THE EFFECTS OF COLIBACILLINUM AND C0-TRIMOXAZOLE ON THE GROWTH AND CELLULAR MORPHOLOGY OF *ESCHERICHIA COLI*

by:

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I, Farhana Moolla, declare that this dissertation represents my own work, both in conception and execution.

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To my grandmother, Momeen, who dedicated her life to the selfless service of her loved ones.

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ABSTRACT

The purpose of this investigation was to determine the effect of Colibacillinum and co-trimoxazole in terms of it's effect on the growth rate and cellular morphology of *E.coli* in order to contribute towards the understanding of the use of Colibacillinum in *E.coli* infections. The strain of *E.coli* used in this study was isolated from a patient with cystitis. The experiment consisted of 2 trials. During the first trial, *E.coli* was tested for sensitivity to co-trimoxazole. The method of choice was the serial-dilution-agar-plating procedure. Samples were removed 0h, 6h, 12h, 24h and 48h after inoculation, serially diluted and incubated for 24h. During the second trial, *E.coli* was tested for sensitivity to Colibacillinum 5CH, 7CH, 9CH, 12CH and 15CH by employing the method stated above. From the data obtained the growth rate, specific growth rate, maximum cell population reached and lag phase were computed. Colibacillinum 7CH proved to be the most effective potency, of the various potencies tested. Colibacillinum 7CH reduced the maximum population by 41.5% and co-trimoxazole reduced the maximum population by 97% relative to the maximum population of the control. The maximum specific growth rate of the *E.coli* treated with Colibacillinum 7CH increased by 0.609% and that treated with co-trimoxazole decreased by 67.33% when compared to the maximum specific growth rate of the control. The in vitro results indicate that co-trimoxazole is a far superior bacteriostatic chemotherapeutic agent than Colibacillinum. This is further supported by the changes in cellular morphology observed during scanning electron microscopy. The *E.coli* treated with co-trimoxazole was found to be shorter in length and irregular when compared to *E.coli* treated with Colibacillinum 7CH. Definitive conclusions could not be drawn from the results therefore Colibacillinum cannot be recommended as a front line agent in the treatment of cystitis. It's use in the treatment of *E.coli* infections, in conjunction with other forms of treatment, either homoeopathic or allopathic, may only be recommended once further studies have been conducted.

UITTREKSEL

Die doel van hierdie ondersoek was om die effek van Colibacillinum en co-trimoxazole te ondersoek in terme van die effek wat hierdie middels op die groei tempo sel morfologie van *E.coli* het, met die doel om 'n bydrae te lewer in verband met die algemene kennis van die gebruik van Colibacillinum en co-trimoxazole in *E.coli* infeksies. Die ondersoek het uit twee eksperimente bestaan. In die eerste is die sensitiwiteit van *E.coli* vir co-trimoxazole getoets. Die gekose metode was die seriaal-verdunde-agar-kultuur metode. Monsters is onderskeidelik 0, 6, 12, 24 en 48 uur na inokulasie geneem, seriaal verdun, en vir 24 uur in 'n broeimasjien geplaas. Die tweede eksperiment het die sensitiwiteit van *E.coli* vir Colibacillinum 5CH, 7CH, 9CH, 12CH and 15CH getoets. Dieselfde metode soos bo is gebruik. Vanaf die data verkry is die groei tempo, spesifieke groei tempo, maksimum sel populasie bereik en vertragings fase bereken. Hieruit blyk dit dat Colibacillinum 7CH die mees effektiewe potensie is. Die maksimum populasie is met 41.5% deur Colibacillinum en met 97% deur co-trimoxazole verlaag in vergelyking met die maksimum populasie van die kontrole. In vergelyking met die maksimum spiesifieke groei tempo van die kontrole het die maksimum spesifieke groei tempo van *E.coli* behandel met Colibacillinum met 0.609% verhoog, en met 67.33% verlaag met die geval van die *E.coli* kultuur met co-trimoxazole behandel.

Die in vitro studie dui aan dat co-trimoxazole 'n baie beter kiem dodende chemotherapeutiese middel is as Colibacillinum. Dit stelling word verder versterk deur die resultate verkry waneer die morfologie onder 'n skandeer elektron mikroskoop ondersoek word. Daar is gevind dat die *E.coli* selle behandel met co-trimoxazole korter in lengte en ongelyk was in die vergelyking met die selle behandel met Colibacillinum 7CH. Aanbevelings aangaande die gebruik van Colibacillinum as 'n uitstaande middel teen blaasinfeksies kan nie gemaak word nie. Die rol en gebruik van Colibacillinum in die behandeling van *E.coli* infeksies, te same met ander vorme van behandeling, hetsy homoeopaties of allopaties, kan slegs gemaak word na verdere ondersoeke.

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LIST OF ABBREVIATIONS

cfu/ml:	colony forming units per millilitre
CH :	Centisimal Hahnemanian
h:	hours
mm:	millimetre
ml :	millilitres
rpm:	revolutions per minute
ug/ml:	micrograms per millilitre
w/v:	weight per volume

INTRODUCTION

E.coli is regarded as part of the normal flora of the human intestinal tract. However, under certain circumstances it may cause disease in man, the most common being urinary tract infections and diarrhoea. About 20-30% of adult women will experience some type of urinary tract infection during their life span (Sanford, 1975; Kunin, 1987). Most of these infections are uncomplicated cystitis, caused in more than 60% of cases by *E.coli* (Naber, et al.,1987; Petersen, 1988). *E.coli* has also been implicated as the causative agent of travellers diarrhoea and diarrhoea in infants.

Co-trimoxazole is often used as a first line agent in the treatment of urinary tract infections since it is effective against a wide range of bacteria including *E.coli.* However, various side effects have been reported (Gilman, et al., 1985; Traunce,1989). These include nausea, vomiting, exfoliative dermatitis and blood disorders. Furthermore co-trimoxazole should be avoided during pregnancy and in patients suffering from folate deficiency. Development of resistant *E.coli* strains to co-trimoxazole has also been reported (Spencer and Cole, 1992). This can be attributed to the increase in systematic prescription of antibiotics for infections. Therefore there is an increasing need for new forms of treatment. Colibacillinum, which is a homoeopathic nosode, may provide an alternative. If Colibacillinum is found to be effective in the treatment of *E.coli* infections, it will offer several advantages over treatment with co-trimoxazole. These include reduced adverse effects, decreased risk of disturbing bacterial flora and selecting resistant microorganisms, improved patient compliance and lower costs.

The aim of this investigation was to determine the relative effect of Colibacillinum and co-trimoxazole on *E.coli* in terms of their effect on the growth rate and cellular morphology of *E.coli* in order to contribute towards the understanding of the use of Colibacillinum in *E.coli* infections.

CHAPTER ONE

1.0. THE PROBLEM AND ITS SETTING

1.1 THE STATEMENT OF THE PROBLEM

The purpose of this study was to evaluate the relative effects of homoeopathic Colibacillinum and an antibacterial agent, co-trimoxazole, on *E.coli* with reference to its impact on the growth rate and cellular morphology of *E.coli* in order to contribute towards the understanding of the role of homoeopathic Colibacillinum in the treatment of *E.coli* infections.

1.2 THE STATEMENT OF THE SUBPROBLEMS

1.2.1. First Subproblem

To evaluate the effects of co-trimoxazole on *E.coli* with reference to its impact on the growth rate and cellular morphology of *E.coli* in order to confirm that the antibacterial agent influences growth of *E.coli* .

1.2.2. Second Subproblem

To evaluate the effect of homoeopathic Colibacillinum on *E.coli* with reference to its impact on the growth rate and cellular morphology of *E.coli* in order to identify whether the different potencies of homoeopathic Colibacillinum influence growth of *E.coli* .

1.2.3. Third Subproblem

To integrate the knowledge of how the different potencies of homoeopathic Colibacillinum and the co-trimoxazole influence growth of *E.coli* in order to contribute towards the understanding

of the role of homoeopathic Colibacillinum in the treatment of *E.coli* infections.

1.3 THE HYPOTHESES

1.3.1. First Hypothesis

Co-trimoxazole will decrease the growth rate of *E.coli* and distort the shape of the bacteria.

1.3.2. Second Hypothesis

The homoeopathic Colibacillinum will decrease the the growth rate of*E.coli* for a longer period than co-trimoxazole and it wil distort the shape of the bacteria.

1.3.3. Third Hypothesis

By understanding the relative effectiveness of the homoeopathic Colibacillinum and the co-trimoxazole on *E.coli* growth , it will be possible to comment on the role of homoeopathic Colibacillinum in the treatment of *E.coli* infections.

1.4. THE DELIMITATIONS

a. This study was limited to a specific strain of *E.coli* isolated from a clinical case of cystitis .

b. This study was limited to the following growth media: Oxoid no. 2 broth, violet red bile agar and nutrient agar. Media were made according to the manufacturers specifications.

c. This study was limited to the following potencies of Colibacillinum :
5CH, 7CH, 9CH, 12CH, 15CH.

d. Temperature or pH ~~was~~ were not ~~be~~ varied. The temperature was kept constant at 37°C and the pH was kept constant at 7.0.

e.This study was limited to a specific antibiotic (co-trimoxazole).

1.5. THE ASSUMPTIONS

1.5.1. First Assumption

The principles of Homoeopathy are valid.

1.5.2. Second Assumption

The electron microscopic techniques used will reveal any changes in cellular morphology.

1.6. DEFINITION OF TERMS

1. Law of Minimum Dose

   The law of minimum dose states that by extreme dilution the medicines curative principles are enhanced and all undesirable side effects are lost.

2. Principle of Potency

   The principle of potency states that the dilution and succussion of medicines greatly enhance their activity.

3. Growth Rate

   Rate of total number increase of a culture. It is not constant but increases as the number of the dividing cells in the culture increase.

4. Specific Growth Rate

Rate of growth per unit organism.

