**A controlled in vitro study of the antimicrobial effectiveness of *Colibacillinum* against *E. coli***

**BY**

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# DECLARATION

This is to certify that the work is entirely my own and not of any other person, unless explicitly acknowledged (including citation of published and unpublished sources). The work has not previously been submitted in any form to the Durban University of Technology or to any other institution for assessment or for any other purpose.

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# DEDICATION

Someone was passing the word of God in 2014 and read this scripture:

**JOHN 11:41-42 English Standard Version (ESV)**

**“[41] So they took away the stone. And Jesus lifted up his eyes and said, “Father, I thank you that you have heard me. [42] I knew that you always hear me, but I said this on account of the people standing around, that they may believe that you sent me.”** This verse always lingers in my heart; as a result, it got me to this point of surpassing all the obstacles meant to prevent me from reaching my destiny. Without you Father I am nothing. For I know, you know my prayer before I even utter it in secret.

**“FOR THE LORD IS MY SHEPERD AND I SHALL NOT WANT”**

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# ABSTRACT

Title: **A controlled in vitro study of the antimicrobial effectiveness of *Colibacillinum* against *E. coli***

## Background

Due to the rise of antibiotic resistant bacterial infections, alternative methods of treatment need to be explored. Homoeopathic medicine is based on the principle of *'like cures like'* (O'Reilly, 1996) i.e. the symptoms the substance can cause, it can cure which is the underlying rationale for this study. *Colibacillinum* is a remedy prepared from an enteropathogenic strain of *E. coli*, this remedy is already used in clinical practice against chronic cystitis (Leupen, 2010) caused by *E. coli*, however, an extensive literature search has revealed there to be no empirical investigation into its antibiotic capacity. This study aimed to show whether or not this remedy demonstrates antimicrobial capacity against *E. coli* in an *in vitro* setting. Parallell homoeopathic potencies of this remedy and one prepared from a uropathogenic strain of *E. coli* were tested for antimicrobial effects against enteropathogenic and uropathogenic strains of *E. coli in* vitro using the disc diffusion method.

## Aim of the study

The aim of this controlled in vitro study is to determine the antimicrobial effectiveness of parallel potencies of the homoeopathic remedy *Colibacillinum* (manufactured from a uropathogenic strain and enteropathogenic strains respectively) against uropathogenic and enteropathogenic cultures of E. coli *in vitro* by means of the disc diffusion assay method.

## Methodology

Measurements were by means of the disc diffusion essay.

For this experiment thirty Mueller-Hinton plates were prepared and inoculated with each test bacteria in turn. Fifteen plates were inoculated with Uropathogenic strain of *E. coli* and the remaining 15 plates were inoculated with Uropathogenic strain of *E. coli*. A sterile

5mm Whatman® filter paper number 4 discs were individually inoculated with test substances 3CH,9CH,30CH and 200CH potencies and the controls, negative (43% ethanol) and positive control (Ciprofloxacin) using a micropipette, before being allowed to dry in the incubator.

A Ciprofloxacin antibiotic (positive control) was included in the experiment with sole purpose of accounting for plate-plate variations in the pharmacological sensitivity of the same specie of bacteria.

The plates were incubated at 37°C, and the zones of inhibition measured with a pair of Vernier calipers at 24 hours.

Data entry was done using the SPSS statistical package. ANOVA was used to compare the differences between the test and control groups, Mauchly's Test of Sphericity for Uropathogenic prepared strain, Mauchly's Test of Sphericity for Enteropathogenic prepared strain, Normality test.

## Results

The results obtained from this study showed that the Homoeopathic remedy *Colibacillinum* prepared from both Uropathogenic and Enteropathogenic strains displayed inhibitory effects against Enteropathogenic and Uropathogenic strains of *E. coli,* and exhibited statistically significance. The control group (ciprofloxacin) had the highest inhibitory effect (42.3±0.58mm) against Enteropathogenic and Uropathogenic E. coli, while the negative control (43% ethanol) had the lowest inhibitory effect (0.67±1.15mm). *Colibacillinun* 200CH prepared from a Uropathogenic strain of *E-coli (Coli-b_U 200CH)* displayed statistically significant antimicrobial effects against uropathogenic *E.coli;* such antimicrobial effects were significantly greater than 43% ethanol (negative control); the antimicrobial effect was however inferior to Ciprofloxacin (positive control). *Colibacillinum* 9CH prepared from Enteropathogenic strain of *E-coli (Coli-b_E 9CH)* also displayed statistically significant antimicrobial effects against enteropathogenic *E.coli* which were significantly greater than 43% ethanol (negative control) but inferior to Ciprofloxacin.

## Conclusion

This study concluded that Colibacillinum prepared from Uropathogenic and Enteropathogenic strains of *E. coli,* are effective in inhibiting the *in vitro* growth of *E.coli* when evaluated by means of disc diffusion. The study further confirmed that the biological (anti-microbial) activity of an ultra-high homoeopathic dilution (*Coli-b_U* 200CH) (1:$10^{400}$) and in the case of *Coli-b_U* the findings support existing literature which suggests that the anti-microbial properties of homeopathic nosodes increase with potency; all hypotheses for this remedy were thus accepted.

This trend was not noted for *Coli-b_E* in which the potency with the greatest anti-microbial effect was the 9CH, thus *Colibacillinum* prepared from Enteropathogenic strain (*Coli-b_E)* did not conform with hypothesies one, two and four that were proposed in chapter one. Despite this the confirmation of significant antimicrobial effects of a substance at this level of deconcentration (1:$10^{18}$) is noteworthy.

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# LIST OF ACRONYMS AND ABBREVIATION

1. **Coli-b_U-** Colibacillinum prepared from Uropathogenic strain of E. coli.
2. **Coli-b_E-** Colibacillinum prepared from Enteropathogenic strain of E. coli.
3. **E. coli-** Escherichia coli.
4. **UTI-** Urinary Tract Infection.
5. **HIV-** Human Immunodeficiency Virus.
6. **AIDS-** Acquired Immune Deficiency Syndrome.
7. **NCCLS-** National Committee for Clinical Laboratory Standards.
8. **RHDC**- Research and Higher Degree Committee.
9. **CH-** Centesimal Hahnemann.
10. **ROH**- Alcohol (ethanol).
11. **Uro-** Uropathogenic strain.
12. **Entero-** Enteropathogenic strain.
13. **Antib-** Antibiotic

## DEFINATION OF TERMS

**Antimicrobia-** An antimicrobial is an agent that kills microorganisms or stops their growth (Martin, E. A. 2015).

**Cystitis-** Inflammation of the urinary bladder (Beers, M. H. 2005).

**Disc diffusion method-** is a test of the antibiotic sensitivity of bacteria. It uses antibiotic discs to test the extent to which bacteria are affected by those antibiotics (Jorgensen *et al*. 2007).

**Pyelonephritis**- Inflammation of both the parenchyma of a kidney and the lining of its renal pelvis especially due to bacterial infection (Beers, M. H. 2005).

**Septicaemia-** Invasion of the bloodstream by virulent microorganisms (as bacteria, viruses, or fungi) from a focus of infection that is accompanied by acute systemic illness (Beers, M. H. 2005).

**Virulence-** The ability of an agent of infection to produce disease. The virulence of a microorganism is a measure of the severity of the disease it causes (Britannica, E. 2009).

**Nosode-** A nosode is a homoeopathic remedy prepared from an infectious disease product either directly from the bacteria or virus, or less directly from a tissue purported to contain it (Klein, L. 2009).

**Dysentery-** Inflammation of the intestine, with pain, diarrhoea, bloody stools, and mucus (Beers, M. H. 2005).

**Homoeopathy-** A system of therapeutics founded by Samuel Hahnemann (1755–1843) in which diseases are treated by drugs that are capable of producing in healthy persons symptoms like those of the disease to be treated, the drug being administered in minute doses (Sankaran, R. 1991).

**Haemolytic uremic syndrome**- A condition characterized by the breakup of red blood cells and kidney failure. “haemolytic" refers to the breakup of red blood cells (Noris and Remuzzi, 2005).

**Law of Similars-** Every substance which is capable of producing a set of symptoms in healthy human being has the capacity to annihilate them when administered to a sick person with similar set of symptoms (Hahnemann and Dudgeon, 2002).

**Law of Infinitesimals-** States that the serial dilution process increases the curative power of homeopathic medications (as the degree of dilution increase) (Hahnemann and Dudgeon, 2002).

**Potentization-** The preparation of a homoeopathic remedy through the process of serial of dilutions and succussions (Ullman and Reichenberg-Ullman, 1994).

**Materia medica-** "Material of medicine" in Latin. A reference that lists the curative indications and therapeutic actions of homoeopathic medicines (Lilley, 2008).

**Mother Tincture-** An alcoholic or water-alcoholic solution, usually referring to a preparation from herbal materials (Blumenthal, M.1998).

