PROJECT

TITLE: RESISTANCE OF ESCHERICHIA COLI FROM URINARY TRACT INFECTIONS:

A STUDY ON WINTOMYLON AND CO-TRIMOXAZOLE

STUDENT: KINGORI NDIRANGU

SUPERVISOR: MR. A.L. PALEKAR

B. Pharm. 1979
ACKNOWLEDGEMENT

A lot of thanks to the following individuals and groups without whose co-operation the whole exercise would not have succeeded.

Mr. L.A. Palekar, my supervisor, who advised me especially at the initial stages when I did not know what I was supposed to do.

The pharmaceutical department technicians and in particular Miss Mwari Kibaya who saw to it that most of the apparatus that I needed were available.

The staff of the Diagnostic Laboratory, Department of Pathology and Microbiology, (Kenyatta National Hospital), for allowing me to use the E.coli specimen collected from hospital in-patients, and for the co-operation they extended throughout the project.

My colleagues who came into the laboratory every so often and gave me words of encouragement.

Last, but not the least my warm thanks is extended to Mrs. R.I. Muturi for typing the manuscript.
SUMMARY

The research was conducted to study the resistance of Escherichia coli from urinary tract infections to both Co-trimoxazole and Wintomylon. The specimens were collected from the Kenyatta National Hospital and as such the results can only be related to this hospital, though it is expected that variations in most other hospitals, especially those in major Kenyan towns would not be very great.

After the research had been conducted and results recorded, it was found that E. coli from urinary tract infections was more sensitive to Wintomylon than to Co-trimoxazole. It was therefore recommended that due to the large difference in the response of the organism to the two drugs, it would be better if Wintomylon were used for those urinary tract infections where E. coli had been diagnosed as the causative agent rather than Co-trimoxazole. This, obviously is subject to the contra-indications involved pertaining to individual patients.

DESCRIPTIONS

E. coli is a gram-negative, motile, non-sporing, non-capsulated bacillus. It is anaerobic and facultatively anaerobic. It produces an antibiotic which causes diarrhoea. It ferments lactose giving a rose-pink colour on MacConkey's media. On subculture a small amount of the colony on a sheet of filter paper previously treated in Ehrlich's reagent, it gives reddish colour due to tyride production.

Ehrlich's reagent contains:

- Cresol methyl reino benzoate
- Alcoholic hydrochloric acid (1:10) - 10ml
- Sodium hydroxide

Drop of three times of Ehrlich's reagent-

- Somatic - O
- Envelope or surface - N
- Flagella - N

K- factor of antigen inhibiting anti O weak
K- is destroyed by heat but O antigen is least stable.
INTRODUCTION

AIM: The aim of the project was to study the sensitivity of E. coli (a common urinary tract pathogen) to two major drugs used to treat its infections. The drugs, Wintomylon and Co-Trimoxazole are frequently used in the treatment of E. coli infections.

As such, the results of the project would ascertain
(i) The extent of the Resistance to each one of the two drugs.
(ii) Which of the two drugs, E. coli is more resistant to in "VITRO".

Escherichia coli:

It is a normal gastrointestinal flora to be found mostly in the lower section of the ileum and in the colon. It is a pathogen in both urinary tract and would be from either endogeneous or exogeneous source. The E. coli cell can survive several days to a few weeks outside the body.

Description:

E. coli is a gram negative, motile, non-sporing, non-capsulated bacillus. It is anaerobic and facultatively anaerobic. It produces an endotoxin which causes diarrhoea. It ferments lactose giving a rose-pink colour on MacConkey's media. On rubbing a small amount of the colony on a piece of filter paper previously soaked in Ehrlich's reagent, it gives reddish colour due to indole production.

Ehrlich's media contains:-
Paradimethyl amino benzalddehyde - 4g.
Hydrochloric acid (sp. gr. = 1.19) - 80ml.
Ethyl alcohol - 380ml.