CHAPTER TWO

2.0. REVIEW OF THE RELATED LITERATURE

2.1. INTRODUCTION

E.coli forms part of the normal flora of the intestinal tract of man and it is usually considered harmless. However, it may cause disease if it is moved from its normal anatomical site and introduced into a normally sterile area. In these cases *E.coli* behaves as an opportunistic pathogen. It can also, under certain circumstances, behave as a true pathogen and cause disease in its normal anatomical site. These *E.coli* strains are not commensals of man's bowels (Jamison, 1984). As a pathogen, *E.coli*, is associated with two clinical syndromes v.i.z., urinary tract infections and diarrhoea. Antibiotics are normally used to treat the above conditions, but due to the adverse effects and the development of resistance, alternative forms of therapy need to be considered. An alternative to the allopathic treatment of *E.coli* infections is homoeopathic treatment using Colibacillinum.

2.2. ESCHERICHIA COLI

The species *E.coli* belongs to the family, Enterobacteriaceae and genus, Escherichia.

E.coli is a short, plump rod about 1 to 3um long and varying in thickness from 1/3 to 1/5 of its length. The cells occur singly but may also form short chains. It is Gram negative and does not form spores. Most strains are motile and bear eight or more flagella peripherally arranged. Different strains of *E.coli* bear different surface structures known as O, K and H antigens (Zinsser and Bayne-Jones, 1939). Fimbrae are also present and are responsible for adherence. For infection to occur the bacteria must adhere to the intestinal mucosa which enables the

organisms to resist expulsion by peristaltic clearing mechanisms. This allows proliferation and colonisation of the gut. Adherence is then followed by toxin production or invasion (Ghosh, et al., 1991). *E.coli* is found in the intestinal tract of warm blooded animals. The optimal temperature for growth is 37°C, with a temperature range of 10°C-40°C. The optimal pH for growth is 7.0-7.5 with the minimum at pH 4.0 and the maximum at pH 8.4.

2.3. INFECTIONS CAUSED BY ESCHERICHIA COLI

2.3.1. URINARY TRACT INFECTIONS (U.T.I.)

The term urinary tract infection includes cystitis, urethritis, prostatitis and pyelonephritis.

Prevalence

Urinary tract infection is a commonly occurring disorder. At least 50% of women experience this problem sometime in their life. 3% of women are affected by the age of 20 (Edwards and Bouchier, 1991). The pathogen most commonly responsible for causing urinary tract infections is *E.coli* (Zinsser and Bayne-Jones, 1939; Trienekens, et al., 1989; Spencer and Cole, 1992). According to Turk and Porter(1978) *E.coli* is responsible for 80% or more of all urinary tract infections that occur outside the hospital.

Factors that predispose to urinary tract infections include:

1. Pregnancy. 5-8% of pregnant women were found to have significant bacteriuria. Coliform bacilli were most often implicated. Furthermore, the incidence of prematurity, perinatal deaths and small birth size is related to significant bacteriuria (Duguid, et al., 1978).
2. Urinary tract infections are more common in women. This may be due to their short urethra and sex hormones. Women also lack prostatic fluid which contains bactericidal substances (Jamison, 1984).
3. Abnormalities of the urinary tract.

4. Diabetic patients.

2.3.1.1. INFECTIONS OF THE LOWER URINARY TRACT

E.coli is responsible for 75% of infections of the lower urinary tract (Edwards and Bouchier, 1991).

Pathogenesis

E.coli possess a certain property which enable them to invade the urinary tract. These fimbrae allow the *E.coli* to adhere to the surface receptors on the urothelium. Patients susceptible to urinary tract infections may possess more surface receptors. At the initial stage the *E.coli* colonize the periurethral zone (Edwards and Bouchier, 1991).

Clinical features

Typical symptoms include dysuria, frequency, burning, pain in the urethra during micturition, supra pubic pain during and after voiding the bladder and an intense desire to pass urine after the bladder has been emptied. The urine may have an unpleasant odour and may be cloudy (Petersen, et al.,1990; Edwards and Bouchier, 1991).

2.3.1.2. INFECTIONS OF THE UPPER URINARY TRACT

About 75% of infections are due to *E.coli* (Edwards and Bouchier, 1991). Pyelonephritis is an example of an infection of the upper urinary tract. Symptoms include pain in the loin, loin tenderness, with high fever, rigors and frequency of micturition.

2.3.2. DIARRHOEA

Four different classes of diarrheic *E.coli* have been identified and they are: Enteropathogenic*E.coli* (EPEC), Enterotoxigenic *E.coli* (ETEC), Enteroinvasive *E.coli* (EIEC) and Enterohaemorrhagic *E.coli* (EHEC).

2.3.2.1. EPEC

EPEC strains have a worldwide distribution and cause diarrhoea year round. It is an important cause of childhood endemic diarrhoea in Singapore. 2.7% of pathogens isolated from infants with diarrhoea, were EPEC (Lim, et al., 1992).

EPEC strains are responsible for 10-40% of diarrhoea cases presenting to hospitals. They have also been implicated in community epidemics (Evans and Brachman, 1991).

The average mortality of EPEC is 5-6% with the age specific mortality rate in the neonatal period of 16% (Kessner, et al.,1962 cited by Evans and Brachman, 1992).

EPEC strains are the cause of diarrhoea in children under 2 years of age (Evans and Brachman, 1991). This is supported by Lim, et al., (1992) who reported that the isolation rate of EPEC was highest amongst infants, accounting for 81,4%.

Females are more prone to EPEC infections, with a prevalence rate of 2.9%, than males with 2.6% (Lim, et al.,1992). But according to Evans and Brachman (1992) males show a higher infection rate.

EPEC adhere to the intestinal mucosa of the small bowel where they cause destruction of the brush border producing a characteristic lesion. There is no invasion of the mucosa. Two types of adherence have been identified, localized and diffuse. Localized adherence is related to pathogenecity among *E.coli* strains (Evans and Brachman, 1991; Knutton, et al., 1992).

The illness lasts for approximately 7 days. Symptoms include fever, diarrhoea, abdominal distention, respiratory symptoms and convulsions. Dehydration may result from excessive loss of fluids. Hypernatremia and metabolic acidosis are also common (Evans and Brachman, 1991).

2.3.2.2. ETEC

ETEC strains have a worldwide distribution. It is an important cause of diarrhoea in neonates in less developed countries and it is also the pathogen most frequently responsible for travellers diarrhoea in these countries (Hitotsubashi, et al., 1992). ETEC has also been implicated in epidemics.

Children are most commonly affected by diarrhoea caused by ETEC.

Adherence of the ETEC strains to the mucosa of the small bowel, is a prerequisite for infection. Adherence is facilitated by fimbrae which are present on the surface of the ETEC strains as well as by the presence of specific receptors for the fimbrae. Thereafter enterotoxins are produced (Ruter, et al.,1975 cited by Evans and Brachman, 1991).Two types of enterotoxins have been implicated in the pathogenesis and they are heat labile enterotoxin and heat stable enterotoxin (Hitotsubashi, et al.,1992).

Travellers diarrhoea usually lasts between 2-3 days. The onset is sudden and typical symptoms include watery diarrhoea, abdominal cramps, vomiting and anorexia. It may be accompanied by a low-grade fever (Edwards and Bouchier, 1991).

2.3.2.3. EHEC

EHEC causes endemic and epidemic diarrhoea in the United States, Canada and Europe (Remis, et al.,1984; Riley, et al.,1983 cited by Evans and Brachman, 1991). EHEC has been found to cause food poisoning, which manifests as haemorrhagic colitis (Edwards and Bouchier, 1991).

EHEC cause disease by adhering to the mucosa of the transverse colon and producing high levels of Shiga-like toxins. Adherence is mediated by a fimbrael antigen (Karch, et al.,1987 cited by Evans and Brachman, 1991).

Symptoms include bloody diarrhoea and cramps.

2.3.2.4. EIEC

This type of diarrhoea is not common.

EIEC penetrate the mucosa of the colon and cause ulcer formation (Evans and Brachman, 1991).

Fever, abdominal pain and bloody mucous stools are common.

The above mentioned conditions are a widespread problem throughout the world. People of every race and socioeconomic class are effected. These infections are routinely treated using antibiotics. The antibiotic of choice is usually co-trimoxazole.

2.4. CO -TRIMOXAZOLE

Co-trimoxazole is a broad spectrum antibiotic which is effective against many gram-positive and gram-negative bacteria. It is made up of two components, trimethoprim and sulphamethoxazole. It is administered in the ratio of 1:5 but the plasma concentration of trimethoprim and sulphamethoxazole is in the ratio of 1:20. The half lives of trimethoprim and sulphamethoxazole are approximately 11 and 12 hours respectively (Gilman, et al., 1985).

2.4.1. Therapeutic uses of co-trimoxazole

Co-trimoxazole is a commonly used antibiotic for the treatment of urinary tract infections (Spencer and Cole, 1992). This is further supported by studies which used 800mg of sulphamethoxazole plus 160mg trimethoprim every 12 hours for 10 days. There was a cure in the majority of cases. It is also used in the treatment of acute diarrhoea due to EPEC (Gilman, et al.,1985).

2.4.2. Mechanism of action

The normal metabolic pathway of *E.coli* is as follows: PABA (para-amino-benzoic acid) is converted to dihydrofolic acid. Dihydrofolate reductase then converts dihydrofolic acid to folinic acid. Folic acid is essential for cell growth.

Sulphamethoxazole, is a sulfonamide, which is chemically similar to PABA. Sulphamethoxazole is erroneously incorporated into the cell, instead of PABA. This prevents the formation of dihydrofolic acid. The trimethoprim component inhibits the enzyme dihydrofolate reductase which in turn prevents the reduction of dihydrofolic acid to folinic acid thus folic acid synthesis is inhibited. But folic acid is essential for DNA and RNA synthesis which are necessary constituents of the nucleus. The nucleus plays an important role in cell division and hence cell division is inhibited.

Co-trimoxazole via its two components v.i.z. sulphamethoxazole and trimethoprim affects two steps of the metabolic pathway, making it an effective bactericidal agent (Penn, 1977; Gilman, et al.,1985; Traunce, 1989).

2.4.3. Side effects

Several side effects have been attributed to the use of co-trimoxazole. Trienekens, et al.,(1989) reported that 27% of patients experienced side effects. 75% of the adverse effects involve the skin (Gilman, et al.,1985). Other commonly occurring symptoms include nausea, vomiting, diarrhoea and vaginal discharge. Central nervous system reactions include depression, hallucinations and headaches which are due to the sulfonamide component. Blood dyscrasias occur occasionally. Stevens-Johnson syndrome is rare (Gilman, et al.,1985; Traunce, 1989; Trienekens, et al.,1989; Petersen, et al.,1990; Spencer and Cole, 1992).