**Potency-** The strength of homoeopathic remedy (Kayne, 2008).

**Succussion-** The system and repeated shaking of a homoeopathic medicine after each serial dilution (Ullman and Reichenberg-Ullman, 1994).

**Avogadro number**-number of units in one mole of any substance (defined as its molecular weight in grams), equal to 6.022140857 × 1023 (Daintith, J. 2008).

**Proving-** homeopathic proving, also called proving trial or pathogenetic trial, a homoeopathically prepared substance is administered to healthy volunteers in order to produce the symptoms specific to that substance and thereby reveal its inherent curative powers (Walach *et al*. 2004).

**German Homoeopathic pharmacopoeia-** The compendium published by the Federal Ministry of Health and Social Security in the Federal Gazette. With monographs and directions for the identification of compound medicines on how to prepare Homoeopathic remedies (Benyunes, 2005).

# CHAPTER ONE

## 1.1 OVERVIEW

*Escherichia coli* (E. coli) has been reported as the major cause of community-acquired urinary tract infection (UTI), affecting mostly women, and has shown resistance to well-known antibiotics (Rogers and Peterson, 2011).

UTIs are among the most common infectious diseases worldwide. Recent studies have reported an increased incidence of antibiotic resistant E. coli, the primary causative agent of UTI. The resistance has emerged even to more potent antimicrobial agents like fluoroquinolone. Fluoroquinolones are preferred as initial agents for empiric therapy because of high bactericidal and clinical cure rates as well as low rates of resistance among uropathogens (Gururaju *et al.* 2015). UTI may vary from mild asymptomatic cystitis to pyelonephritis and septicemia. The commonest bacterial agent involved in causation of UTIs is E. coli; being the principal pathogen both in the community as well as in the hospital environment (Gururaju et al. 2015). Treatment of UTI constitutes a great portion of prescription of antibiotics; urinary pathogens have shown a changed pattern of susceptibility to antibiotics, resulting in an increase in resistance to commonly used antibiotics (Gururaju *et al.* 2015).

Although *E. coli* can be an innocuous resident of the gastrointestinal tract, it also has the pathogenic capacity to cause significant diarrheal and extra-intestinal diseases. Pathogenic variants of E. coli (pathotypes) cause much morbidity and mortality worldwide. Globally, one in ten child deaths result from diarrhoeal disease during the first 5 years of life, resulting in about 800 000 fatalities worldwide annually, most occurring in sub-Saharan Africa and south Asia in this regard diarrhoeal mortality remains unacceptably high (Kotloff *et al.* 2013).

Consequently, pathogenic E. coli outbreaks are common in developed and developing countries, and they sometimes have fatal consequences. Many of these pathotypes (enterogenic and pathogenic strains) are a major public health concern, the seriousness of pathogenic E. coli is exemplified by dedicated national and international surveillance programs that monitor and track outbreaks, unfortunately, this surveillance is often lacking in developing countries (Croxen *et al.* 2013).

The decreasing effectiveness of antibiotics in treating common infections has quickened in recent years, and with the arrival of untreatable strains of carbapenem-resistant and Enterobacteriaceae, healthcare is potentially at the ‘dawn’ of a postantibiotic era (Mathers *et al.* 2015). Resistance arises as a consequence of mutations in microbes and selection pressure from antibiotic use that provides a competitive advantage for mutated strains, resistance genes are borne on chromosomal, and increasingly, on transmissible extrachromosomal elements. The resulting resistant clones—e.g., meticillin-resistant Staphylococcus aureus, Escherichia coli and Klebsiella are disseminated rapidly worldwide (Mathers *et al.* 2015).

Even before penicillin was introduced, resistant strains of bacteria had been detected. The selection pressure caused by the use of millions of tonnes of antibiotics over the past 75 years since antibiotics were introduced has made almost all disease-causing bacteria resistant to antibiotics commonly used to treat them (Mathers *et al.* 2015).

South Africa has the highest prevalence of HIV/AIDS in the world (Aids Foundation, 2014), this has resulted in more people suffering from acute cystitis and more complicated UTI’s such a pyelonephritis and prostatitis, this in the context of increasing drug resistance warrants the exploration of alternative treatments (Rogers and Peterson, 2011).

Homoeopathy has been around for more than 200 years and has shown to be effective in the treatment of various conditions throughout the world (Koithan *et al.* 2015). The Homoeopathic remedy *Colibacillinum* (manufactured from an enteropathic strain of E. coli) is already used in clinical homoeopathic practice against chronic cystitis (Leupen, 2010) and has proved to be effective against accordingly. Some studies have shown that higher the homoeopathic potency the greater the antimicrobial capacity (Joshi *et al.* 2016).

Thus this study sought to investigate the antimicrobial capacity of two forms of homoeopathic *Colibacillinum* in parallel potencies of 3CH, 9CH, 30CH and 200CH; one form, the traditional *Colibacillinum* manufactured from an enteropathogenic strain of *E. Coli* and the second, a novel form of *Collibacillinum* manufactured from a Uropathogenic strain of *E. Coli.*

It was anticipated that should such an *in vitro* study yield positive results it would justify a larger *in vivo* study and possibly lead to an alternative intervention for UTI caused by *E. Coli*. Further evidence supporting the effectiveness of homoeopathic dilutions specifically, ultra-high dilutions against gram positive and gram negative bacteria would support the findings of Invernezzi (2002) and Witt (2007) and the more recent findings of Joshi *et al.* (2016).

Homoeopathic Mycobacterium nosode (Emtact- polyvalent nosode) prepared according to the Indian Homoeopathic Pharmacopoeia in a potency range of 1CH to 30CH determined that the antimicrobial effect was directly proportionate to the level of dilution i.e. as the potency increased (higher dilution) the antimicrobial effect increased (Joshi *et al.* 2016).

De Waard (1995) tested homoeopathic *Staphylococcinum* 5CH, 9CH, 15CH potencies against *S Aureus in* vitro and found that the 15Ch potency did show an effect on the growth parameters of the organism although inferior to the positive control (antibiotic), it was interesting to note that statistical significance was only achieved at the highest potency.

The action of Methicillin on the *in vitro* growth of methicillin-resistant Staphylococcus aureus previously treated with homeopathic dilutions was assessed; Methicillin-resistant Staphylococcus aureus (MRSA) is a known cause of nosocomial infection and is considered as to be a worldwide epidemic. Given the magnitude of the MRSA problem new strategies to fight against MRSA that do not generate resistant strains to antibiotics should be sought. Homoeopathy has been explored as one of these new strategies and may play a pivotal role in the treatment of MRSA; a study was conducted on the action of homeopathy on growth of MRSA bacteria *in vitro*. The results showed a decrease in growth of bacterial strains with homeopathic dilutions of *Belladonna* and the *S. aureus* nosode. The significant changes in the growth was observed and compared to the control (30% alcohol), cultures treated with Belladonna 6cH and the antibiotic in the dilution 4 µg/mL showed a decrease of 40% of the growth, while in the 30cH group the decrease was 75%. Cultures treated with the *S. aureus* nosode 30cH and the antibiotic at 4 µg/mL dilution, showed a decrease of 60% in bacterial growth *in vitro* (Passeti *et al*. 2015) these concur with those of Joshi *et al.*

(2016) i.e. the antimicrobial effect increases with the increase in homoeopathic potency.

## 1.2 AIM OF THE STUDY

The aim of this controlled in vitro study is to determine the antimicrobial effectiveness of parallel potencies of the homoeopathic remedy *Colibacillinum* (manufactured from a uropathogenic strain and enteropathogenic strains respectively) against uropathogenic and enteropathogenic cultures of E. coli *in vitro* by means of the disc diffusion assay method.

## 1.3 OBJECTIVES

### 1.3.1 Objective one

To evaluate the antimicrobial effectiveness of *Colibacillinum* 3CH, 9CH, 30CH, 200CH using the homoepathic principle “like cures like”. The potencies will be manufactured from a uropathogenic strain of *E. coli* and against cultures of uropathogenic and enteropathogenic *E. coli in vitro* in terms of the disc diffusion assay method.

### 1.3.2 Objective two

Using the ‘Law of similars” determine the antimicrobial effectiveness of *Colibacillinum* 3CH, 9CH, 30CH, 200CH manufactured from an enteropathogenic strain of *E. coli* and against cultures of uropathogenic and enteropathogenic E. *coli in vitro* in terms of the disc diffusion assay method.

### 1.3.3 Objective three

To determine the antimicrobial capability of a negative control (43% ethanol) against cultures of uropathogenic and enteropathogenic E. *coli* in terms of the disc diffusion assay method.

### 1.3.4 Objective four

To determine the antimicrobial efficacy of a positive control Ciprofloxacin against cultures of uropathogenic and enteropathogenic E. *coli* in terms of the disc diffusion assay method.