E. coli has three kinds of antigens:

- Somotic - O
- envelope or surface - K
- flagella - H

K- masks O antigen inhibiting anti O serum
K- is destroyed by heat but O antigen is heat stable.
Pathogenesis:

E. coli causes:

(i) Acute gastroenteritis in infants up to two years of age but rarely in adults.

(ii) Urinary tract infections particularly amongst married women but also in young girls and elderly men with prostatic enlargement.

(iii) It could also be the causal organism in appendicular abscess, peritonitis, cholecystitis, and wound infections.

The main factors predisposing urinary infections in women by these bacteria are pregnancy and sexual intercourse.

It is accepted that bacterial concentration greater than 100,000/ml in clean voided urine suggests a urinary tract infection. In causing the urinary tract infection, E. coli can travel from intestinal tract to urinary tract and kidneys through the haematogenous route, lymphatic route and the "ascending" route which is mostly followed. The ascending route is from the urethra via the bladder to kidney. The infection is usually in pregnant women, infants (during diaper period), in patients with obstructive lesions of the urinary tract or neurological diseases affecting micturition. In kidneys, primary lesions occur in medulla rather than the cortex, the reasons being that inflammatory response in the medullary tissues is lower than in cortex. The resulting delay in mobilisation of phagocytic cells appear to be sufficient to allow bacteria invading the medulla to establish a firm foothold. When confined to bladder, antibacterial therapy is enough. But in the kidney, once suppuration has occurred, lesions may continue to progress despite treatment and may eventually lead to scarring and to destruction of the tubules and glomeruli. Chronic pyelonephritis of this kind may cause hypertension and frequently terminates in fatal uremia.
In peritonitis, appendicitis and infections of gall bladder and biliary tract, the colon bacillus is often found in the exudate along with other enteric bacterial.

In infants, certain E. coli belonging to antigenic types (e.g., O-55, O-111, O-127) cause outbreaks of acute gastroenteritis and sometimes meningitis. These are resistant to phagocytosis suggesting that antiphagocytic surface factors may be involved in their pathogenicity.

Progressive infection due to colon bacillus may give rise to bacteremia, resulting terminally in a severe form of shock.

Laboratory Diagnosis:

The pathogenic E. coli do not differ in morphology, cultural characteristics and appearance, and biochemical activities from commensal strains in the gut or strains isolated from non-enteric pathological material. They must be examined serologically to determine whether an enteropathogenic strain belonging to a certain O-group and possessing -antigen is present.

Cultural characteristics

E. coli is aerobic but facultatively anaerobic. It grows within 12-24 hours at temperatures ranging from 20-40°C. It ferments glucose lactose, maltose, and other sugars to give acid and gas.

E. coli does best in MacConkey's agar which has the following constituents.

1. Peptone water
2. Sodium taurocholate (0.5%) or other bile salt to inhibit growth of non-intestinal organism.
3. Lactose (10% aqueous solution) with neutral red as an indicator of lactose fermentation.

The MacConkey's agar is made by solidifying the peptone water with agar containing the other two constituents.
Chemotherapy:

Like most enterobacteria, E. coli is usually resistant to ordinary levels of most penicillins but may be sensitive to sulfonamides, ampicillin, streptomycin, the tetracyclines, and various other drugs. However, resistance to one or more of these is common, especially in strains from antibiotic treated patients and when septicaemia or some other serious infections has to be treated urgently without waiting for results of sensitivity tests on the causative E. coli strain, a drug which it is unlikely to be resistant should be used e.g. Kanamycin, Gentamycin, Nitrofurantoin or Nalidixic acid may be useful for treating a urinary tract infection.

5. ENZYMATIC INACTIVATION OF THE ANTIBACTERIAL AGENT

An example of this is the elaboration of penicillinase, that attacks the penicillin molecule. The penicillin molecule may be attacked in 2 ways.

\[ \text{AMIDASE} \]

\[ R - C - N - H \]

\[ \text{AMIDASE GIVES} \]

\[ R - C - N - H \]

\[ \text{OTHERWISE PENICILLINASE GIVES} \]

\[ R - C - N - H \]
DEVELOPMENT OF RESISTANCE IN BACTERIAL

Bacterial can become resistant to drug in either of the following ways:

1. **Production of enzymes capable of inactivating the antibacterial agent.** These enzymes may be either intracellular or extracellular.