Furthermore the combination should not be administered to patients who have a history of sensitivity to either component. It should be avoided during pregnancy, breast feeding period and in

infants under two months of age. Caution should be exercised when administering co-trimoxazole to patients with folate deficiency, immunocompromised patients, elderly patients and patients with an impaired renal function (Wade, 1977; Gilman, et al.,1985).

2.4.4. Resistance

Antibiotics are prescribed more and more frequently which has lead to the development and spread of drug resistance. Spencer and Cole (1992) reported that 9.4% of pathogens responsible for urinary tract infections, were resistant to co-trimoxazole. This is supported by Singh, et al.,(1992) who reported that 9.3% of *E.coli* isolates were resistant to co-trimoxazole. Furthermore, Trienekens, et al.,(1989) found that failure of patients to respond to co-trimoxazole was due to the resistance of *E.coli* to the drug.

With the development of drug resistance, there is an increasing need for new forms of treatment. There have been no reports of resistance against Colibacillinum.

2.4.5. The effect of co-trimoxazole on E.coli growth

Co-trimoxazole has a bactericidal effect on *E.coli*. However, it was found to have a bacteriostatic effect on *E.coli* in a study done previously (Lewis, et al., 1974). At a concentration of 1.6ug/ml trimethoprim and 23ug/ml sulphamethoxazole, the bacterial count at 0 h, 2 h, 4 h, 6 h and 24 h was >10, 3x10, 2x10 and 8x10 respectively.

When conducting the literature search, an abundance of literature was available on co-trimoxazole and it's effect on the growth rate and cellular morphology of *E.coli* . However no information could be obtained on the effect of Colibacillinum on the growth rate and cellular morphology of *E.coli* . This finding emphasizes the need for research to be carried out in this particular field.

2.5. COLIBACILLINUM

An alternative to the allopathic treatment of *E.coli* infections is a nosode. The homoeopathic nosode in this case is Colibacillinum. Colibacillinum is prepared from the lysate obtained from a culture made up of a mixture of several *E.coli* stocks. The nosode is then prepared to the correct potencies, in this case 5CH, 7CH, 9CH, 12CH and 15CH, according to the law of minimum dose and the principle of potency.

2.5.1. The clinical pathogenesis

This includes urinary tract symptoms and diarrhoea. Babies and young children are most commonly affected by the diarrhoea which is profuse and weakening.

Urinary tract symptoms include frequency, burning pain at the end of micturition and an intense desire to pass urine after the bladder has been voided. There may be pain along the urethra, kidney and costo-lumbar region. The urine may be dark coloured and have a bad smell. Symptoms are aggravated by cold humid weather. Haematuria may also be present (Julian, 1982).

2.5.2. Resistance and side effects

There are no known side effects of Colibacillinum. Side effects of medication influence patient compliance, the fewer the side effects, the greater the patient compliance. Therefore improved patient compliance is expected with Colibacillinum.

Furthermore there have been no reports of resistant strains to Colibacillinum.

2.6. SUMMARY

1. The history, morphology and habitat of *E.coli* is described in section 2.2.

2. *E.coli* is a common cause of urinary tract infections and diarrhoea. The prevalence, pathogenesis and clinical features of these conditions are described in section 2.3.

3. Co-trimoxazole is the most commonly prescribed antibiotic for *E.coli* infections. The therapeutic uses, mechanism of action, side effects and resistance of *E.coli* to co-trimoxazole is discussed in section 2.4. The effect of co-trimoxazole on the growth rate of *E.coli* is also discussed in this section.

4. In section 2.5. the clinical pathogenesis of Colibacillinum is presented.

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1. Preparation of Media

Violet Red Bile Agar (oxoid) , Nutrient Agar (oxoid) and Nutrient Broth (oxoid) were the media of choice. They were prepared according to the manufacturer's specifications.

3.2. Isolation of *E.coli*

A single strain of *E.coli* isolated from a clinical case of cystitis was obtained from Addington hospital. The strain of *E.coli* was maintained on Nutrient Agar.

3.3. Verification of *E.coli*

Specific tests were conducted for the verification of *E.coli* as well as to check for contamination. These tests include Gram stain, API 20E and agar culture.

3.3. Preparation of Inoculum

A loopful of growth from the agar slant was transferred to 9ml sterile Nutrient Broth (Oxoid) and incubated at 37°C for 24 h. Following incubation the broth culture was centrifuged at 3000 rpm for 10 min and the supernatant decanted. Cells were then washed with sterile physiological solution (0.8% w/v saline), separated from solution by centrifugation and resuspended in sterile physiological solution.

3.4. Effect of Co-trimoxazole on *E.coli*

Nutrient Broth was dispensed, in 45 ml aliquots, into 4 Erlenmeyer flasks (100ml volume). Flasks were autoclaved at 121° C for 15 minutes. After addition of 5% haemolysed horse

blood to the 4 flasks , 0.5ml of sterile distilled water was added to 2 flasks. This formed the control group. To the remaining flasks, 0.5ml of a filter sterilised aqueous solution of co-trimoxazole (Wellcome Ltd.) was added to achieve a final concentration of 1,6 ug/ml trimethoprim and 3,2 ug/ml sulphamethoxazole in the broth. The co-trimoxazole was prepared by a pharmacist.

A 0.5ml aliquot of resuspended cells was introduced into each of the flasks at time 0 h. Samples were removed immediately from both flasks containing sterile distilled water. These were labelled T_0. All flasks were then incubated on an orbital shaker at 136 rpm and 37° C. Further samples were removed from the control series and test series containing co-trimoxazole at 6, 12, 24 and 48 hours after inoculation (T6, T12, T24 and T48) .

The number of viable cells were enumerated in all samples using the serial-dilution-agar-plating procedure (Cuppuccino and Sherman. 1992) Samples from each time interval were serially diluted to 10^{-8}. Aliquots of 1ml were removed in duplicate from each dilution and transferred into 90 mm petri dishes. To each was added 20 ml of violet red bile agar at 50°C .Plates were swirled and the agar was allowed to solidify before incubation at 37°C for 48 hours. Colonies were enumerated on plates containing 30 - 300 colony forming units.

Nutrient Agar plates were used to screen for contamination. The plating procedure was conducted as above at dilutions 10^{-2} and 10^{-5}

3.5. Effect of Colibacillinum on *E.coli*

Nutrient Broth was dispensed in 45 ml aliquots, into 12 Erlenmeyer flasks and sterilised as described above. Haemolyzed horse blood (5%) was added.

An aliquot of 0.5ml of sterile distilled water was added to each of 2 flasks. This formed the control group. Five further series, each consisting of 2 flasks, were prepared with the addition of 0.5 ml of Colibacillinum at potencies of 5CH, 7CH, 9CH, 12CH and 15CH. The Colibacillinum was prepared from the *E.coli* strain used in this experiment, according to the laws of homoeopathy,by a qualified homoeopath and pharmacist. Distilled water was used as the solvent.

The flasks in both the control and test groups were inoculated with a suspension of *E.coli* prepared as described above. Samples from both flasks in the control group were assayed immediately in order to determine initial cell counts (T_0). Further samples were then removed from the control and test series and viable cell numbers determined as described in 3.3. at 6, 12, 24 and 48 hours.

3.6. Scanning Electron Microscopy

Scanning electron microscopy was conducted on samples removed from the control and test series at 24 hours. See Appendix 1 for preparation of samples.

3.7. Processing of Data

From the growth curves obtained, estimations of growth rate, specific growth rate, maximum cell population reached and length of lag phase were computed (Appendix 4).

CHAPTER FOUR

4.0. RESULTS

4.1. The Effect of Co-trimoxazole on *E.coli*

Growth of *E.coli* in the presence of co-trimoxazole was reduced, compared with the growth in the absence of co-trimoxazole (Figure 1).