## 1.4 HYPOTHESES

### 1.4.1 Hypothesis one

*Colibacillinum* 3Ch and 9Ch (low potency) prepared from enteropathogenic and uropathogenic strains will have limited antibacterial effects on the *in vitro* growth of enteropathogenic and uropathogenic *E. coli*

### 1.4.2 Hypothesis two

*Colibacillinum* 30Ch and 200Ch (high potency) prepared from enteropathogenic and uropathogenic strains will have significant antibacterial effects on the *in vitro* growth of enteropathogenic and uropathogenic *E. coli*

### 1.4.3 Hypothesis three

*Colibacillinum* prepared from uropathogenic and enteropathogenic strains below 30CH will have limited antibacterial effects compared to positive control (Ciprofloxacin).

### 1.4.4 Hypothesis four

*Colibacillinum* prepared from uropathogenic and enteropathogenic strains above 30CH will have significant antibacterial effects compared to positive control (Ciprofloxacin).

### 1.4.5 Hypothesis five

Parallel potencies of enteropathogenic and uropathogenic *Colibacillinum* will have significant antibacterial effects compared to the negative control (43% ethanol) on the growth of uropathogenic and enteropathogenic strains of *E.Coli.*

### 1.4.6 Hypothesis six

Antibacterial effects of respective forms (enteropathogenic and uropathogenic) of homoeopathic *Colibacillinum* will be greater against corresponding *E. Coli* strains.

## 1.5 DELIMITATIONS

1.5.1 This study was limited to only one specie of bacteria i.e. *E. coli.*

1.5.2 This study was limited to a specific strains of *E. coli* viz. Enteropathogenic and Uropathogenic strains of *E. coli*.

1.5.3 Only Mueller-Hinton agar was used as a media growth.

1.5.4 This was an *in vitro* study; the findings of which should be confirmed *in vivo.*

# CHAPTER 2

## 2.1 History of antibiotics and drug resistance

The chromosomally encoded drug efflux mechanisms that are ubiquitous in gram-negative bacteria contribute to antibiotic resistance and present a major challenge for antibiotic development (Li *et al*. 2015). As a result of this as the years have progressed, bacteria have developed resistance to a number of antibiotics causing diseases that were previously treatable to be become potentially fatal again (World Health Organization, 2014). This global emergence of multidrug-resistant Gram-negative bacteria is a growing threat to antibiotic therapy (Li *et al*. 2015).

During the past 10 years, multidrug-resistant Gram-negative Enterobacteriaceae have become a substantial challenge to infection control. It has been suggested by clinicians that the effectiveness of antibiotics is in such rapid decline that, depending on the pathogen concerned, their future utility can be measured in decades or even years. Unless the rise in antibiotic resistance can be reversed, we can expect to see a substantial rise in incurable infection and fatality in both developed and developing regions (Wellington *et al*. 2013).

There are a number of bacteria which have been found to be commonly resistant to antibiotics many of which are nosocomial (Wright, 2010). *Staphylococcus aureus*, *Pseudomonas aerigenosa, Enterococci faecalis, Klebselia pneumonia* and *Escherichia coli* are some of these bacteria which are found to be commonly bacterial resistant and nosocomial (Guilefoile, 2007).

## 2.2 Escherichia Coli

*E. coli*, the most significant species in the genus Escherichia, was first described by Escherich in 1885. It was first considered to be a harmless member of the colon resident biota (Mahon *et al.* 2014). It is now recognized as an important human pathogen frequently associated with wide range of clinical syndromes, including

urinary tract infections (UTIs), diarrhea diseases, and central nervous system infections (Mahon *et al*. 2014).

## 2.3 Morphology and identification

*E. coli* strains are usually motile and may express fimbriae, they grow well on non-selective media and usually ferment lactose and produce large red colonies on MacConkey agar (Greenwood, 2012). This bacterium has ability to utilize certain sugars (Greenwood, 2012), and on certain selective and differential media such as MAC (MacConkey) or EMB (Eosin methylene blue) agars *E. coli* has a distinctive morphology (Mahon *et al*. 2014). It usually appears as a lactose-positive (pink) colony with a surrounding area of precipitated bile salt on MAC agar and a green metallic sheen on EMB agar (Mahon *et al*. 2014).

## 2.4 Properties of *E. coli*

***E. Coli* is known to exhibit the following typical properties:**

- It is able to ferment glucose, lactose, trehalose, and xylose.
- It produces indole from tryptophan.
- Fermentation of glucose is by mixed acid pathway: methyl red- positive and Voges-Proskauer- negative.
- It does not produce hydrogen sulphide, DNase, urease, or phenylalanine deaminase.
- Cannot use citrate as a sole carbon source

(Mahon *et al*. 2014).

## 2.5 Epidemiology of *E. coli*

This gram-negative rod-shaped member of the coliform group inhabits the intestines and is found in sewage, contaminated water and can contaminate raw food. *E. coli* infections are typically mild however the condition can be serious sometimes fatal in the very young, elderly or immunocompromised patient. Belongs to the Family of enterobacteriaceae (Mangialavori *et al*. 2009).

Infection with *E. coli* often leads to bloody diarrhoea and occasionally to kidney failure. In certain cases, particularly children under 5 years of age and the elderly, the infection can also cause a complication called haemolytic Uremic Syndrome (HUS) in which erythrocytes are destroyed and kidney failure may result (Mangialavori *et al.* 2009).

Although *E. coli* is a commensal of human intestine, it can cause variety of important infections including infections of the gastrointestinal tract, urinary tract, biliary tract, lower respiratory tract, septicaemia, haemolytic-uremic syndrome, haemorrhagic colitis and neonatal meningitis (Elliott *et al*. 1997).

Urinary tract infect occurs more frequently in female than in men, because of the shorter, wider, female urethra appears to be less effective in preventing access of the bacteria to the bladder. High incidence in pregnant women due to impaired urine flow, the prevalence of various serotypes of *E. coli* in urinary tract infections varies with geographical location (Mahon *et al.* 2014).

## 2.6 *E. coli* infection

*E. coli* usually only becomes infectious when it reaches areas outside the intestines, and is known to cause infections such as Urinary tract infection, biliary tract infection tract, haemolytic- uremic syndrome, pyelonephritis, cystitis, lungs, meninges and other areas (Mahon *et al*. 2014). This bacterium is considered to be opportunistic and usually affect infants, elderly people, immunocompromised patients and also patients with terminal diseases and in severely ill patients it is capable of causing septicemia.

*E. coli* may possess lipopolysaccharide endotoxins in their cell walls and may sometimes produce exotoxins of clinical importance (Mahon *et al*. 2014).

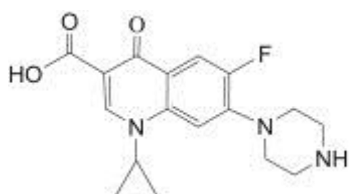
## 2.7 Antimicrobial sensitivity

In the absence of acquired resistance, *E. coli* is susceptible to many antibiotics including ampicillin, cephalosporins, tetracyclines, quilones, aminoglycosides, trimethoprim, and sulphonamides. Many strains however have acquired plasmids conferring resistance to one or more of the above drugs (Mahon *et al.* 2014). Uncomplicated cystitis usually responds to treatment with oral antibiotics such as

trimethoprim or nitrofurantoin but more serious infections require specific antimicrobial therapy (Mahon *et al.* 2014).

## 2.8 Ciprofloxacin

The discovery of ciprofloxacin was a crucial medical breakthrough and it opened the door for any analysis, development, and promoting of recent category of antibiotics. Ciprofloxacin was the primary fluoroquinolone dropped at the market(Forrest, *et al.* 1993). Ciprofloxacin has been extensively studied and its safety profile is well documented in additional than 32,000 publications(Forrest, *et al.* 1993). This antibiotic has been typically effective, and its use has been in the course of comparatively few adverse effects. However, ciprofloxacin as a single agent has exhibited variable clinical success against organisms like staphylococci, streptococcus pneumoniae, and pneumonia specie (Forrest, *et al.* 1993). Improved dosing ways might increase the probabilities of success against these microorganisms. Common pathogens exhibit a good vary of susceptibilities to antibiotic drug (Forrest, *et al.* 1993).




Chemical structure of Ciprofloxacin (El-Kemary, *et al.* 2010)

## 2.9 Strains of *E. coli*

**2.9.1 Uropathogenic** strains are widely recognized as the most common cause of UTIs in humans. It originates from large intestines as a resident biota and can exist either as the predominant *E. coli* population or as a small part of the *E. coli* strain in the large intestines (Mahon *et al.* 2014). The primary virulence factor associated with the ability of *E. coli* to cause UTIs is the production of pili, which allow Uropathogenic strains to adhere to epithelial cells and not be washed out by urine flow (Mahon *et al.* 2014).

