | R | R | R | R | 18 | 8 | 7 | 11 |
| 914 | F | 22 Years | - | - | Acid No gas | - | Shigella | R | R | R | R | R | 16 | 8 | R |
| 569 | M | 3 Years | - | - | Acid + H ₂ S gas | + | Salmonella | R | R | R | R | 22 | 15 | 10 | 12 |
| 571 | F | 7 Months | - | + | No acid No gas | + | E. coli | R | R | R | R | 24 | 16 | 12 | 8 |
| 561 | M | 4 days | - | - | Acid No gas | - | Shigella | R | R | R | R | 18 | 14 | 10 | 12 |

| Number | Sex | Age | Gram Stain | Lactose Fermenter | Reaction on TSIA | Motility | Micro-organism | [REDACTED] | | | | | | | | | | |
|--------|-----|-----------------------|------------|-------------------|------------------------------|----------|----------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| 350 | M | 8 Weeks | - | - | Acid H ₂ S gas | + | Salmonella | [REDACTED] | [REDACTED] | [REDACTED] | [REDACTED] | [REDACTED] | [REDACTED] | [REDACTED] | [REDACTED] | [REDACTED] | [REDACTED] | [REDACTED] |
| 435 | M | 1 $\frac{3}{4}$ Years | - | + | No Acid No Gas | + | E. coli | R | R | R | R | R | 15 | 8 | 6 | | | |
| 436 | M | 1 Year | - | - | Acid No Gas | - | Shigella | R | R | R | R | 17 | 13 | 8 | 12 | | | |
| 433 | M | Adult | - | + | No Acid No Gas | + | E. coli | 27 | R | 18 | 16 | 19 | 14 | 10 | 12 | | | |
| 432 | M | Paediatric | - | + | No Acid No Gas | + | E. coli | 25 | 22 | 15 | 15 | 20 | 16 | 8 | 12 | | | |
| 907 | M | Paediatric | - | + | Acid No Gas | - | Shigella | R | R | R | R | R | 12 | 8 | R | | | |