2. **A change in the permeability of the organism to the antibacterial agent.**

3. **A change at the site of action of the antibacterial agent, rendering the site no longer susceptible to the drug.**

4. **Utilisation of an alternative metabolic pathway to pass a blocked reaction.**

**1. ENZYMATIC INACTIVATION OF THE ANTIBACTERIAL AGENT**

An example of this is the elaboration of penicillinase, that attacks the penicillin molecule. The penicillin molecule may be attacked in 2 ways.

![Diagram of penicillin molecule and penicillinase reaction](attachment:penicillin_diagram.png)
In bacteria that are capable of producing penicillinase, the presence of penicillin, the substrate for the enzyme, greatly increases enzyme production.

The E.coli however becomes resistant to penicillin by exclusion of the drug from the site of mucopeptide synthesis. Chloramphenical can be inactivated enzymatically, in particular by E.coli (Smith and Worrel, 1950. Shaw 1966).

2. RESISTANCE RESULTING FROM CHANGES IN PERMEABILITY OF THE ORGANISM TO ANTIBACTERIAL AGENT

It has been proved that many types of drug resistance are due to decreased permeability of the outer layers of the cells (Watanabe, 1963, Okamoto and Mizumo, 1964, Franklin and Godfrey, 1965).

In most of these cases of resistance to chloramphenicol or tetracycline, the system for the synthesis of ribosomal protein in resistant cells was still sensitive to chloramphenicol or tetracycline in "cell-free preparations". E.coli have been trained to be resistant to quaternary ammonium compounds by the method of serial subculture. It was found that the resistance was due to increase in lipid content on cell. Resistant cells, on treatment with lipase, lost resistance. (Chaplin, 1951 and 1952).

But this same kind of resistance, can be due to a decrease in phospholipid content in some cases. Polymyxin resistance in P. aeruginosa is probably related to decrease in phospholipid content. Since the phospholipid is an important uptake site for this antibiotic, the reduction in phospholipid leads to an increase in resistance (Brown and Watkina, 1970).

Resistance can also be due to changes in cytoplasmic membrane. Acriflavine resistance in E.coli has been attributed to changes in the cytoplasmic membrane resulting in a reduced cellular accumulation of acriflavine.
3. **Resistance arising from a change at the site of action of the agent**

The target enzyme for sulphonamide action is almost certainly tetrahydrofolic acid synthetase (Brown et al, 1961).

\[
\text{Z-amino-4, hydroxy-5, 6, 7 tetrahydropteridine} + \text{P-amino Benzoic acid (PABA)} \]

**ENZYME**

\[
\text{Tetrahydropteroic acid} \]

The sulphonamide is a structural analogue of the natural enzyme substrate, PABA. One type of sulphonamide resistant mutant overproduces the natural competitor (PABA), thus overcoming the competitive block (Brown, 1962). Others synthesize a modified synthetase enzyme (Hotchkiss and Evans, 1960, Woff and Hotchkiss, 1963). And yet some others alter permeability to the drug (Watanabe, 1963). Resistance to streptomycin in E. coli has been attributed to a change in ribosomal protein structure (Funatsu et al, 1972).

4. **Utilisation of an alternative metabolic pathway to pass a blocked reaction**

This is largely a theoretical mechanism, supportive evidence is poor. The theoretical model is:

\[
A \xrightarrow{e_1} B \xrightarrow{e_2} C \xrightarrow{e_3} D \xrightarrow{e_4} E \xrightarrow{e_5} \text{essential cellular component.} \]
If the antimicrobial agent is specifically active against $e_3$, preventing synthesis of $D$, resistance may occur via a biochemical shunt.

Evidence: Sevag and Green (1944) showed that a strain of staph. aureus resistant to sulphonamides in presence of glucose, could not grow if pyruvate was substituted as a sole source of carbon.