**2.9.2 Enteropathogenic** strains usually affect people from tropical countries (Greenwood, 2012).and have been known to cause infantile diarrhoea since the 1940s (Mahon *et al*. 2014). **Enterotoxigenic** strains are a major cause of infant bacterial diarrhoea especially in tropical and subtropical areas in developing countries where it is also known to cause 'traveller's diarrhoea' (Mahon *et al.* 2014).

**2.9.3 Enteroinvasive** strains are rare and is seen less commonly in developing countries than other strains of *E. coli*. It produces dysentery with direct penetration, invasion, and destruction of the intestinal mucosa (Mahon *et al*., 2014). The dysentery is similar to that produced by Shigella spp, in patients of all ages (Greenwood, 2012).

**2.9.4 Enterohemorrhagic** strains were first recognized during an outbreak of haemorrhagic diarrhoea and colitis. Also associated with haemolytic uremic syndrome, producing a watery diarrhoea that progresses to bloody diarrhoea with abdominal cramps and possible fever (Mahon *et al*. 2014).

## 2.10 Homoeopathy

### 2.10.1 Law of Similars

The homoeopathic system of medicine is based on the SIMILIA SIMILIBUS CURENTUR which means "let like be cured by likes", every substance which is capable of producing a set of symptoms in healthy human being has the capacity to annihilate them when administered to a sick person with similar set of symptoms (Vithoulkas, 2002).

### 2.10.2 Isopathy

One of the earliest and most remarkable developments of homeopathy, mentioned even in the later releases of the Organon, is isopathy or isotherapy (Bellavite *et al*. 2005). The term was most likely authored by the veterinarian Wilhelm Lux something close to 1831– 33, subsequent to beginning to treat his animals with the homeopathic technique, he ended up persuaded that each infectious malady bears inside itself the methods whereby it tends to be the cure (Bellavite *et al*. 2005). He saw that the

technique of dilution and dynamization of an infectious substance (bacterium, infection or tainted discharges, and natural material) would set such a substance in a place to apply a helpful activity on the illness coming about because of the disease. The law of similars 'Similia similibus curentur' hence moves toward becoming 'Aequalia aequalibus curentur' or the law of sameness. Three creators overwhelm the historical background of Isopathy, and every one of the three were homeopaths: Constantine Hering, Wilhelm Lux and Denys Collet (Bellavite *et al*. 2005).

### 2.10.3 Homeopathy's "Law of Infinitesimals"

Homeopathic remedies are prepared through a process called potentization. This involves a series of systematic dilutions and succussions (vigorous shaking) These actions eliminate chemical toxicity and enable the therapeutic effect. Homeopathic potencies are designated by a combination of a number and a letter (for example, 3X or 3C). The number refers to the number of dilutions that the tincture has undergone in series to prepare the remedy. The letter refers to the proportions (scale) used in each dilution of the series (the Roman Numeral X means 10, and the Roman Numeral C means 100) and the number of succussions that the vial of solution undergoes at each successive stage (Sagar, 2007).

The law of infinitesimals in homeopathy states that the serial dilution process increases the curative power of homeopathic medications (as the degree of dilution increase). This means that a 1-part-per-million solution of a substance is more medicinally powerful than a 1-part-per-thousand solution, which in turn has more curative power than a 1-part-per-hundred solution (Sagar, 2007). Most homeopathic remedies come in 30X or 200C dilutions. A substance diluted to 30X contains 1 part of the substance in 1029 parts water. A very important concept in chemistry is the Avogadro number, which is determined by X-ray diffraction of crystals. It has a value of $6.0221367 \times 10^{-23}$ as calculated by the International Council of Scientific Unions. Taking this constant into account, the limit imposed upon dilution—that is, the dilution that can be made without losing the original substance altogether—is 12C or 24X (Sagar, 2007).

### 2.10.4 Homoeopathic Nosodes

A nosode is a homoeopathic remedy prepared from an infectious disease product either directly from the bacteria or virus, or less directly from a tissue purported to contain it. Due to potentization- dilution and succussions nosodes lose their infectious nature even while their energetic possibilities are increased (Mangialavori *et al*. 2009).

### 2.10.5 Bowel Nosodes

The supply material for the bowel nosodes are certain groups of microbes that are particularly found as a part of the microorganism lining the intestinal flora of the human bowel special in all states of sickness, and at specific stages of the illness process (Saxton, J. 2012).They belong to the cluster generally known as 'Non Lactose Fermenting Bacilli process'(NLFB), however among that classification individual members were known specifically by their ability or otherwise to ferment three other sugars, particularly glucose, saccharose, and dulcitol(Saxton, J. 2012). Morden medical microbiology has modified hugely in respect of each technique and word since the times of the first on the gut nosodes, and they are currently classed as being among the genera *Klebsiella, Enterobacter, Serratia, Proteus* and others (Saxton, J. 2012).

The initial work that led ultimately to the event of the remedies transpire within the strictly standard field. Over years the names of many workers are coupled to the gut nosodes, and though a lot of valuable work are contributed by range of individuals two names of major importance, those of "Bach" and "Paterson"(Saxton, J. 2012). Dr. Edward Bach (1886-1936) was the cause within the early years and once he left the field in 1930 to start his work on Flower Remedies, Dr. John Paterson (1886-1954) and his wife Dr. Elizabeth Paterson (died in 1963) in Glasgow carried the work forward (Saxton, J. 2012).

### 2.10.6 Colibacillinum (Nosode)

**Proving of *Colibacillinum***

*Colibacillinum* is a relatively unknown nosode. The Homeopathic School of Verona organized a proving of the homeopathic drug which was conducted between February

and May 2011. The subjects were recruited from students and teachers of the school: The proving included 15 provers, 6 supervisors, 1 coordinator and 1 director. The proving consisted of a triple blind test with a 20% placebo control sample. 30CH (6), 200CH (3) and MK (3) potencies were used together with a placebo (3), the substance was taken in liquid form (hydroalcoholic solution), 5 drops 4 times a day for a maximum of 7 days; it is stopped as soon as the first unusual symptom appears. The observation period lasted 30 days (Dominici *et al*. 2011).

### 2.10.7 Materia medica of *Colibacillinum* - Female urinary/reproductive system

The proving of *Colibacillinum* yielded the following homoeopathic clinical indications for the remedy:

- Shooting pain in the left pelvis which radiates to the left sacro-iliac joint and spontaneously disappears after a few minutes.
- Pain originating from the uterus and radiating to the pubis and the right hip, lower abdominal and back pain.
- Sudden urge to urinate, abundant and light coloured urine.
- The urge to urinate during the night and in the morning. In the evening diuresis increased.

In conclusion the proving fully fulfilled the objectives that had been set, both in terms of training and experimental research. As a result of the proving, the pathogenesis of the nosode *Colibacillinum,* for which there had been a serious lack of data, has been enriched with valuable symptoms which facilitate or enable its prescription (Dominici *et al*. 2011).

### 2.10.8 Manufacture of *Colibacillinum*

For the preparation of the mother tincture see **appendix A**

According to the German Homoeopathic pharmacopoeia (Benyunes,. 2005) *Colibacilinum* is manufactured in the following way:

The first centesimal dilution (1CH) is made with

- 10 parts of mother tincture and
- 90 parts of ethanol 30%,
- Succuss 10 times than label as 1CH.

The 2nd centesimal dilution (2CH) with

- 1 part of the 1st centesimal dilution(1CH) and
- 99 parts of ethanol 43%.
- Succuss 10 times than label as 2CH.

Subsequent dilutions will be made this way until the desired potencies are reached (Benyunes, 2005).

#### 2.10.9 Clinical uses of *Colibacillinum*

Existing homoeopathic literature suggests the use of *Colibacillinum* for:

- repeated cystitis
- kidney infections
- cramping and spasms in the bladder
- pain on urination or close to urination
- turbid offensive urine
- chronic kidney infection
- glomerular nephritis
- kidney stones
- pain in the lower back with kidney infection and Haemolytic- uremic syndrome heat and burning after urination
- painful ejaculation with burning in the urethra after coition
- in females burning of the vagina preventing coition
  (Mangialavori *et al*. 2009:388).

## 2.11 Related *in vitro* research involving homeopathic nosodes

A review of the literature revealed a significant body of knowledge surrounding the i*n vitro* application of homoeopathic nosodes in various disease contexts:

*Psorinum* is a homoeopathic remedy prepared from the fluid of blisters from scabies infested skin. Once potentised none of the original fluid remains but the energetic effects of the remedy will treat a range of complaints (Sankar and Jadhav, 2017)

Potential anticancer effects of homeopathic *Hepatitis C* 30C (Hep C 30) were tested on three cancer cell lines, HepG2 (liver cancer), MCF-7 (breast cancer) and A549 (lung cancer) and one normal liver cell line WRL-68 cells further scientific protocols was undertaken on HepG2 cells (against WRL-68 cells as the normal control). Hep C 30C induced apoptosis, caused distortion of cell structure, increased reactive oxygen species generation and produced increased DNA incisions. Further it enhanced pro-apototic signal proteins like Bax, cytochrome c and inhibited anti-apoptotic signal proteins. The study concluded that Hep C 30C has obvious anticancer effects against liver cancer cells in vitro (Mondal *et al*. 2016).