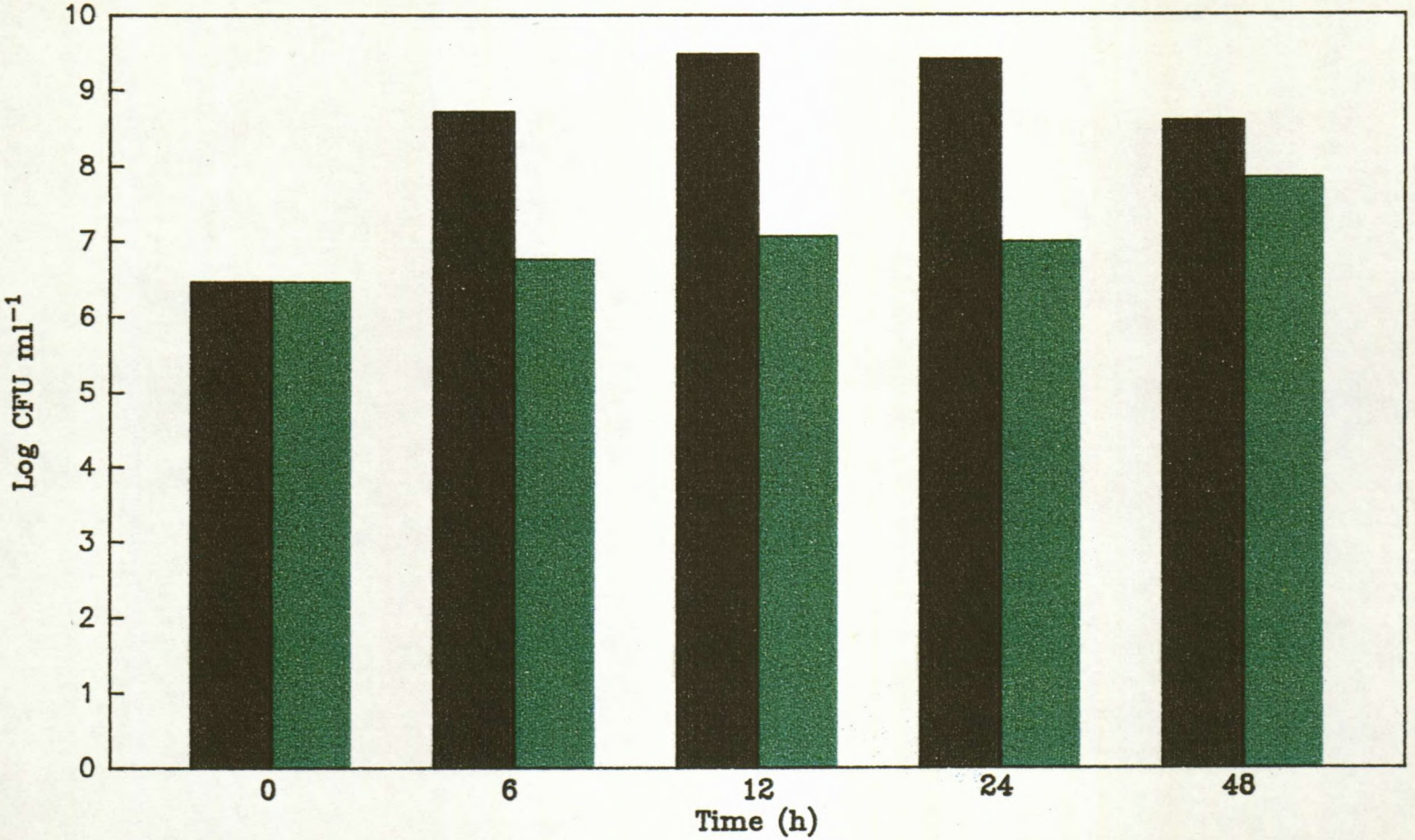


Fig. 1. Effect of Co-trimoxazole on E. coli, control ■, cotrimoxazole ■

4.2. The Effect of Colibacillinum on *E.coli*

The *E.coli* was exposed to various potencies of Colibacillinum over a period of 48 hours. No definitive trend was evident amongst the different potencies. The results are presented in Figure 2.

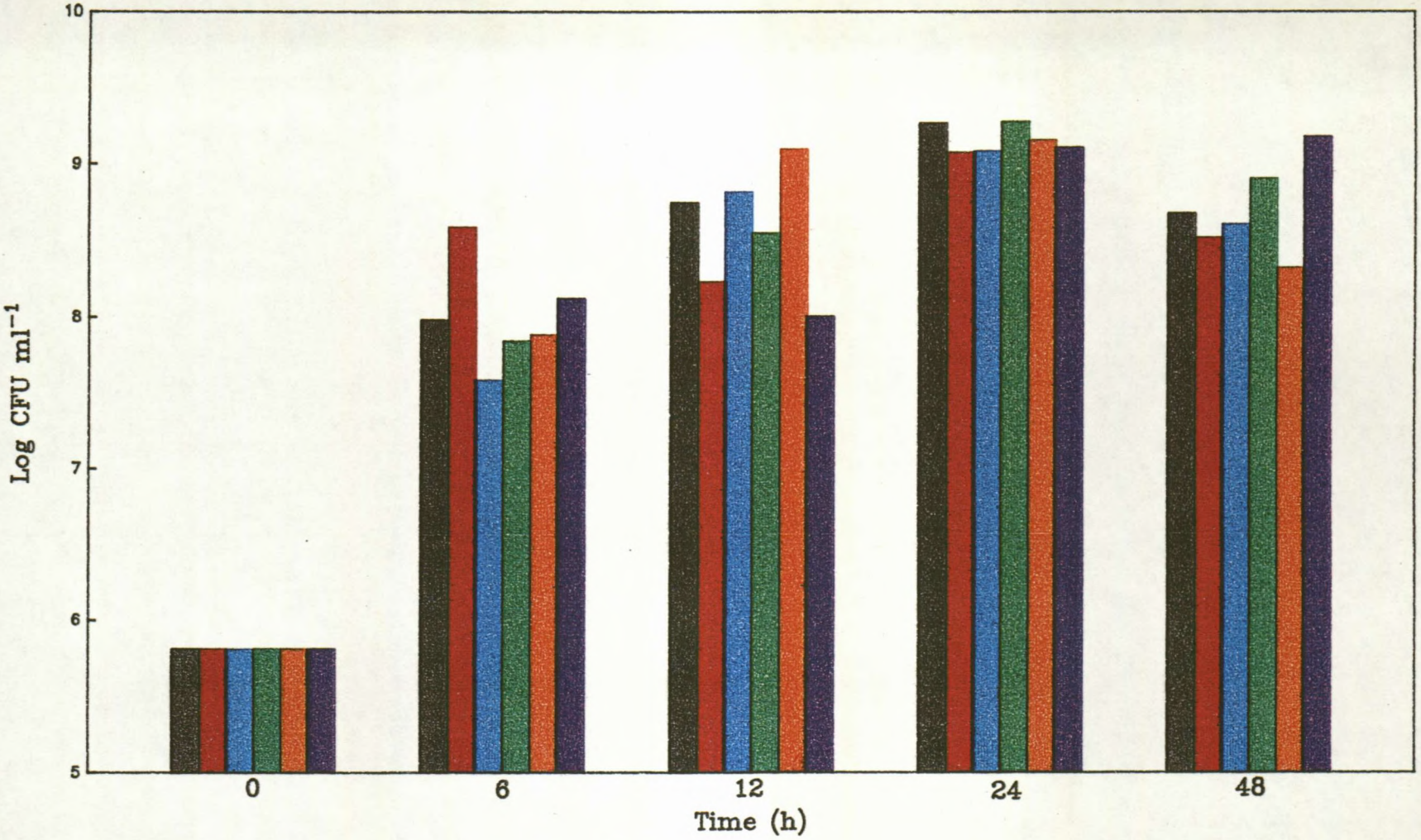


Fig. 2. Effect of Colibacillinum on E. coli, control, colibacillinum 5CH, 7CH, 9CH, 12CH, 15CH

4.3. The Effect of Co-trimoxazole and Colibacillinum on the specific growth rates, maximum population achieved and the time taken to reach the maximum specific growth rates of *E.coli.*

In the presence of co-trimoxazole the maximum population was depressed by 97% when compared to the control. Colibacillinum 7CH was found to be the most effective potency, depressing the maximum population by 41.5% while Colibacillinum 5CH was the least effective potency retarding the maximum population by 7.3%.

The maximum specific growth rate of *E.coli*, in the presence of co-trimoxazole, was depressed by 67.33% when compared to the control. An increase in the maximum specific growth rate of *E.coli* resulted when exposed to various potencies of Colibacillinum. The results are presented in Table 1.

Table 1: Growth parameters of *E.coli*.

	max. specific growth rate	% difference in max. population relative to control	time(hrs) taken to reach max. specific growth rate
Control 1 (co-trimoxazole)	0.329		6
co-trimoxazole	0.108 (-67.33%)	97%	12
Colibacillinum 5CH	0.332 (+1.89%)	7.30%	6
Colibacillinum 7CH	0.327 (+0.609%)	41.50%	6
Colibacillinum 9CH	0.326 (+0.026%)	16%	6
Colibacillinum 12CH	0.331 (+1.55%)	13%	6
Colibacillinum 15CH	0.330 (+1.44%)	11%	6
Control 2 (Colibacillinum)	0.325		6

( ) indicates the percentage difference in the maximum specific growth rate relative to the appropriate control.
- indicates a decrease when compared to the control
+ indicates an increase when compared to the control

4.4. The effect of co-trimoxazole and Colibacillinum on the cellular morphology of *E.coli.*

In the presence of co-trimoxazole the *E.coli* appeared to be irregular and ellipsoidal in shape (Fig. 3). The bacteria were smaller when compared to the control. The *E.coli* in the control group were rod-shaped and more regular (Fig. 4). When exposed to Colibacillinum 7CH, the *E.coli* appeared regular and rod-shaped, similar to the control (Fig. 5).

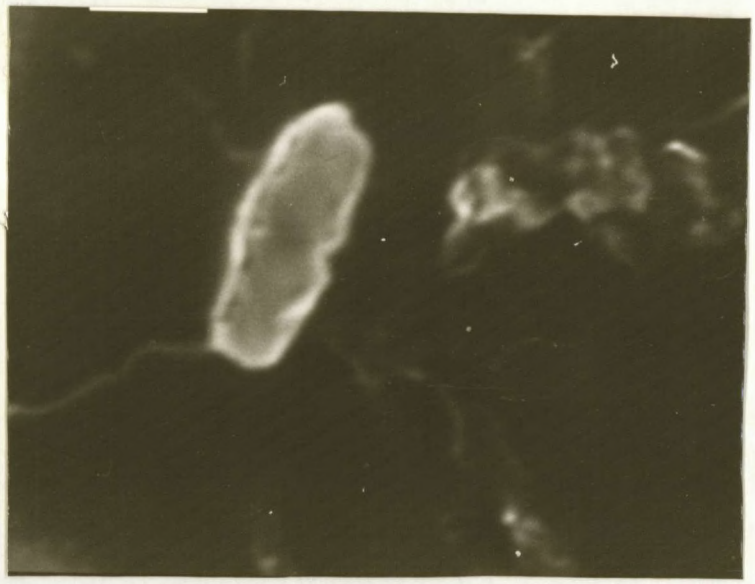


Fig.3. Scanning electron micrograph of*E.coli* in the presence of co-trimoxazole.

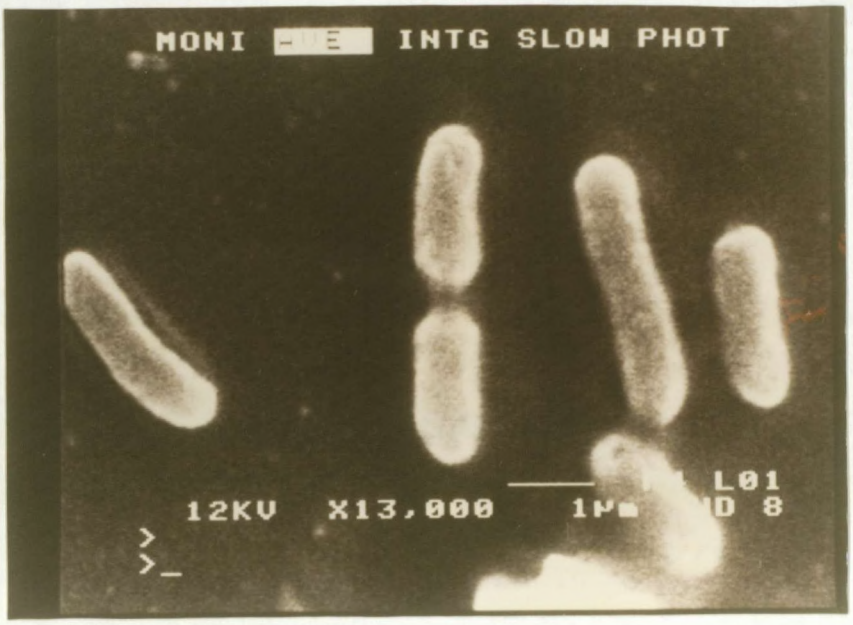


Fig.4. Scanning electron micrograph of *E.coli* in the absence of co-trimoxazole and Colibacillinum.