Thus resistance to the two drugs that we have can be explained by the above mechanisms. The Co-trimoxazole is a combination of a sulphonamide and an antifolic and resistance to both of these drugs can be explained by the mechanism involving a change at the site of action of the antibacterial agents rendering the site no longer susceptible to the drug.

Linomycin acts by inhibiting the protein synthesis and resistance to it may be due to a change in the permeability of the organism to the antibacterial agent.
The combination of these two drugs in **CO3TRIM0XAZ0LE**, makes use of sequential blockade in its activity. Thus inhibiting two steps in an essential metabolic pathway, which is the synthesis of tetrahydrofolic acid.

Sulphamethoxazole inhibits synthesis of dihydropteroic acid while Trimethoprim inhibits the conversion of dihydrofolic acid to Tetra-hydrofolic acid.

**SULPHAMETHOXAZOLE**

- **H₂N-**
- **SO₂-NH-**
- **CH₃**

**TRIMETHOPRIM**

- **H₂N-**
- **CH₂-**
- **OCH₃**
- **OCH₃**
- **OC₃**

It is well absorbed from the upper alimentation of the gastro-intestinal tract like many other sulphonamides. The peak level is reached by 2-4 hours.

It is bound to plasma proteins particularly albumin to appreciable extent. This bound is less readily reversible and the free form is reabsorbed faster. Thus concentration is reached by selective transfer, including the oedematous placenta. It even manages to cross the placenta and reaches the foetus.

It can replace bilirubin from albumin. This could raise the level of the bilirubin in the foetus and cause kernicterus.

The raised levels in the plasma can be explained by both protein binding and renal elimination.

The combination of these two drugs in CO3TRIM0XAZ0LE, makes use of sequential blockade in its activity. Thus inhibiting two steps in an essential metabolic pathway, which is the synthesis of tetrahydrofolic acid.

Sulphamethoxazole inhibits synthesis of dihydropteroic acid while Trimethoprim inhibits the conversion of dihydrofolic acid to Tetra-hydrofolic acid.
Thus sulphamethoxazole inhibits PABA and Trisemophrin inhibits NADPH + H⁺ such that it can not donate its 'hydrogens'.

Sulphamethoxazole Pharmacokinetics:

- It is well absorbed from the upper section of the gastrointestinal tract like many other sulphonamides. The peak level is achieved in four hours.
- It is bound to plasma proteins particularly albumin to appreciable extent. The bond is loose and reversible and the free form of the drug is available. Most organs are reached by bacteriostatic levels including the brain and the placenta. It even manages to cross the placenta and reaches the foetus.
- It can replace bilirubin from albumin. This could raise the level of the bilirubin in the foetus and cause kernicterus.
- The prolonged levels in the plasma can be explained by both protein binding and the high degree of reabsorption from glomerular filtrate.
- It is excreted through the urine, by glomerular filtration. In kidney malfunction, excretion may be impaired leading to toxic plasma levels.

Pharmacology:

- It is employed for both systemic and urinary tract infections. Due to the high percentage of acetylated relatively insoluble form of the drug in urine (like all sulphonamides) precautions should be taken to avoid crystalluria.
**Trimethoprim**

**Pharmacokinetics**

Absorption is rapid and peak levels are achieved in three to four hours. Plasma half life is six to twelve hours according to the degree of protein binding and the rate of urinary excretion. Almost all of it will be retrieved in urine up to five days after administration. The kidney excretes trimethoprim by glomerular filtration at an increasing rate as the urine pH falls. Tubular secretion may also be taking part in excretion.

**Pharmacology**

It is one of the most active agents which exerts a supra-additive effect when used with a sulphonamide. It is an effective inhibitor of some species of gram-negative bacterial but repeated exposure lead to resistance development. The effectiveness of the combination is due to the sequential blockade exhibited by the two drugs.

Cross-resistance between trimethoprim and sulphonamides does not develop because they are not structurally related.