Current research into the preparation, standardization and in vitro safety testing of Mycobacterium nosodes (Emtact- polyvalent nosode) was conducted in India, where Mycobacterium was potentised and sucuccused applying the Indian Homoeopathic Pharmacopeia, 30 potencies were prepared from 1Ch to 30Ch using a centesimal scale (Ch),and the results showed no inhibition of bacterial growth from 1Ch to 4Ch and from 5ch to 30Ch there was no growth; the results suggested that the higher the potency the greater the antimicrobial effect (Joshi *et al*. 2016).

De Waard (1995) tested homoeopathic *Staphylococcinum* 5CH, 9CH, 15CH potencies against *S Aureus in* vitro and found that the 15Ch potency did show an effect on the growth parameters of the organism although inferior to the positive control (antibiotic), it was interesting to note that statistical significance was only achieved at the highest potency.

The action of *Methicillin* on the "in vitro" growth of methicillin-resistant Staphylococcus aureus previously treated with homeopathic dilutions was assessed; - Methicillin-resistant Staphylococcus aureus (MRSA) causes nosocomial infections, and it has

been considered as a worldwide epidemic. The medical system seeks new strategies to fight against MRSA that do not generate resistant strains to antibiotics. Homeopathy has been explored as one of these new strategies, which may play a pivotal role in the treatment of MRSA. In this context, the study was conducted on the action of homeopathy on growth of MRSA bacteria *in vitro*. The results showed a decrease in growth of bacterial strains with homeopathic dilutions of *Belladonna* and the *S. aureus* nosode. The significant changes in the growth was observed, compared to the control (30% alcohol), cultures treated with Belladonna 6cH and the antibiotic in the dilution 4 μg/mL showed a decrease of 40% of the growth, while in the 30cH the drop was of 75%. Cultures treated with the *S. aureus* nosode 30cH and the antibiotic at 4 μg/mL dilution, showed a decrease of 60% in bacterial growth in vitro (Passeti *et al*. 2015) the outcome of this study suggests that the higher the homoeopathic potency the greater it antimicrobial efficacy which is in keeping with the findings of De Waard (1995)

## 2.12 Disc diffusion method

The principle of disk diffusion testing has been used in microbiology laboratories for over 70 years. Alexander Fleming used a variant of this technique when working with penicillin in the 1950s. At that time, there were as many different procedures in use as there were microbiologists. Drs. Bauer, Kirby, Sherris, and Turck painstakingly tested all of the variables used in the procedure, such as the media, temperature, and depth of agar. In 1966, they published their landmark paper describing the test that is used today. NCCLS adopted the basic procedural steps in the Bauer paper as the disk diffusion reference method. These steps must be followed precisely to obtain accurate results (Jorgensen *et al*. 2007). (See appendix E)

Agar plates are innoculated with an institutionalized inoculum of the test microorganism. At that point, filter paper disc (around 6 mm in breadth), containing the test substance at a desired concentration, are put on the agar surface (Balouiri *et al*. 2016). The Petri dishes are brooded under appropriate conditions, for the most part, antimicrobial agents diffuse into the agar and hinders germination and development of the test microorganism and then the diameters of inhibition growth zones are measured (Balouiri *et al*. 2016). Antibiogram gives subjective results by ordering

microbes as susceptible, intermediate or resistant. In this way, it is a composing apparatus dependent on the resistance phenotype of the microbial strain tested,its results additionally manage clinicians in the proper choice of beginning empiric medications, and anti-microbials utilized for individual patients specifically circumstances (Balouiri *et al*. 2016).

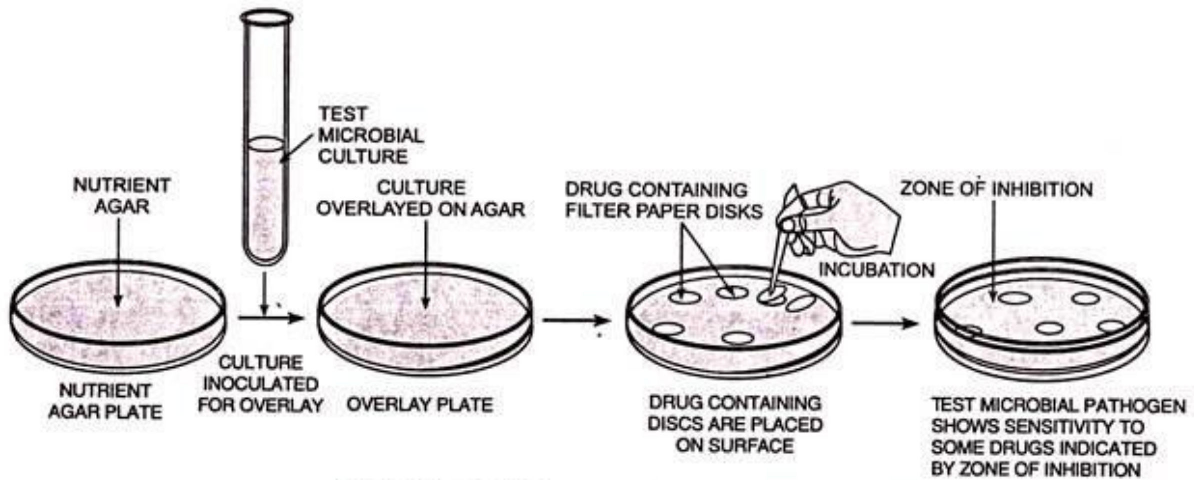


FIG. 46.11. Antimicrobial drug susceptibility test by agar diffusion technique.

Biology Discussion :online ( accessed 10 October 2018)

# CHAPTER 3

## METHODOLOGY

### 3.1 Introduction

This research was an *in vitro* microbial study using a quantitative quasi-experimental design method.

### 3.2 *E. coli* source material

Both Enteropathogenic and Uropathogenic strains of *E. coli* were obtained from the culture collection of Nelson Mandela School of medicine, University of KwaZulu Natal. The study was done at National Health Laboratory at King Edward Hospital under the Supervision of Dr Pratisha Mahabeer who is a qualified Medical Microbiologist.

Clinical and Laboratory Standards Institute(CLSI) guidelines were followed as precaution when dealing with infectious microbes.

- M2 Performance Standards for Antimicrobial Disk Susceptibility Tests were followed as per CLSI guidelines.
- M11 Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria as per CLSI guidelines.
- Development of In Vitro Susceptibility testing Criteria and Quality Control Parameters as per CLSI guidelines.

www.clsi.org (Accessed 10 October 2018)

### 3.3 Preparation of *Colibacillinum*

The test substances were prepared (diluted and succussed in 30% ethanol) as per the protocol required by German Homoeopathic Pharmacopoeia method 43 (Benyunes, 2005*)*. The respective parallel potencies were prepared from both Enteropathogenic and Uropathogenic strains of *E. coli*.

### 3.3.1 Method 43: Mother tinctures and liquid dilutions

Method 43 is applied for the manufacture of homoeopathic remedies originating from animal or human organs or parts of organs that are subject to pathological changes. One part of minced starting material (raw comminuted material), which must comply with the 'Test for sterility' in the German Pharmacopoeia is dispersed in 10 parts glycerol 85% then stored in a dark place with at a temperature of 25°C for not less than 5 days, with maceration every morning before filtering using a muslin cloth. The mother tincture was prepared at National Health Laboratory in King Edward Hospital.

In this regard the researcher dispersed 2 ml of each respective strain of *E. Coli* into (2 ml of *E. coli* strain into 20ml of 85% glycerol and placed into a 50ml amber glass bottle which was sealed, labelled and stored for 5 days at 25°C, the researcher agitated the container each morning and on the 6th days filtered the solution using muslin cloth. At the end of this stage the researcher had prepared two mother tinctures of Colibacillinum i.e. of uropathogenic origin and one of enteropathogenic origin.

### 3.3.2 Preparation of homoeopathic potencies

For each type of *Colibacillinum* (uropathogenic and enteropathogenic) the researcher followed the following method, method 43 of the German Homeopathic Pharmacopoeia (Benyunes, 2005*)* to potentize and reach the desired end test potencies i.e. 6CH, 12CH, 30CH, 200CH:

The first centesimal dilution (1CH) was made with

- 10 parts of mother tincture combined with
- 90 parts of ethanol 30%,
- Succuss 10 times than label as 1CH.

The 2nd centesimal dilution (2CH) was made with

- 1 part of the 1st centesimal dilution(1CH) and
- 99 parts of ethanol 43%.