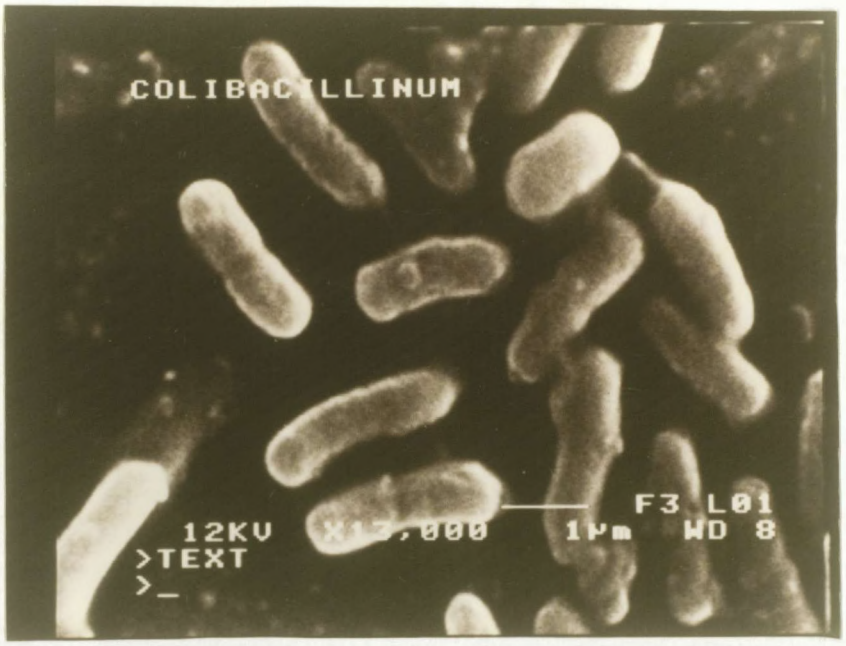


Fig.5. Scanning electron micrograph of *E.coli* in the presence of Colibacillinum 7CH.

CHAPTER FIVE

5.0. DISCUSSION

The method of choice in this investigation was the serial-dilution-agar plating procedure as opposed to the disc diffusion method. The reason being that diffusion tests using either a single disc containing two antibiotics or two discs superimposed are not valid since it has been found that the effect obtained is that produced by the antibiotic producing the wider zone of inhibition when acting alone (Ericson, H. , 1964).

Co-trimoxazole is an effective bactericidal agent (Traunce,1989) but in this study it was found to have a bacteriostatic effect. In a previous study co-trimoxazole was also reported to have had a bacteriostatic effect on *E.coli* (Lewis, et al.1974). This bacteriostatic effect could have resulted for the following reasons. Firstly, it could be attributed to the choice of the concentration of trimethoprim and sulphamethoxazole. The concentrations chosen for this study were the same as those previously used (Lewis, et al. 1974). Secondly, this *E.coli* strain could be resistant to either component of co-trimoxazole. Various reports of resistance of *E.coli* to co-trimoxazole have been cited (Trienekens, et al., 1989; Singh, et al., 1992; Spencer and Cole, 1992).

In the presence of co-trimoxazole, the maximum population was depressed by 97% when compared to the control and the maximum specific growth rate was depressed by 67.33% when compared to the control (Table 1). A marked difference was also noted in the cellular morphology of *E.coli* treated with co-trimoxazole, when compared with the control. There was a decrease in the length of the bacteria and they appeared irregular in shape.

Various potencies (5CH, 7CH, 9CH, 12CH, 15CH) of Colibacillinum were tested against *E. coli.*

Colibacillinum 7CH was found to be the most effective potency, depressing the maximum population by 41.5% when compared to the control while Colibacillinum 5CH was the least effective potency, depressing the maximum population by 7.3% when compared to the control (Table1). An inverse relationship between the potency of Colibacillinum and its efficacy in depressing the maximum population was noted. However this was only true for potencies 7CH, 9CH, 12CH and 15CH.

The maximum specific growth rate for the various potencies of Colibacillinum increased, when compared to the control. With an increase in potency there was an increase in the maximum specific growth rate. Colibacillinum 7CH was found to be the most effective potency and Colibacillinum 5CH the least effective potency. Furthermore a significant differrence in the cellular morphology of *E.coli*, when treated with Colibacillinum 7CH, could not be noted.

7CH is considered a medium potency and is thought to have an effect on the metabolic processes in the body. Colibacillinum 7CH may also have an effect on the metabolic processes of *E.coli.* However information regarding the mechanism of action of potencies could not be cited.

As stated previously co-trimoxazole reduced the maximum population by 97%, and Colibacillinum 7CH reduced the maximum population by 41.5% relative to the maximum population of the control. Similarly the maximum specific growth rate of co-trimoxazole was depressed by 67.33% and that of Colibacillinum 7CH increased by 0.609% when compared to the maximum specific growth rate of the control.Although Colibacillinum 7CH has depressed the maximum population significantly, the depression of the maximum population by co-trimoxazole is far superior in vitro, a difference of 55,5%. This difference suggests

that, when compared to Colibacillinum, co-trimoxazole is the treatment of choice in *E.coli* infections.

In summary, the findings of this study have shown that :

a) co-trimoxazole portrayed a bacteriostatic effect on the growth of *E.coli*,
b) co-trimoxazole was superior to Colibacillinum as an antibacterial agent,
c) significant changes in the cellular morphology of *E.coli*, treated with co-trimoxazole, was noted and
d) the most effective potency of Colibacillinum was found to be 7CH.

It is, therefore, suggested that a more comprehensive study including in vivo application of Colibacillinum be conducted as the homoeopathic preparation may have an effect on the human immune system.

REFERENCES

Cappucino, J. G ., Sherman , N . 1992. Microbiology : a laboratory manual. 3rd edition. New York : Benjamin /Cummings.

Duguid, T.P., Marmian, B.P. and Swain, R.H.A. 1978. Medical Microbiology. 13th edition. London: Churchill Livingstone.

Edwards, C.R.W, and Bouchier, I.A.D. 1991. Davidsons principles and practices of medicine. London: Churchill Livingstone.

Evans, A.S. and Brachman, P.S. 1991. Bacterial infections of humans. 2nd edition . New York: Plenum.

Gilman, A.G., Goodman, L.S., Rall, T.W. and Murad, F. 1985. The Pharmacological Basis of Therapeutics. 7th edition. New York: Macmillan.

Ghosh, A.R., Nair, G.B., Naik, T.N., Paul, M., Pal, S.C., and Sen, D.1991. Enteroadherent *Escherichia coli* is an important diarrhoeogenic agent in infants aged below 6 months in Calcutta, India. Journal of medical microbiology. 36:264-268.

Hawker, L. E. and Linton, A. E. 1979. Micro-organisms function, form and enviroment. 2nd edition. London : Edward Arnold Publishers.

Hitotsubashi,S., Fujii,Y., Yamanaka, H., and Okamato, K.1992. Some properties of purified *E. coli* heat-stable enterotoxin ii. Infection and Immunity. 60(11):4468-4474.

Jamison, J.R.1984. Man Meets Microbe. Durban: Butterworths.

Julian, O.A.1982. Materia Medica of the Nosodes. New Delhi: Jain.

Knutton, S. ,Show, R.K. , Bhan, M.K. , Smith, H.R., McConnall, M.M., Cheasty, T., Williams, P.H., and Baldwin, T.J.1992. Ability of Enteroaggregative *E.coli* strains to adhere in vitro to human intestinal mucosa. Infection and Immunity. 60(5):2083-2091.

Kuchler, R. and Koch, U. J. 1973. The in vitro effect of the Efficacy of Trimethoprim as an Antibacterial agent in a comparative bacteriological study on the effects of

Trimethoprim, Sulfamethoxazole and the combination Trimethoprim/Sulfamethoxazole.Chemotherapy. 18:242-252.

Lewis, E.L. ,Anderson, T.D. and Lacey, R.W. 1974. A reappraisal of the antibacterial action of co-trimoxazole in vitro. Journal of Clinical Pathology. 27:87-91.

Lim, Y.S., Nigan, C.C.L., Tay, L.1992. Enteropathogenic *Escherichia coli* as a cause of diarrhoea among children in Singapore. Journal of Tropical Medicine and Hygiene. 95:339-342.

Neethling, D. C. 1972. The physiology of the production of cyclopiazonic acid by *Penicillium cyclopium* westling. Cape Town. Thesis (Ph.D.) - U.C.T.

Peterson, E.E., Wingen, F., Fairchild, K.L., Halfhide, A., Hendrischk, A., Links, M., Schad,M., Scholtz, H.R., Schurmann, N., Siegmann, S., Yassin, A.J.1990. Single dose pefloxacin compared with multiple dose co-trimoxazole in cystitis. Journal of antimicrobial chemotherapy. 26, suppl.B:147-152.

Renn, R.G. 1977. Pharmacology. 2nd edition. New York: Macmillan.

Singh, M., Choudhry, M.A, Yadava, T.N.S., and Sanyal, S.C.1992. The spectrum of antibiotic resistance in human and veterinary isolates of *Escherichia coli* collected from 1984-1986 in Northern India. Journal of Antimicrobial Chemotherapy. 29:159-169.

Spencer, R.C. and Cole, T.P.1992. Ofloxacin versus trimethoprim and co-trimoxazole in the treatment of uncomplicated urinary tract infections in general practice. British Journal of Clinical Practice .46(1):30-33.