**Nalidixic Acid**

![Chemical structure of Nalidixic Acid]

It inhibits DNA synthesis

**Pharmacokinetics:**

Absorption from the gastro-intestinal tract is dependable and plasma levels of free plasma fall steadily after one hour, due to reversible protein binding and rapid excretion by the kidney. Some of the drug is altered in the liver but several of its derivatives have been found to retain antibacterial activity even in urine. Concentration of unaltered drug in the urine are reduced by a high pH.
Pharmacology

It is orally active and acts by inhibiting DNA synthesis. It is excreted predominantly in urine.

Although it exists in active form in the blood, the concentration of the drug in the body fluids is generally too low to be effective against systemic infections. It is active against many gram-negative. Micro-organisms including members of escherichia, aerobacter, klebsiella, and proteus all of which are involved in urinary tract infections.

Pseudomonas aeruginosa is resistant. It is useful in treating acute and chronic infections caused by sensitive bacteria.

However, sensitivity bacteria can become resistant in as few as five days.

Side effects are nausea, vomiting, skin rashes, and photosensitivity reactions.

Central nervous system side effects include:

- Dizziness
- Visual disturbance and occasionally convulsions particularly in patients with predisposition to convulsive disorders.

Although rarely seen, cholestatic jaundice and blood dyscrasias indicate the value of hepatic function tests and blood cell counts in patients treated for more than two weeks.

Intracranial hypertension due to nalidixic acid has been recorded in young children and the drug should not be used in children under one month of age.
MATERIALS AND METHOD

The apparatus and materials needed to carry out the project were:

1. PETRI DISHES
2. MACCONKEY'S AGAR
3. NUTRIENT BROTH
4. SENSITIVITY DISCS OF (a) WINTOMYLON
   (b) CO-TRIMOXAZOLE
5. STANDARD E.coli SPECIMEN
6. E.coli SPECIMENS FROM PATIENTS
7. AUTOCLAVE
8. LYSOL (2% SOLUTION)
9. SPECIMEN BOTTLES
10. REFRIGERATOR
11. INCUBATOR (OVEN)

The apparatus and materials needed to carry out the project were:

1. PETRI DISHES
2. MACCONKEY'S AGAR
3. NUTRIENT BROTH
4. SENSITIVITY DISCS OF (a) WINTOMYLON
   (b) CO-TRIMOXAZOLE
5. STANDARD E.coli SPECIMEN
6. E.coli SPECIMENS FROM PATIENTS
7. AUTOCLAVE
8. LYSOL (2% SOLUTION)
9. SPECIMEN BOTTLES
10. REFRIGERATOR
11. INCUBATOR (OVEN)

Technique

A source of the antibiotic (sensitivity disc) is applied to a plate of solid medium.

The diffusion of the antibiotic through the medium inhibits the growth of a sensitive organism placed in or on it to a degree partly dictated by the susceptibility of the organism. It is known however that a number of other factors will influence the size of the zones of inhibition and these also require control. Such factors are:

1. Choice of the Medium,
2. Effect of pH
3. Size of the inoculum - that is the greater the size of the inoculum, the smaller the zones.
The discs should be stored in a cool and dry place. When applied to the medium, they are pressed firmly to ensure proper contact and thus even diffusion. The incubation time should be the minimum required for the normal growth of the organism. Prolonged incubation may result in the subsequent growth of the organism which have been prevented from reproducing but which are not killed.

The control used was standard E. coli. The distance measured was the diameter of the inhibition zone.

**Interpretation of the results**

The test should be compared to the control zones of inhibition.

1. A zone diameter of the same size as, or larger than, or not smaller than the control by more than three millimeters = SENSITIVE.

2. A zone of diameter greater than three millimeters but less than control by more than three millimeters = MODERATELY SENSITIVE.

3. A zone diameter of two millimeters or less = RESISTANT.
EXPERIMENTAL WORK

The project started with the collection of a standard E. coli specimen from the Diagnostic Laboratory, culturing it in nutrient broth, and then on to the MacConkey's agar plate so that it was available whenever needed arose. Both the nutrient broth and the MacConkey's agar were available in powder form and as such had to be prepared before use.