- Succuss 10 times than label as 2CH.
- Subsequent dilutions were made by repeating this method until the parallel desired end test potencies were reached, which are 3CH, 9CH, 30CH and 200CH.

At the end of this step the researcher had produced the following remedies:

- *Colibacillinum* of uropathogenic origin (*Coli-bac_U)* in 3CH, 9CH, 30CH and 200CH liquid potencies (43% ethanol)
- *Colibacillinum* of enteropathogenic origin *(Coli-bac_E)* in 3CH, 9CH, 30CH, 200CH liquid potencies (43% ethanol)

### 3.4. Preparation of the media

Test media were prepared according to Clinical and Laboratory Standards Institute (Jorgensen *et al*. 2007).

1. Prepare according to the manufacturer's directions.

2. Agar dilution plates, immediately after autoclaving, the agar was allowed to cool to 45 to 50°C in a water bath before aseptically adding antimicrobial solutions and heat-labile supplements, and poured into plates.

3. The pH of each batch of Mueller-Hinton agar was checked before the medium was prepared, the agar medium had a pH of 7.4 at room temperature, and was checked after gelling. Which is the standard PH for Mueller-Hinton agar according to the Clinical and Laboratory Standards Institute guidelines (CLSI)

- Enough agar was macerated to submerge the tip of a pH electrode.
- A small amount of agar was allowed to solidify around the tip of a pH electrode in a beaker.
- The surface of the electrode was used.

### 3.5. Preparation of the inoculums

Inoculums were prepared according to respective colonies of each uropathogenic and enteropathogenic strains of *E.Coli* were tested and used to inoculate 30Mueller-Hinton

agar plates, and allowed to incubate for 24 hours at $37^0$C. At this stage the researcher has prepared 15 plates inoculated with Uropathogenic *E.Coli* and 15 plates with Enteropathogenic *E. Coli.*

### 3.6. Sourcing and preparation of controls

The study applied two controls against which the antimicrobial effects of parallel potencies of *Colibacillinum* were compared.

The Positive control was Ciprofloxacin batch number 17850 (purchased from sigma Aldrich) and the Negative control- Ethanol 43% batch number 896187 (purchased from **DALGEN**) i.e. the same percentage and batch of used to manufacture the test substances described in 3.3.2.

### 3.7 Preparation of saline test cultures

A few colonies from the overnight Mueller- Hinton agar cultures of the selected bacterial strains were suspended in 10ml sterile solution (8.5 g/l) and the solution adjusted to 0.5 McFarland Equivalence Turbidity Standard, this was done to be able to withdraw 2ml of Enteropathogenic and Uropathogenic strains of *E. coli* to prepare the mother tincture from both strains.

### 3.8 Antimicrobial activity testing

Antimicrobial testing was performed according to Clinical and Laboratory Standards Institute (Jorgensen *et al*. 2007)

Ethanol 43% acted as the negative control and Ciprofloxacin (an antibiotic) as the positive control. The disc diffusion method was done to test the antimicrobial activity of the controls and respective parallel homoeopathic potencies of *Colibacillinum* on the agar plates inoculated with the respective strains of *E. Coli* bacteria. Subsequent comparisons in the zones of inhibition between the various potencies of *Colibacillinum* and controls was then made using vernier calipers to gauge the extent of antibiotic activity.

### 3.8.1 Preparation of medicated discs and ethanol only dry discs

Discs were prepared according to Clinical and Laboratory Standards Institute (Jorgensen *et al.* 2007)

#### 3.8.1.1 Preparation of medicated discs

Preparation of *Colibacillinum* in 43% ethanol base dry discs

- Sterile, 5mm, Whatman® filter paper number 4 discs were evenly placed upon the bottom of a sterile dish using a pair of sterile forceps, so that each petri dish contained 6 discs.
- 10 microliters of respective *Colibacillinum* potencies was pipetted onto each disc using a calibrated micropipette.
- The petri dishes were then placed in a dark incubator at $37^0$C, and the discs allowed to dry.
- The dry discs were then labelled, placed in a sterile jar until used.

#### 3.8.1.2 Preparation of 43% v/v ethanol negative control dry discs

1. 43% v/v ethanol (negative control) was prepared according to the German Homoeopathic Pharmacopoeia (Benyunes, 2005*)*.

2. 2.5L of 99% v/v ethanol was purchased from **DALGEN** products, was diluted to 150 millilitres of 43% v/v ethanol. The concentration of ethanol was verified using a hydrometer.

- Sterile 5mm, Whatman® filter paper number 4, discs were evenly placed upon the bottom of a sterile petri dish using a sterile forceps, so that each petri dish contained 6 discs.
- 10 microliters of 43% ethanol were pipetted onto each disc using a calibrated micropipette.

- The petri dishes were then placed in a dark incubator at $37^0$, and discs allowed to dry.
- The dry discs were then stored in labelled sterile jar until used.

#### 3.8.1.3 Preparation of the Ciprofloxacin positive control discs

- Sterile, 5mm, Whatman® filter paper number 4 discs were evenly placed upon the bottom of a sterile dish using a pair of sterile forceps, so that each petri dish contained 6 discs.
- 100mg of Ciprofloxacin was diluted by 2ml of distilled water.
- 10 microliters of respective Ciprofloxacin solution were pipetted onto each disc using a calibrated micropipette.
- The petri dishes were then placed in a dark incubator at $37^0$C, and the discs allowed to dry.
- The dry discs were then labelled, placed in a sterile jar until used.

### 3.8.2 Disc diffusion method

- A marker pen was used to label the side of the agar plate with a number to denote which bacteria were streaked on the plate. Twenty four plates containing Uropathogenic *E.Coli* were marked with 'Uro' ( for uropathogenic strain) and twenty four plates containing Enteropathogenic *E. Coli* were marked with 'Entero' (for Enteropathogenic strain)
- The marker pen was then used on the under surface of the agar plates, referring to a particula disc, namely:

Group A

1) *Colibacillinum* Enteropathogenic strain potency 43% ethanol vehicle (3CH Entero, 9CH Entero, 30CH Entero, 200CH Entero)

2) 43% ethanol (ROH)

3) Ciprofloxacin (Antib)

Group B

1) *Colibacillinum* Uropathogenic strain potency in 43% ethanol vehicle (3CH Uro, 9CH Uro, 30CH Uro, 200CH Uro)

2) 43% ethanol (ROH)

3) Ciprofloxacin (Antib)

- With sterile forceps filtered paper discs were distributed over the agar surface.
- All the plate cultures were inoculated in an inverted position for 24 hours at 37®C.

Following incubation, the plates were examined for the presence of growth inhibition, indicated by a clear zone surrounding each disc. The susceptibility of an organism was determined by the size of this.

### 3.8.3 Test combinations

The researcher applied the disc diffusion method using the following test combinations:

- *Coli-bacillinum* of enteropathogenic origin *(Coli-bac_E)* 3CH, 9CH, 30CH, 200CH tested against enteropathogenic *E.coli* (*E.coli_E)*
- *Coli-bacillinum* of enteropathogenic origin *(Coli-bac_E)* 3CH, 9CH, 30CH, 200CH tested against uropathogenic *E.coli* (*E.coli_U)*
- *Coli-bacillinum* of uropathogenic origin *(Coli-bac_U)* 3CH, 9CH, 30CH, 200CH tested against uropathogenic *E.coli* (*E.coli_U)*
- *Coli-bacillinum* of uropathogenic origin *(Coli-bac_U)* 3CH, 9CH, 30CH, 200CH tested against enteropathogenic *E.coli* (*E.coli_E)*

- Ciprofloxacin (positive control) against uropathogenic *E.coli* (*E.coli_U)* & enteropathogenic *E.coli* (*E.coli_E)*
- 45% ethanol (negative control) against uropathogenic *E.coli* (*E.coli_U)* & enteropathogenic *E.coli* (*E.coli_E)*

### 3.9 Data collection process and analysis

Measurements of the zone of inhibition was done using a Vernier caliper and was written in data collection sheets (see appendix J), and the data analysis was done by comparing the zones of inhibition of the positive control Ciprofloxacin which is the standard antibiotic recommended for the treatment of cystitis (European Committee on Antimicrobial Susceptibility Testing) against *Colibacillinun* potencies (zones created by *Colibacillinun* potencies impregnated discs. (see appendix J)

### 3.10 Ethical considerations

This study was done at National health laboratory under the supervision of a qualified Medical Microbilologist Dr Pratisha Mahabeer, with the permission from Professor Koleka Mlisana who is the Head of National Health Laboratory at King Edward Hospital. The approval for the study was given by the Faculty of Health Sciences RHDC, since no human subjects were recruited for the study was exempt from ethical review.