HISTOGRAM OF SENSITIVE SALMONELLA



KEY

Y-axis → Number of Sensitive Salmonella

X-axis → Antibiotic used

1 → Ampicillin

2 → Tetracycline

3 → Trimethoprim/Sulphamethoxazole

4 → Streptomycin

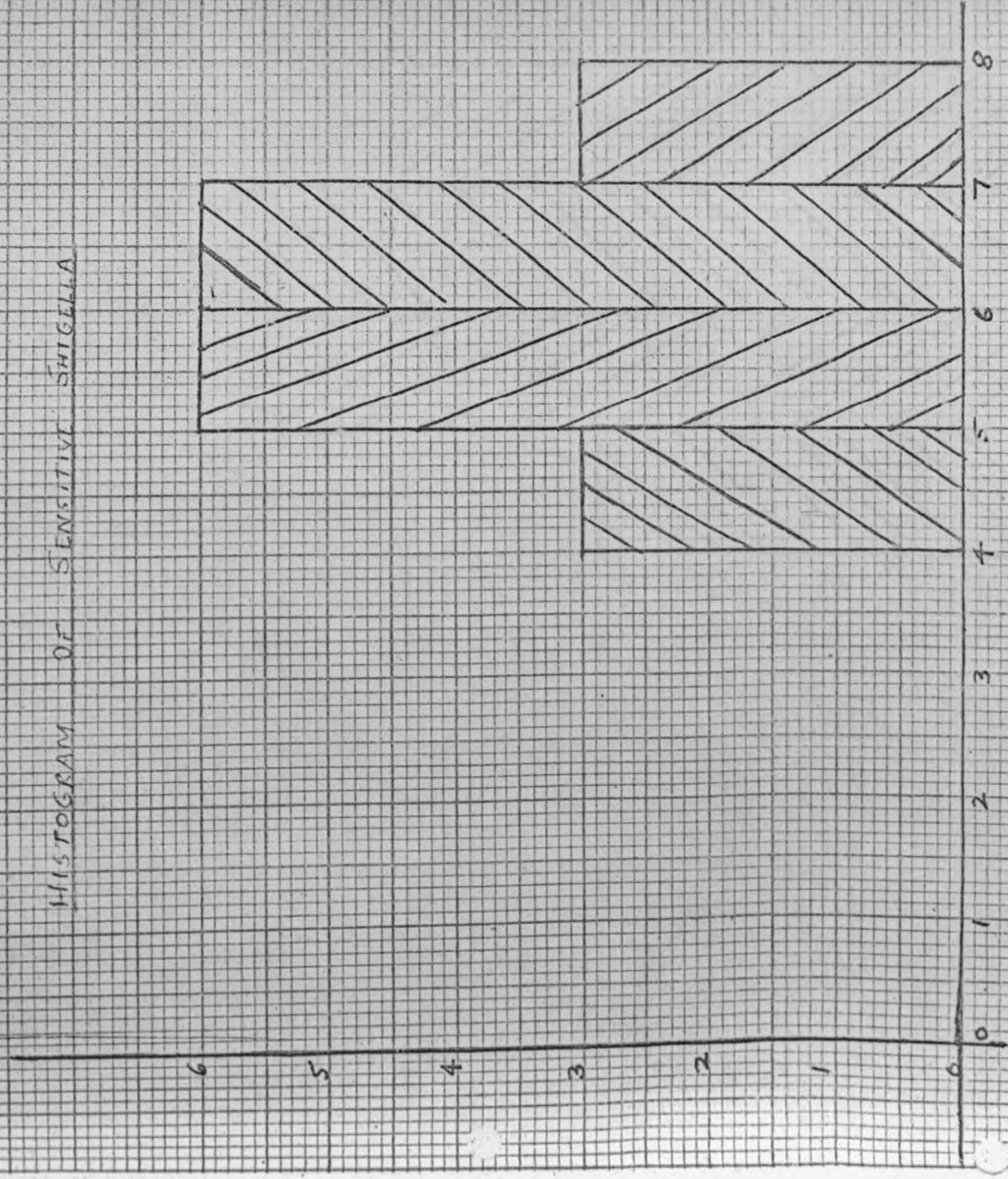
5 → Kanamycin

6 → Gentamicin

7 → Polymyxin B 50g

8 → Neomycin

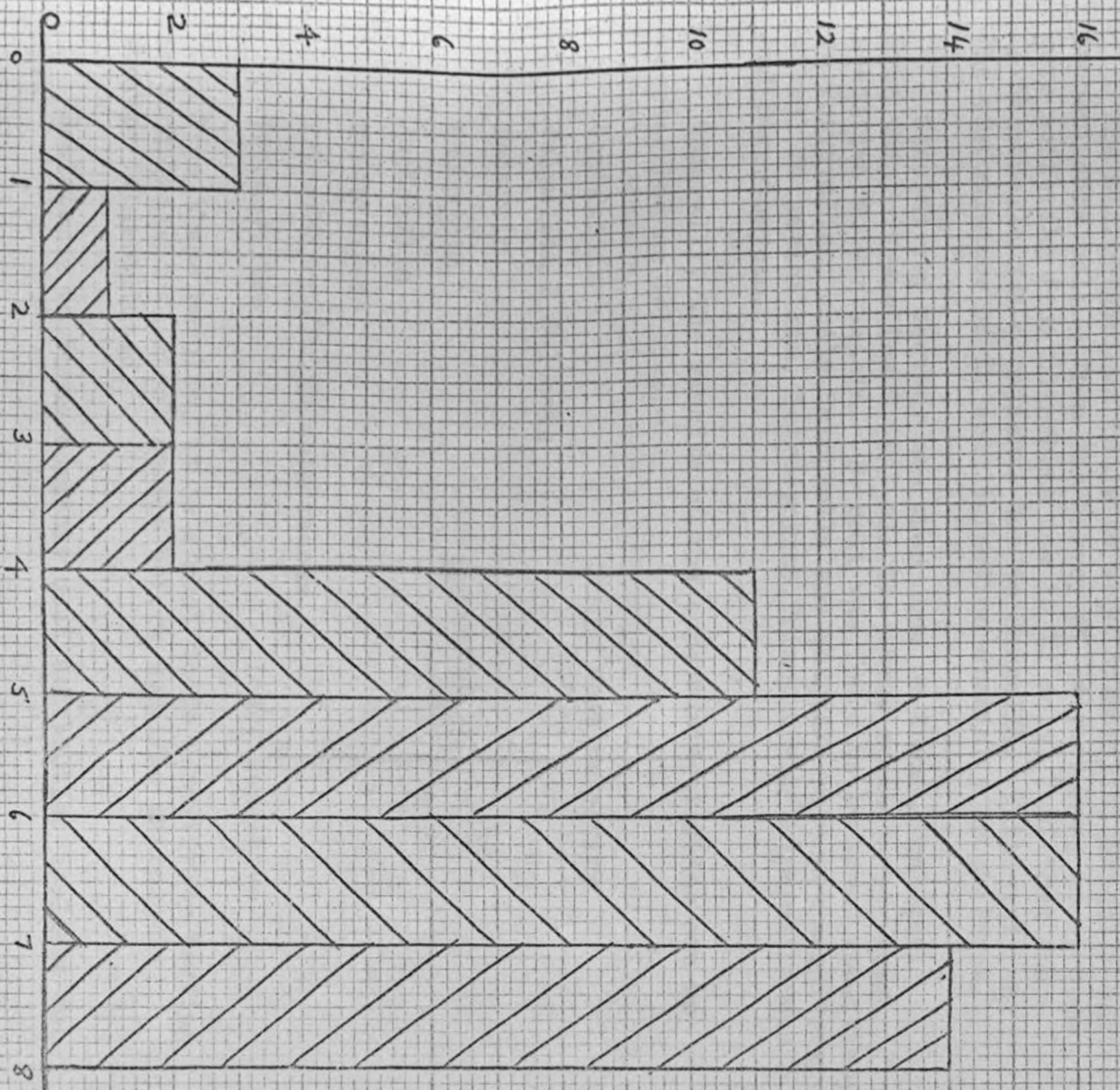
HISTOGRAM OF SENSITIVE SHIGELLA



KEY

- 1 -> Ampicillin
 - 2 -> Tetracycline
 - 3 -> Trimethoprim / Sulphamethoxazole
 - 4 -> Streptomycin
-
- 5 -> Kanamycin
 - 6 -> Gentamicin
 - 7 -> Polymyxin B SO₄
 - 8 -> Neomycin

HISTOGRAM OF SENSITIVE E.coli



KEY X-axis → Number of E. coli sensitive

Y-axis → Antibiotics Used

- 1 → Ampicillin
- 2 → Amikacin
- 3 → Streptomycin
- 4 → Gentamicin
- 5 → Polymixin B

DISCUSSION AND CONCLUSION

Stool samples from patients in the Adult and Padiatric Observation wards at Kenyatta National Hospital were used to study the microbial flora and their sensitivity to various antibiotics.

The microorganisms isolated were Salmonella, Shigella and Escherischia coli. From the survey of 24 patients, 16 of them were suffering from diarrhoea due to E. coli, 2 of them due to Salmonella and 6 patients due to Shigella. This confirms the predominance of E. coli infection specially in children.

Sensitivity testing was then done on these organisms. From this, it was found that all the three species were most sensitive to Gentamicin. E. coli was found to be least sensitive to Tetracycline, while Salmonella and Shigella were not sensitive at all to Ampicillin, Tetracycline, Trimethoprim/Sulphomethaxazole and Streptomycin.

Generally, the results of the sensitivity testing can be represented as follows:-

| | |
|--------------------------------|-----------------|
| Gentamicin | Most sensitive |
| Polymixin B Sulphate | |