Preparation of nutrient broth

This involved dissolving the powder in water in the proportions given in literature:

13gms per litre.

After complete dissolution, it was put into several specimen bottles, and then autoclaved at 15 pound pressure for 15 minutes. The specimen could then be inoculated after the media cooled down in the bottles.

Preparation of MacConkey's agar

This involved putting the powder in water in the proportions given in literature:

52gms per litre.

The suspension was then given a few boils to make sure that the powder completely dissolved. The media was then transferred to several specimen bottles when still hot and autoclaved at 15 pound pressure for 15 minutes. The sterile hot media was then transferred into petri dishes that had already been autoclaved.

It was then let to settle undisturbed, so that we ended up with a flat smooth surface.

Both the nutrient broth and the MacConkey's agar were then put into the refrigerators until required.
The cultures came from the Diagnostic Laboratory on plates of MaConkey's agar. Using a wire loop which was sterilised by flame, the specimens were cultured in nutrient broth overnight.

On the following day, the culture from the nutrient broth were swabbed onto the MacConkey's agar using sterile swabs.

The sensitivity discs were placed on the plate and pressed to ensure diffusion of the drug. The plates were then incubated overnight at 30°C. The following day, the zones of inhibition were measured. Standard E.coli grown on one plate was used as the control for all specimens.

On each of the plates, sensitivity discs of Wintomylon and Co-trimoxazol were placed and pressed after swabbing and incubated overnight.

The zones of inhibition were measured the following day and compared to those of Standard E.coli for the respective sensitivity discs. For each specimen, three plates were cultured and their inhibition zones measured. The average size of the zones was then considered.

The following results were obtained.
### RESULTS

**CO-TRIMOXAZOLE**

<table>
<thead>
<tr>
<th>Source of organism (By Patient number)</th>
<th>Diameter of Inhibition zone (mm)</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard E.coli</td>
<td>18.5</td>
<td></td>
</tr>
<tr>
<td>1. D 572</td>
<td>22.5</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>2. D 571</td>
<td>18</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>3. D 754</td>
<td></td>
<td>RESISTANT</td>
</tr>
<tr>
<td>4. D 750</td>
<td>22.5</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>5. D 757</td>
<td></td>
<td>RESISTANT</td>
</tr>
<tr>
<td>6. D 899</td>
<td></td>
<td>RESISTANT</td>
</tr>
<tr>
<td>7. C 153</td>
<td></td>
<td>RESISTANT</td>
</tr>
<tr>
<td>8. C 176</td>
<td></td>
<td>RESISTANT</td>
</tr>
<tr>
<td>9. C 148</td>
<td>24</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>10. D 260</td>
<td></td>
<td>RESISTANT</td>
</tr>
<tr>
<td>11. D 157</td>
<td>24</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>12. D 261</td>
<td>21</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>13. C 180</td>
<td>17</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>14. D 747</td>
<td></td>
<td>RESISTANT</td>
</tr>
<tr>
<td>15. D 267</td>
<td>14</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>16. D 278</td>
<td>15</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>17. D 276</td>
<td></td>
<td>RESISTANT</td>
</tr>
<tr>
<td>18. D 279</td>
<td></td>
<td>RESISTANT</td>
</tr>
<tr>
<td>19. D 277</td>
<td>17</td>
<td>RESISTANT</td>
</tr>
<tr>
<td>20. D 318</td>
<td>19</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>21. D 319</td>
<td>23</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>22. D 320</td>
<td>15</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>23. D 301</td>
<td></td>
<td>RESISTANT</td>
</tr>
<tr>
<td>24. D 315</td>
<td>21</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>25. D 317</td>
<td></td>
<td>RESISTANT</td>
</tr>
</tbody>
</table>