### 3.11 Data analysis

A mixed factorial ANOVA was used to test for the difference across strain (concentration) in the inhibitory effect of an antibacterial formulation that was prepared from Uropathogenic and Enteropathogenic strain, respectively. Mauchly's Test of Sphericity on both Uropathogenic and Enteropathogenic strains was done.

# CHAPTER 4

## RESULTS

This chapter presents the results of the pilot and main phases of this study. In the pilot phase, a mixed factorial ANOVA was used to test for the difference across strain potency in the inhibitory effect of an antibacterial formulation that was prepared from Uropathogenic and Enteropathogenic strain, respectively. Specifically, the pilot phase attempts to evaluate the effect of different potencies on the antibacterial ability to inhibit *E. coli* growth. In the main phase, a comparison of the prepared antibacterial formulation with 43% ethanol (negative control) and Ciprofloxacin (control) was done in order to evaluate the effectiveness of the inhibitory properties of the prepared antibacterial. Here, One-way ANOVA was used to compare the differences between the test and control groups. This chapter concludes with a summary of the data that was analysed.

The results obtained from this study showed that the Homoeopathic remedy Colibacillinum ready from each Uropathogenic and Enteropathogenic strains displayed restrictive effects against Enteropathogenic and Uropathogenic strains of E. coli, and exhibited statistically significance. The management cluster (ciprofloxacin) had the very best restrictive impact (42.3±0.58mm) against Enteropathogenic and Uropathogenic E. coli, whereas the negative management (43% ethanol) had the bottom restrictive impact (0.67±1.15mm). Colibacillinun 200CH ready from a Uropathogenic strain of E-coli (Coli-b_U 200CH) displayed statistically vital antimicrobial effects against uropathogenic E.coli; such antimicrobial impacts were considerably bigger than forty third grain alcohol (negative control); the antimicrobial effect was but inferior to antibiotic drug (positive control). Colibacillinum 9CH ready from Enteropathogenic strain of E-coli (Coli-b_E 9CH) conjointly displayed statistically vital antimicrobial effects against enteropathogenic E.coli that were considerably larger than forty third grain alcohol (negative control) however inferior to antibiotic drug.

### 4.1. Mauchly’s Test of Sphericity for Uropathogenic prepared strain

Table 4-1 showed the test for sphericity for the inhibitory effect of antibacterial formulation prepared from Uropathogenic strain. The Mauchly test has a p-value of

0.875, which shows evidence of homogeneity of covariance. This suggests that sphericity of the data is assumed.

**Table 4. 1: Mauchly's Test of Sphericity for Colibacillinum prepared from Unropathogenic strain**

| Mauchly's Test of Sphericity[a] | | | | | | | |
|---|---|---|---|---|---|---|---|
| Measure: | | | | | | | |
| Within Subjects Effect | Mauchly's W | Approx. Chi-Square | Df | Sig. | Epsilon[b] | | |
| | | | | | Greenhouse-Geisser | Huynh-Feldt | Lower-bound |
| Potency | 0.504 | 1.867 | 5 | 0.875 | 0.762 | 1.000 | 0.333 |

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.

a. Design: Intercept + Strain

Within Subjects Design: Concentration

b. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table

## 4.2 The effectiveness of *Colibacillinum* prepared from Uropathogenic strain (*Coli-b_U)*

### 4.2.1 Descriptive Statistics by potency prepared from Uropathogenic strain

Table 4-2 showed the inhibitory effect of the prepared antibacterial formulation by potency against Uropathogenic and Enteropathogenic *E. coli*. From the table below, it can be gathered that the higher the potency, the better the inhibitory effect against Uropathogenic strain, while the lower the potency the better the inhibitory effect against Enteropathogenic strain. For example, 200CH potency (14.67±1.15) had the best inhibitory effect against Uropathogenic strain, but was less effective against Enteropathogenic strain (1.0±1.73). In contrast, 9CH potency (13.00±7.55) had the

best inhibitory effect against Enteropathogenic strain and also showed an improved inhibitory effect against Uropathogenic strain (8.00±13.86).

**Table 4. 2: Inhibitory effect of *Coli-b-U* by potency**

| Strain | | Mean | Std. Deviation | N |
|---|---|---|---|---|
| 3CH | Uropathogenic | 8.6667 | 7.57188 | 3 |
| | Enteropathogenic | 10.0000 | 8.88819 | 3 |
| | Total | 9.3333 | 7.42069 | 6 |
| 9CH | Uropathogenic | 8.0000 | 13.85641 | 3 |
| | Enteropathogenic | 13.0000 | 7.54983 | 3 |
| | Total | 10.5000 | 10.34891 | 6 |
| 30CH | Uropathogenic | 7.6667 | 13.27906 | 3 |
| | Enteropathogenic | 6.3333 | 7.76745 | 3 |
| | Total | 7.0000 | 9.75705 | 6 |
| 200CH | Uropathogenic | 14.6667 | 1.15470 | 3 |
| | Enteropathogenic | 1.0000 | 1.73205 | 3 |
| | Total | 7.8333 | 7.60044 | 6 |

### 4.2.2 ANOVA tests of within-subjects and between effects for *Colibacillinum* prepared from Uropathogenic strain (*Coli-b_U*)

As shown in Table 4-3, the mean inhibitory effect of the prepared antibacterial formulation with respect to potency failed to show significant differences beyond the 0.05 level: $F (3,12) = 0.169$; $p>0.05$. Partial eta squared = 0.041 representing a medium effect. There was no significant effect between the potency and *E. coli* strain: $F (3, 12) = 1.144$; $p > 0.05$. Partial eta squared =0.222 representing a large effect.

**Table 4. 3: Tests of within-subjects effects by potency prepared from Uropathogenic strain**

| Source | | Type III Sum of Squares | Df | Mean Square | F | Sig. | Partial Eta Squared |
|---|---|---|---|---|---|---|---|
| Potency | Sphericity Assumed | 43.667 | 3 | 14.556 | 0.169 | 0.915 | 0.041 |
| Potency* Strain | Sphericity Assumed | 294.833 | 3 | 98.278 | 1.144 | 0.371 | 0.222 |
| Error(potency) | Sphericity Assumed | 1031.000 | 12 | 85.917 | | | |

As shown in Table 4-4, the mean inhibitory effects for the *E. coli* strain by potency showed no significant differences beyond 0.05 level: $F(1, 4) = 0.508$; $p > 0.05$. Partial eta squared = 0.113 representing a large effect. This suggests that the effect of the potency of *Coli-b_U* against both Uropathogenic and Enteropathogenic microorganisms were similar.

**Table 4. 4: Test of between-subjects effects by potency prepared from Uropathogenic strain**

| Source | Type III Sum of Squares | Df | Mean Square | F | Sig. | Partial Eta Squared |
|---|---|---|---|---|---|---|
| Intercept | 1802.667 | 1 | 1802.667 | 32.529 | .005 | .890 |
| Strain | 28.167 | 1 | 28.167 | .508 | .515 | .113 |
| Error | 221.667 | 4 | 55.417 | | | |

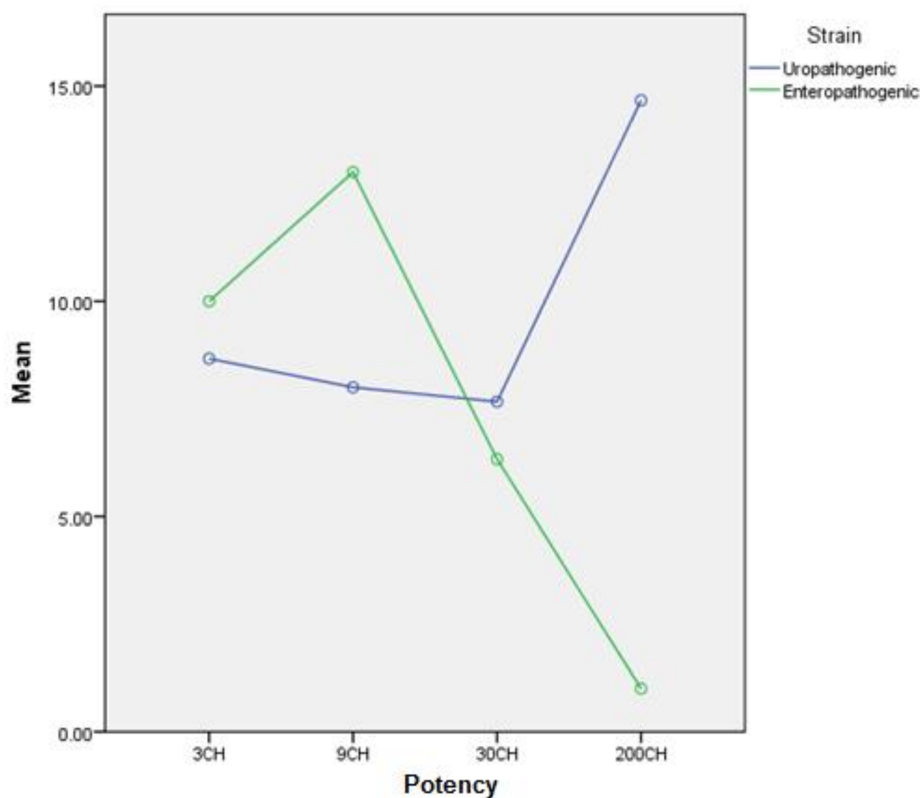



**Figure 4. 1: Differences in the mean inhibitory effects by concentration prepared from Uropathogenic strain**

Figure 4-1 further confirms the inhibitory effects of *Colibacillinum* prepared from Uropathogenic strain. The difference in the inhibitory effects against Uropathogenic and Enteropathogenic *E. coli* strain is evident amongst the potencies. It can be

observed from the graph that the potency of 30CH displayed the lowest inhibitory effect against Uropathogenic strain. The inhibitory effect, however, increases as the potency increasse to 200CH. In contrast, a reverse trend was observed for the Enteropathogenic strain. It can be glanced from Figure 4-1 that 9CH potency had the highest inhibitory effect against Enteropathogenic strain. This effect diminishes as the potency increases to 200CH. It is interesting to note that the greatest inhibitory effect of *Coli-b_U* was against the uropathogenic strain of *E. Coli*.