Trauce, J.R.1989. Clinical Pharmacology for Nurses. New York: Churchill Livingstone.

Trienekens, T.A.M., Stobberingh, E.E., Winkens, R.A.G.and Houben, A.W.1989. Different lengths of treatment with co-trimoxazole for acute uncomplicated urinary tract infections in women. British Medical Journal. 229:1319-1321.

Turk, D.C., Porter, I.A. 1978. A short textbook of Medical Microbiology. 4th edition. London: Hodder and Stoughton.

Wade, A.1977. Martindale, The Extra Pharmacopoeia. 27th edition. London. The Pharmaceutical Press.

Zinsser, H. and Bayne-Jones, S.1939. Textbook of bacteriology. London: D-Appleton-Century.

APPENDICES

APPENDIX 1

Preparation of bacteria for S.E.M.

1. Samples were removed from the appropriate test tubes at 24 hours.
2. Each sample (1ml) was centrifuged at 3000 rpm for 5 mins.
3. Supernatants were decanted and cells were resuspended in 1ml of 3% gluteraldehyde (25% stock gluteraldehyde was diluted in 0.1 M phosphate buffer in the ratio 1:8.33).
4. Cells were fixed for 30 mins on ice. Thereafter cells were washed in 0.1 M phosphate buffer and recentrifuged at 3000 x g for 5 mins.
5. Cells were then resuspended in 1ml of distilled water. A drop of this suspension was placed on a glass cover slip and allowed to dry at room temperature.
6. A cover slip was mounted onto a brass stub with nail varnish and sputter-coated with gold in a Polaron coating unit E5000.
7. Cells were viewed using a S.E.M. (Philips)

APPENDIX 2

Effect of co-trimoxazole on *E.coli*

SAMPLING TIME	SAMPLE TYPE	CFU/ML	LOG CFU/ML	MEAN LOG CFU/ML	STANDARD DEVIATION
0	Control 1	2.19E+06	6.34044411	6.46	0.17
		2.20E+06	6.34242268		
	Control 2	3.00E+06	6.47712125		
		5.00E+06	6.69897		
6	Control 1	6.00E+08	8.77815125	8.71	0.14
		7.30E+08	8.86332286		
	Control 2	3.50E+08	8.54406804		
		4.50E+08	8.65321251		
	Antibiotic 1	7.40E+06	6.86923172	6.76	0.16
		6.90E+06	6.83884909		
	Antibiotic 2	3.83E+06	6.58319877		
12	Control 1	3.50E+09	9.54406804	9.47	0.07
		2.63E+09	9.41995575		
	Control 2	3.30E+09	9.51851394		
		2.45E+09	9.38916608		
	Antibiotic 1	1.33E+07	7.12385164	7.06	0.12
		1.56E+07	7.1931246		
	Antibiotic 2	8.30E+06	6.91907809		
		1.00E+07	7		

SAMPLING TIME	SAMPLE TYPE	CFU/ML	LOG CFU/ML	MEAN LOG CFU/ML	STANDARD DEVIATION
24	Control 1	2.65E+09	9.42324587	9.4	0.07
		3.10E+09	9.49136169		
	Control 2	2.20E+09	9.34242268		
		2.26E+09	9.35410844		
	Antibiotic 1	7.80E+06	6.8920946	6.99	0.08
		9.00E+06	6.95424251		
	Antibiotic 2	1.09E+07	7.0374265		
		1.17E+07	7.06818586		
48	Control 1	4.60E+08	8.66275783	8.59	0.11
		2.90E+08	8.462398		
	Control 2	3.50E+08	8.54406804		
		4.90E+08	8.69019608		
	Antibiotic 1	7.70E+07	7.88649073	7.84	0.23
		1.08E+08	8.03342376		
	Antibiotic 2	3.86E+07	7.5865873		

APPENDIX 3

Effect of Colibacillinum on *E.coli*

SAMPLING TIME	SAMPLE TYPE	HOMOEOPATHIC POTENCIES (CH*)	CFU/ML	LOG CFU/ML	MEAN LOG CFU/ML	STANDARD DEVIATION
0	Control 1		6.70E+05	5.8260748	5.81	0.02
			6.10E+05	5.78532984		
	Control 2		6.60E+05	5.81954394		
			6.30E+05	5.79934055		
6	Control 1		9.40E+07	7.97312785	7.54	0.50
			9.50E+07	7.97772361		
	Control 2		1.08E+07	7.03342376		
			1.56E+07	7.1931246		
	Colibacillinum	5CH	3.40E+08	8.53147892	8.24	0.40
			4.40E+08	8.64345268		
	Colibacillinum	5CH	7.00E+07	7.84509804		
			9.00E+07	7.95424251		
	Colibacillinum	7CH	3.20E+07	7.50514998	7.80	0.26
			4.60E+07	7.66275783		
	Colibacillinum	7CH	9.10E+07	7.95904139		
			1.20E+08	8.07918125		
	Colibacillinum	9CH	6.00E+07	7.77815125	7.70	0.21
			8.10E+07	7.90848502		
	Colibacillinum	9CH	5.10E+07	7.70757018		
			2.60E+07	7.41497335		

SAMPLING TIME	SAMPLE TYPE	HOMOEOPATHIC POTENCIES (CH*)	CFU/ML	LOG CFU/ML	MEAN LOG CFU/ML	STANDARD DEVIATION
	Colibacillinum	12CH	9.60E+07	7.98227123	8.11	0.29
			5.90E+07	7.77085201		
	Colibacillinum	12CH	2.24E+08	8.35024802		
			2.25E+08	8.35218252		
	Colibacillinum	15CH	1.39E+08	8.1430148	8.13	0.05
			1.27E+08	8.10380372		
	Colibacillinum	15CH	1.52E+08	8.18184359		
			1.20E+08	8.07918125		
12	Control 1		6.00E+08	8.77815125	8.75	0.04
			5.30E+08	8.72427587		
	Colibacillinum	5CH	1.50E+08	8.17609126	8.23	0.07
			1.90E+08	8.2787536		
	Colibacillinum	7CH	5.30E+08	8.72427587	8.82	0.14
			8.30E+08	8.91907809		
	Colibacillinum	9CH	5.00E+08	8.69897	8.55	0.21
			2.50E+08	8.39794001		
	Colibacillinum	12CH	1.27E+09	9.10380372	9.09	0.01
			1.21E+09	9.08278537		
	Colibacillinum	15CH	4.90E+07	7.69019608	8.00	0.44
			2.06E+08	8.31386722		

SAMPLING TIME	SAMPLE TYPE	HOMOEOPATHIC POTENCIES (CH*)	CFU/ML	LOG CFU/ML	MEAN LOG CFU/ML	STANDARD DEVIATION
24	Control 1		2.00E+09	9.30103	9.16	0.12
			1.68E+09	9.22530928		
	Control 2		1.25E+09	9.09691001		
			1.07E+09	9.02938378		
	Colibacillinum	5CH	1.17E+09	9.06818586	9.14	0.08
			1.17E+09	9.06818586		
	Colibacillinum	5CH	1.62E+09	9.20951501		
			1.58E+09	9.19865709		
	Colibacillinum	7CH	1.04E+09	9.01703334	8.91	0.21
			1.38E+09	9.13987909		
	Colibacillinum	7CH	6.20E+08	8.79239169		
			4.70E+08	8.67209786		
	Colibacillinum	9CH	1.91E+09	9.28103337	9.04	0.27
			1.82E+09	9.26007139		
	Colibacillinum	9CH	7.00E+08	8.84509804		
			5.90E+08	8.77085201		
	Colibacillinum	12CH	1.40E+09	9.14612804	9.11	0.05
			1.44E+09	9.15836249		
	Colibacillinum	12CH	1.15E+09	9.06069784		
			1.19E+09	9.07554696		
	Colibacillinum	15CH	1.16E+09	9.06445799	9.08	0.06
			1.37E+09	9.13672057		
	Colibacillinum	15CH	1.03E+09	9.01283722		
			1.33E+09	9.12385164		

SAMPLING TIME	SAMPLE TYPE	HOMOEOPATHIC POTENCIES (CH*)	CFU/ML	LOG CFU/ML	MEAN LOG CFU/ML	STANDARD DEVIATION
48	Control 1		4.20E+08	8.62324929	8.93	0.30
			5.40E+08	8.73239376		
	Control 2		1.44E+09	9.15836249		
			1.65E+09	9.21748394		
	Colibacillinum	5CH	3.00E+08	8.47712125	8.41	0.13
			3.60E+08	8.5563025		
	Colibacillinum	5CH	1.88E+08	8.27415785		
			2.17E+08	8.33645973		
	Colibacillinum	7CH	3.90E+08	8.59106461	8.75	0.17
			4.30E+08	8.63346846		
	Colibacillinum	7CH	8.10E+08	8.90848502		
			7.70E+08	8.88649073		
	Colibacillinum	9CH	7.70E+08	8.88649073	8.70	0.23
			8.10E+08	8.90848502		
	Colibacillinum	9CH	3.07E+08	8.48713838		
			3.33E+08	8.52244423		
	Colibacillinum	12CH	2.30E+08	8.36172784	8.51	0.23
			1.89E+08	8.2764618		
	Colibacillinum	12CH	4.70E+08	8.67209786		
			5.50E+08	8.74036269		
	Colibacillinum	15CH	1.54E+09	9.18752072	9.12	0.06
			1.42E+09	9.15228834		
	Colibacillinum	15CH	1.24E+09	9.09342169		
			1.16E+09	9.06445799		

* Centisimal Hahnemanian potencies

APPENDIX 4

SAMPLE TYPE	CELL DENSITY (CD)	TIME (T)	GROWTH RATE*	SPECIFIC GROWTH RATE†
Control	3.10E+06	0	88316666.7	0.32947833
	5.33E+08	6	406166667	0.23189647
	2.97E+09	12	-35000000	-0.0126812
	2.55E+09	24	-89666667	-0.0608322
	3.98E+08	48		
Antibiotic	3.10E+06	0	490000	0.10722101
	6.04E+06	6	960000	0.10762332
	1.18E+07	12	-162500	-0.0150115
	9.85E+06	24	2693750	0.06387078
	7.45E+07	48		
Control	6.43E+05	0	8876166.67	0.32547409
	5.39E+07	6	85183333.3	0.27527333
	5.65E+08	12	77916666.7	0.07546408
	1.50E+09	24	-20416667	-0.0162683
	1.01E+09	48		
C5	6.43E+05	0	39059500	0.3315142
	2.35E+08	6	-10833333	-0.0534979
	1.70E+08	12	101666667	0.13034188
	1.39E+09	24	-46833333	-0.056562
	2.66E+08	48		