| Neomycin | |
| Kanamycin | |
| Ampicillin | |
| Streptomycin | |
| Trimethoprim/Sulphomethaxazole | |
| Tetracycline | Least sensitive |

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From these results, it is a must to do sensitivity testing in each individual stool sample before specific antibiotic therapy is instituted.

Resistance to these commonly used antibiotics could be due to:-

- (1) Development of resistant strains of Salmonella, Shigella and E. coli

(2) Destruction of the antimicrobial agents by the micro-organisms

(3) Previous contact of the patients with the antimicrobial agents.

Although Gentamicin is the most effective antibiotic, it should not be used as a first line measure. It has serious side effects, one of which is the damage of the 8th Cranial nerve. Thus generally Antibiotics should not be used as a first line of measure.

The single most important factor in the management is the correction of the fluid and electrolyte imbalance - Oral Rehydration Therapy (ORT).

The constituents of Oral rehydration fluids are as follows:-

| | |
|-------------|----------------|
| Sodium | 90 mmol/litre |
| Pottasium | 20 mmol/litre |
| Chloride | 80 mmol/litre |
| Bicarbonate | 20 mmol/litre |
| Glucose | 111 mmol/litre |

This formulation has been adopted to meet the varying needs of rehydration therapy in all ages. These fluids are given every 1 - 2 hours as per needs. In severe cases, Intra-venous fluids replacement therapy is instituted.

To minimize cases of these infections, certain preventive measures must be observed. These include:-

- (1) Proper sanitary disposal of excreta
- (2) Elimination of flies
- (3) Protection of food and water
- (4) Proper cooking of food obtained from animal sources.

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