Out of the 25 specimens tested 14 were sensitive and 11 were resistant to Co-trimoxazole.
<table>
<thead>
<tr>
<th>Source of organisms (by patient number</th>
<th>Diameter at Inhibition zone (mm)</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard E.coli</td>
<td>22.5</td>
<td></td>
</tr>
<tr>
<td>1. D 572</td>
<td>22</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>2. D 571</td>
<td>22</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>3. D 754</td>
<td>24</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>4. D 750</td>
<td>18.5</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>5. D 757</td>
<td>21.5</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>6. D 899</td>
<td>29</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>7. C 153</td>
<td>24</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>8. C 176</td>
<td>23</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>9. C 148</td>
<td>21</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>10. D 260</td>
<td>18</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>11. D 157</td>
<td>23</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>12. D 261</td>
<td>21</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>13. C 160</td>
<td>20</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>14. D 747</td>
<td>16</td>
<td>MODERATELY SENSITIVE</td>
</tr>
<tr>
<td>15. D 267</td>
<td>19</td>
<td>RESISTANT</td>
</tr>
<tr>
<td>16. D 278</td>
<td>24</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>17. D 276</td>
<td>19</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>18. D 279</td>
<td>34</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>19. D 277</td>
<td>10</td>
<td>MODERATELY SENSITIVE</td>
</tr>
<tr>
<td>20. D 318</td>
<td>24</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>21. D 319</td>
<td>17</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>22. D 320</td>
<td>18</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>23. D 301</td>
<td>24</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>24. D 315</td>
<td>22</td>
<td>SENSITIVE</td>
</tr>
</tbody>
</table>

24 out of 25 specimens were sensitive and one RESISTANT.
<table>
<thead>
<tr>
<th>Source of organism (by patient number)</th>
<th>Co-Trimoxazole zone of Inhibition (mm)</th>
<th>Sensitivity</th>
<th>trimoxazole zone of Inhibition (mm)</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard E. coli</td>
<td>18.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. D 572</td>
<td>25</td>
<td>SENSITIVE</td>
<td>23</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>2. D 571</td>
<td>18</td>
<td>SENSITIVE</td>
<td>22</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>3. D 754</td>
<td>-</td>
<td>RESISTANT</td>
<td>24</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>4. D 750</td>
<td>22.5</td>
<td>SENSITIVE</td>
<td>18.5</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>5. D 757</td>
<td>-</td>
<td>RESISTANT</td>
<td>21.5</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>6. D 899</td>
<td>-</td>
<td>RESISTANT</td>
<td>29</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>7. C 153</td>
<td>-</td>
<td>RESISTANT</td>
<td>24</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>8. C 173</td>
<td>21</td>
<td>SENSITIVE</td>
<td>23</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>9. C 148</td>
<td>24</td>
<td>SENSITIVE</td>
<td>21</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>10. D 260</td>
<td>-</td>
<td>RESISTANT</td>
<td>18</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>11. D 157</td>
<td>24</td>
<td>SENSITIVE</td>
<td>23</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>12. D 261</td>
<td>21</td>
<td>SENSITIVE</td>
<td>21</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>13. C 180</td>
<td>17</td>
<td>SENSITIVE</td>
<td>20</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>14. D 747</td>
<td>-</td>
<td>RESISTANT</td>
<td>16</td>
<td>MODERATELY SENSITIVE</td>
</tr>
<tr>
<td>15. D 267</td>
<td>14</td>
<td>SENSITIVE</td>
<td>-</td>
<td>RESISTANT</td>
</tr>
<tr>
<td>16. D 278</td>
<td>15</td>
<td>SENSITIVE</td>
<td>19</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>17. D 276</td>
<td>-</td>
<td>RESISTANT</td>
<td>24</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>18. D 279</td>
<td>-</td>
<td>RESISTANT</td>
<td>19</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>19. D 277</td>
<td>-</td>
<td>RESISTANT</td>
<td>34</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>20. D 318</td>
<td>19</td>
<td>-</td>
<td>10</td>
<td>MODERATELY SENSITIVE</td>
</tr>
<tr>
<td>21. D 319</td>
<td>23</td>
<td>SENSITIVE</td>
<td>24</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>22. D 320</td>
<td>15</td>
<td>SENSITIVE</td>
<td>17</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>23. D 301</td>
<td>-</td>
<td>RESISTANT</td>
<td>18</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>24. D 315</td>
<td>21</td>
<td>SENSITIVE</td>
<td>24</td>
<td>SENSITIVE</td>
</tr>
<tr>
<td>25. D 317</td>
<td>-</td>
<td>RESISTANT</td>
<td>21</td>
<td>SENSITIVE</td>
</tr>
</tbody>
</table>
DISCUSSION

From the results, it is clear that the Escherichia coli bacteria are more resistant to Co-trimoxazole than to Wintomylon.