C7	6.43E+05	0	11942833.3	0.3274566
	7.23E+07	6	101283333	0.26926315
	6.80E+08	12	16500000	0.021181
	8.78E+08	24	-11583333	-0.0156743
	6.00E+08	48		
C9	6.43E+05	0	8976166.67	0.32555961
	5.45E+07	6	53416666.7	0.24873884
	3.75E+08	12	73750000	0.09021407
	1.26E+09	24	-29375000	-0.0323691
	5.55E+08	48		
C12	6.43E+05	0	25059500	0.33050652
	1.51E+08	6	181500000	0.26096334
	1.24E+09	12	5000000	0.00393701
	1.30E+09	24	-39166667	-0.0471888
	3.60E+08	48		

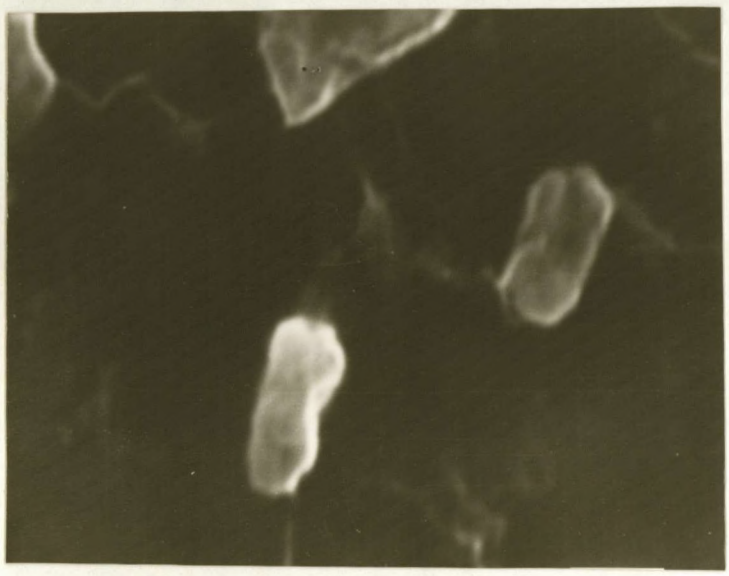
C15	6.43E+05	0	22392833.3	0.33017308
	1.35E+08	6	-1166666.7	-0.008872
	1.28E+08	12	91000000	0.13501484
	1.22E+09	24	5000000	0.00390625
	1.34E+09	48		

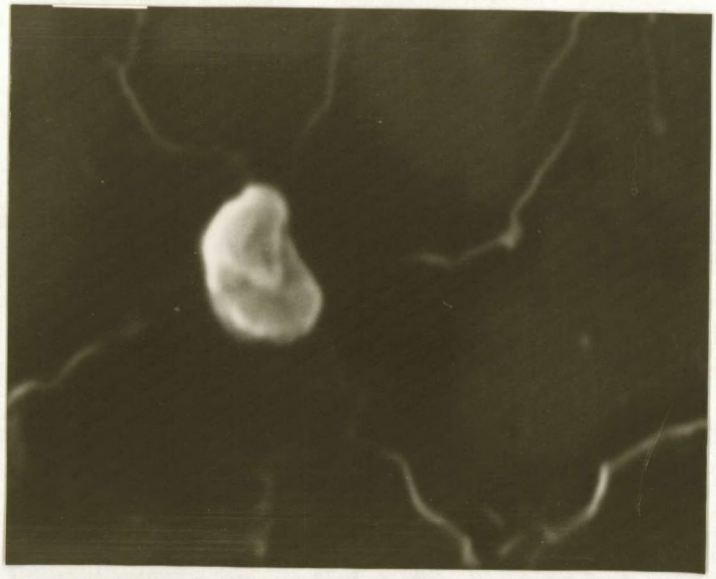
*Growth rate = $\frac{CD2 - CD1}{T2 - T1}$

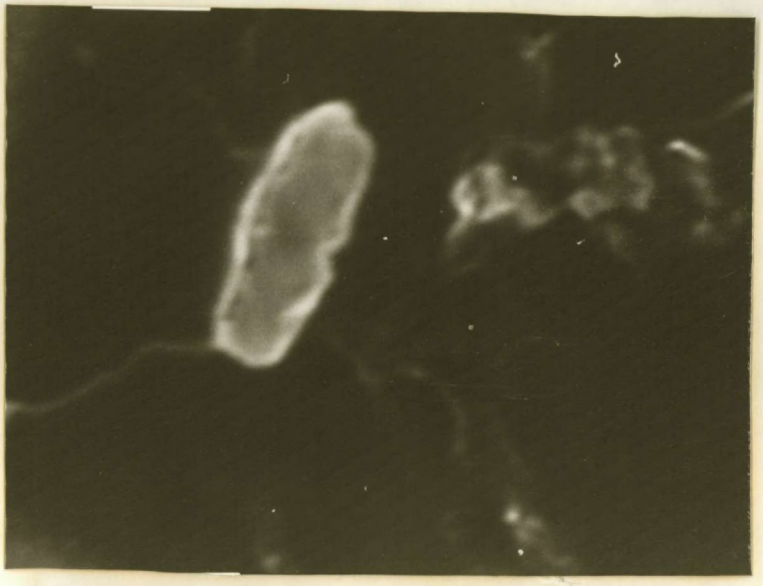
†Specific growth rate = $\frac{\text{growth rate}}{1/2\ (CD2 + CD1)}$

APPENDIX 5

The effect of co-trimoxazole on the cellular morphology of *E.coli*

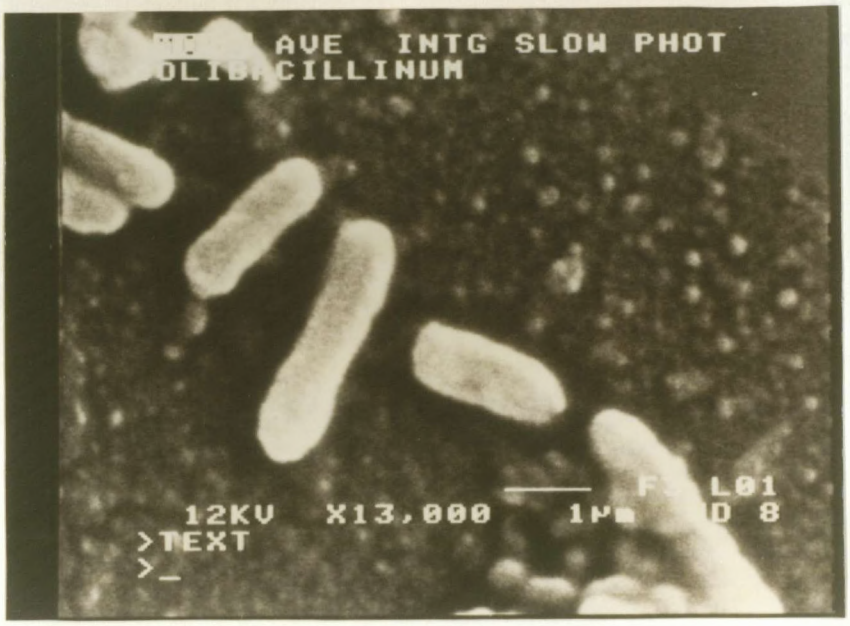


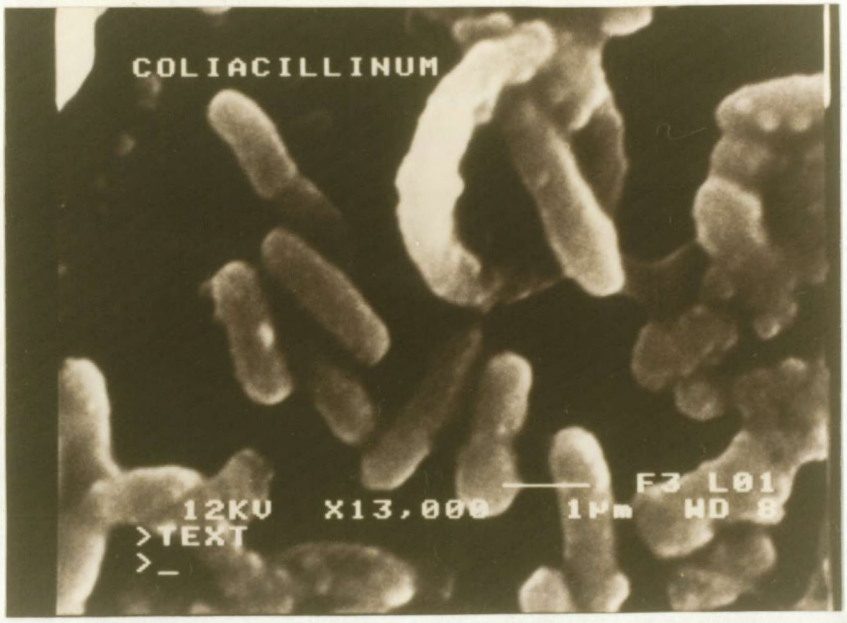


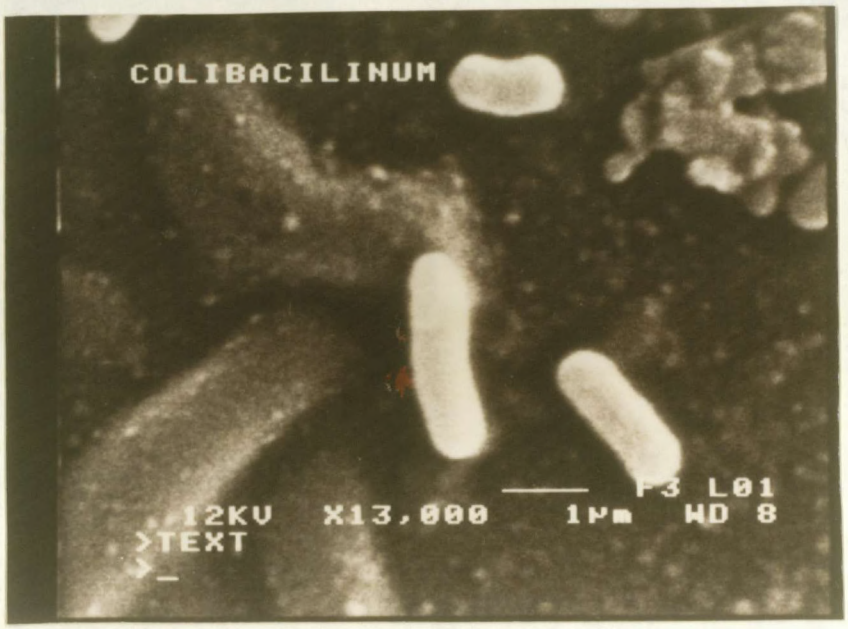


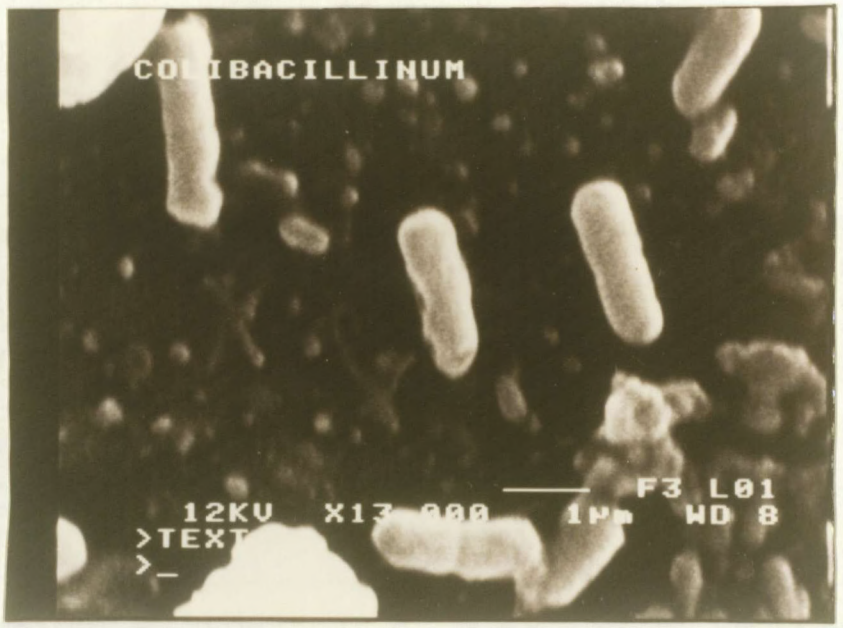
APPENDIX 6

The effect of Colibacillinum 7CH on the cellular morphology of *E.coli*



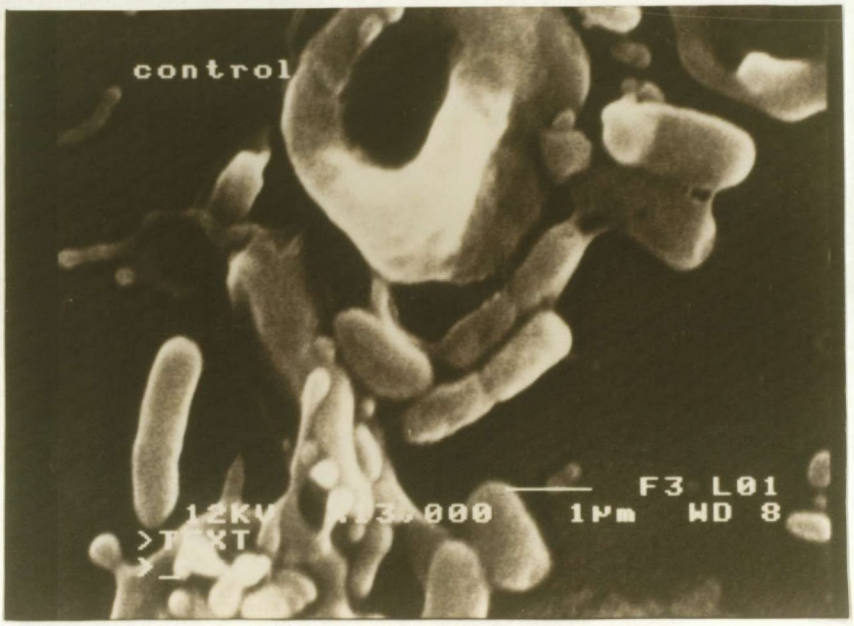


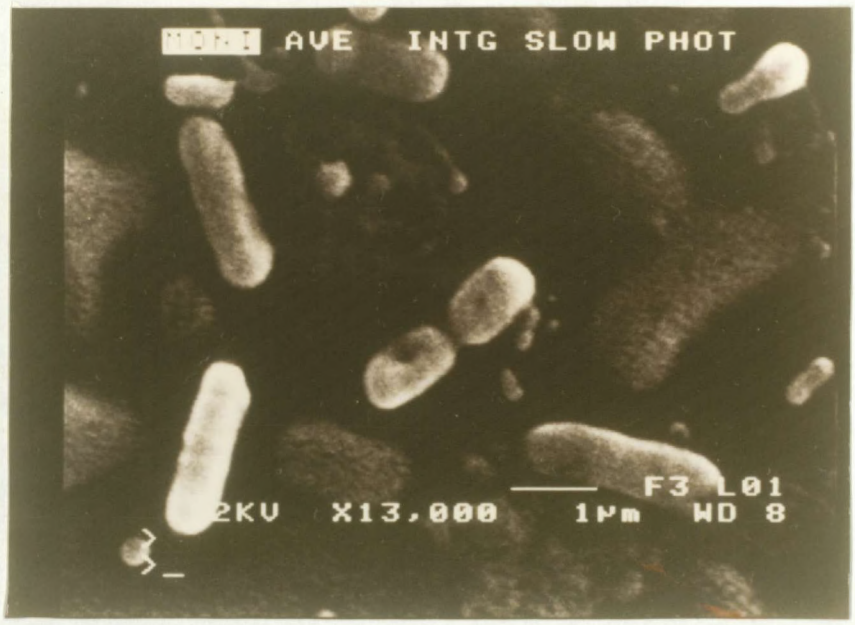

COLIBACILINUM
F3 L01
12KV X13,000 1µm WD 8
>TEXT
>_

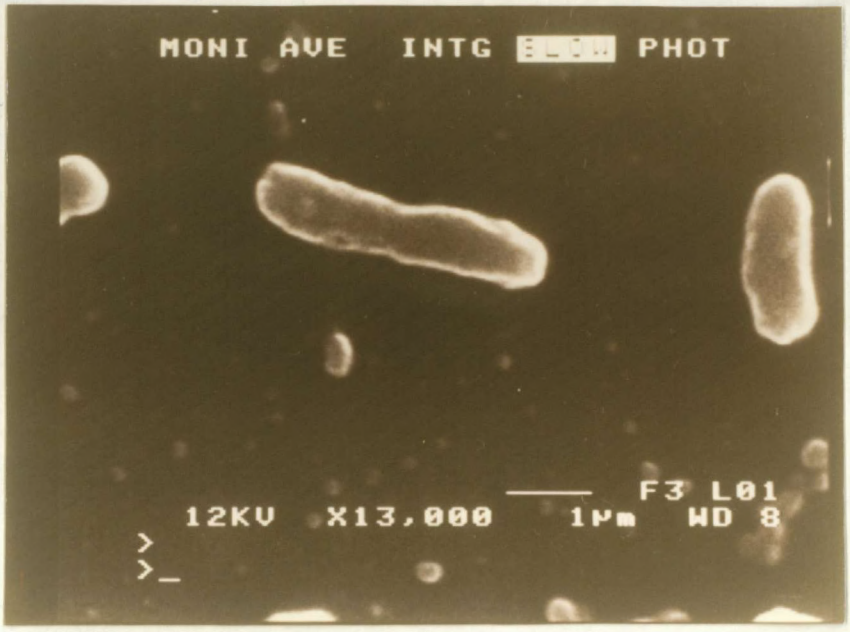

COLIBACILLINUM
F3 L01
12KV X13,000 1µm WD 8
>TEXT
>_

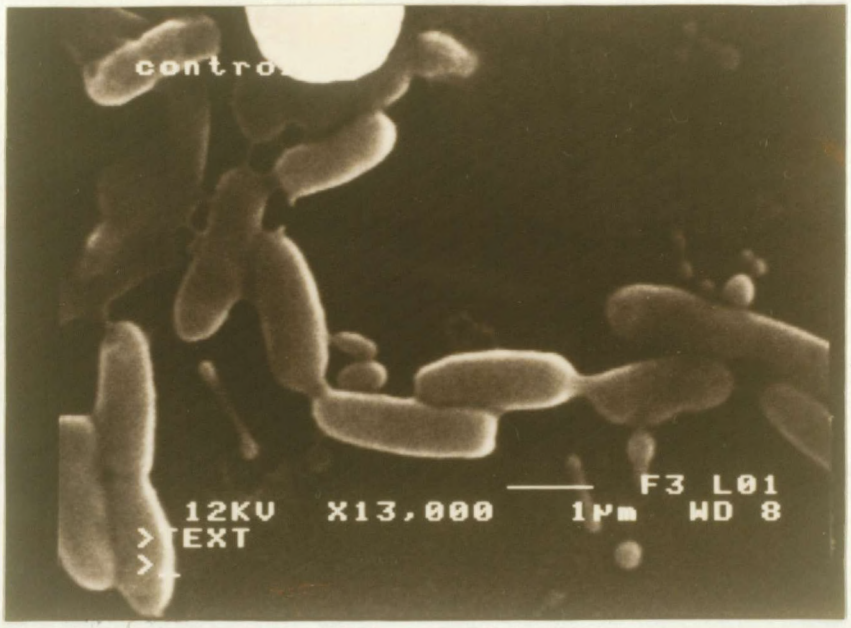
APPENDIX 7

The control group






MONI AVE INTG SLOW PHOT
F3 L01
12KV X13,000 1μm WD 8
>


F3 L01
12KV X13,000 1μm WD 8
>TEXT