Thus, of the 25 specimens that were tested, 11 were found to be resistant to Co-trimoxazole. This represents 44% of the sample.

With this information, it can be argued that for every 100 patients infected with E. coli urinary tract infection at Kenyatta National Hospital, 44 will be carrying resistant species whereas as 56 will be carrying sensitive species to Co-trimoxazole.

As concerns the sensitivity of Escherichia coli to Wintomylon, it was very high.

Of the 25 specimens that were tested, only one was resistant which represents only 4%. Following the same argument as above it can be said that out of 100 E. coli urinary tract infections at Kenyatta National Hospital, 96 will be carrying bacteria sensitive to Wintomylon, whereas only 4 will be carrying bacteria resistant to Wintomylon.

As far as excretion is concerned, glomerular filtration plays a part in removing Co-trimoxazole and tubular secretion may be taking part too. In the case of Wintomylon the glomerular filtration is done triple but unlike the Co-trimoxazole its metabolites are also active.

Thus it can be seen that the excretion of the two drugs depends on both the in vitro results as the in vivo variations.
Comparison

From this argument, the Escherichia coli from urinary tract infections seems to be more sensitive to Wintomylon than to Co-trimoxazole.

It is a fact that the success of the two drugs depends on the individual variation to a certain extent. As it is also true that pharmacokinetics of the drug and the variation of individual response to drugs have not been considered.

Thus whereas both constituents of Co-trimoxazole (sulphamethoxazole and Trimethoprim) are rapidly absorbed from the gut, the absorption of Wintomylon is dependable. As such, the success of Wintomylon will depend on the individual being treated. If the absorption in a particular patient is rapid, the above "in vitro test" results and argument holds but if the absorption is slow then the argument would no longer hold.

The recommendation, therefore is that if pathogenic organism in such a case, the Co-trimoxazole plasma level would be high and as such kill most of the organisms whereas that of the Wintomylon would be low and hence kill less organisms.

As far as excretion is concerned, glomerular filtration takes part as concerns Co-trimoxazole and tubular excretion may be taking part too. In the case of Wintomylon the glomerular filtration is more rapid but unlike the Co-trimoxazole its metabolites are also active.

Thus it can be seen that the success of the two drugs depends on both the in vitro results and the in vivo variations.
RECOMMENDATION

It is a fact that the success of the two drugs depends on the individual variation to a certain extent. It is also true that the success will also depend on the formulation of tablets and other factors. However, from this experiment it is clear that the difference in the "IN VITRO RESULTS" is so great that it would need a lot of inhibition by other factors for the difference to favour the reverse.

Thus, it can still be argued that Wintomylon is better than Co-trimoxazole for E.coli urinary tract infection.

The recommendation, therefore is that if pathogenic organism in a urinary infection has clearly been diagnosed as the Escherichia coli, the Wintomylon would rather be used than Co-trimoxazole.

This argument however holds only for Kenyatta National Hospital where the research was conducted.

REFERENCES


7. NAKAMURA, H. (1968). Acriflavine resistance in E.coli; the role of the cell membrane. Memoir's of the Konan University, Science Series, No. 11 Article 57 Pg. 43.


10. FUNATSU, G. et al. (1972) J. Mol. Biol. 64, 201 - 9

"Review of Medical Microbiology" 10, 196 - 198
Lange Medical publications, Los. altos, California.

13. LYNCH K.J. (1976)
"Lynch's Medical laboratory Technology" 2, 1, 628 - 630

The English Language Book Society and Churchill Livingstone, 23 Ravelston Terrace, Edinburgh.