## 4.3. Mauchly's Test of Sphericity for Enteropathogenic prepared strain

Table 4-5 showed the test for sphericity for the inhibitory effect of antibacterial formulation prepared from Enteropathogenic strain. The Mauchly test has a p-value of 0.311 showed no evidence against the homogeneity of covariance. This suggests that sphericity of the data is assumed.

**Table 4. 5: Mauchly's Test of Sphericity for *Colibacillinum* prepared from Enteropathogenic strain**

| Mauchly's Test of Sphericity[a] | | | | | | | |
|---|---|---|---|---|---|---|---|
| Measure: | | | | | | | |
| Within Subjects Effect | Mauchly's W | Approx. Chi-Square | Df | Sig. | Epsilon[b] | | |
| | | | | | Greenhouse-Geisser | Huynh-Feldt | Lower-bound |
| Potency | 0.101 | 6.230 | 5 | 0.311 | 0.501 | 0.938 | 0.333 |

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.

a. Design: Intercept + Strain

Within Subjects Design: Potency

b. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table

#### 4.3.1 The effectiveness of the antibacterial formulation (*Colibacillinum*) prepared from Enteropathogenic strain *(Coli-b_E)*

#### 4.3.2 Descriptive Statistics by potency prepared from Enteropathogenic strain

Table 4-6 showed the inhibitory effect of the prepared antibacterial formulation by potency against Uropathogenic and Enteropathogenic *E. coli*. 9CH potency had the best inhibitory effect against both Uropathogenic (12.33±1.54) and Enteropathogenic (22.67±6.11) *E. coli*. It is interesting to note that all respective potencies of *Coli-b_E* had consistently greater antimicrobial effects against Enteropathogenic *E. coli* than it had against Uropathogenic *E. coli.*

**Table 4. 6: Inhibitory effect of *Coli-b_E* by potency**

| Strain | | Mean | Std. Deviation | N |
|---|---|---|---|---|
| 3CH | Uropathogenic | 9.0000 | 13.07670 | 3 |
| | Enteropathogenic | 14.0000 | 0.00000 | 3 |
| | Total | 11.5000 | 8.71206 | 6 |
| 9CH | Uropathogenic | 12.3333 | 1.15470 | 3 |
| | Enteropathogenic | 22.6667 | 6.11010 | 3 |
| | Total | 17.5000 | 6.89202 | 6 |
| 30CH | Uropathogenic | 7.0000 | 6.08276 | 3 |
| | Enteropathogenic | 8.3333 | 7.63763 | 3 |
| | Total | 7.6667 | 6.21825 | 6 |
| 200CH | Uropathogenic | 9.0000 | 9.53939 | 3 |
| | Enteropathogenic | 9.6667 | 8.38650 | 3 |
| | Total | 9.3333 | 8.04156 | 6 |

#### 4.3.2 ANOVA Tests of within-subjects and between effects for *Colibacillinum* prepared from Enteropathogenic strain (*Coli-b_E)*

As shown in Table 4-7, the mean inhibitory effect of the prepared antibacterial formulation with respect to potency failed to show significant differences beyond the 0.05 level: $F(3,12) = 1.590$; $p>0.05$. Partial eta squared = 0.284 representing a large effect. There was no significant effect between the potency and *E. coli* strain: $F(3, 12) = 0.423$; $p > 0.05$. Partial eta squared =0.096 representing a medium effect. This

suggests that the effect of potency against both Uropathogenic and Enteropathogenic microorganisms were similar.

**Table 4. 7: Tests of Within-Subjects Effects by Potency prepared from Enteropathogenic strain**

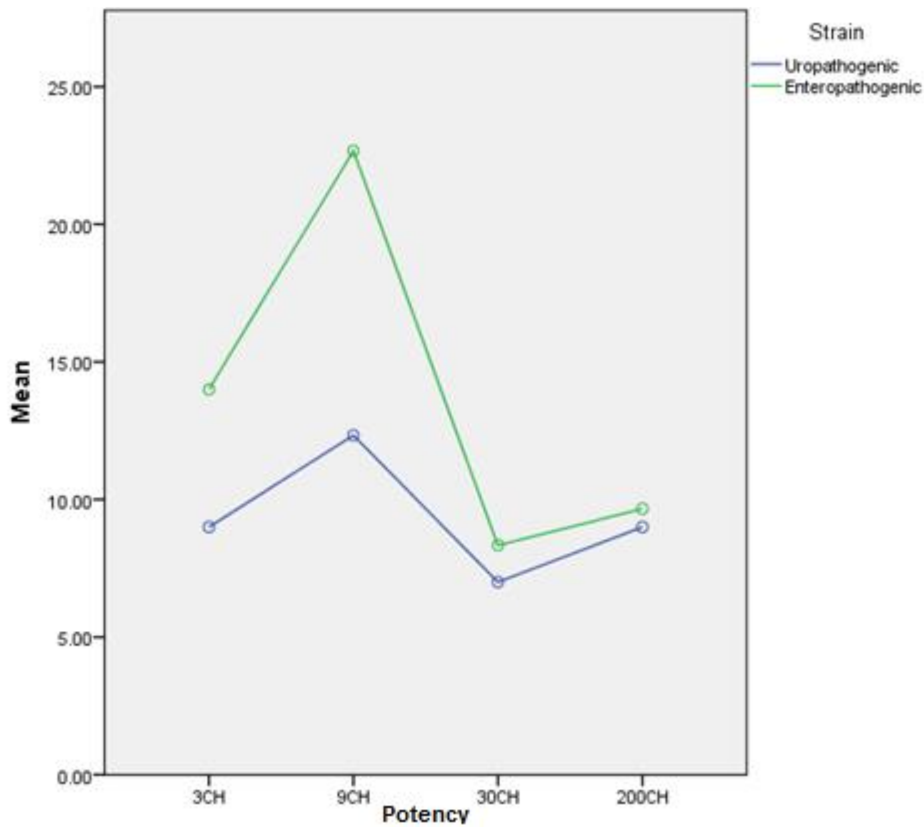
| Source | | Type III Sum of Squares | Df | Mean Square | F | Sig. | Partial Eta Squared |
|---|---|---|---|---|---|---|---|
| Potency | Sphericity Assumed | 332.333 | 3 | 110.778 | 1.590 | 0.243 | 0.284 |
| Potency * Strain | Sphericity Assumed | 88.333 | 3 | 29.444 | 0.423 | 0.740 | 0.096 |
| Error(Potency) | Sphericity Assumed | 835.833 | 12 | 69.653 | | | |

As shown in Table 4-8, the mean inhibitory effects for the *E. coli* strain by potency showed no significant differences beyond 0.05 level: $F(1, 4) = 4.654$; $p > 0.05$. Partial eta squared = 0.538 representing a large effect. This suggests that the effect of potency against both Uropathogenic and Enteropathogenic microorganisms were similar.

**Table 4. 8: Test of between-subjects effects by potency prepared from enteropathogenic strain**

| Source | Type III Sum of Squares | Df | Mean Square | F | Sig. | Partial Eta Squared |
|---|---|---|---|---|---|---|
| Intercept | 3174.000 | 1 | 3174.000 | 131.112 | 0.000 | 0.970 |
| Strain | 112.667 | 1 | 112.667 | 4.654 | 0.097 | 0.538 |
| Error | 96.833 | 4 | 24.208 | | | |

**Figure 4-2** illustrates the inhibitory effects of *Colibacillinum* prepared from Enteropathogenic strain. All the potency showed a better inhibitory effect against Enteropathogenic *E. coli* strain when compared against Uropathogenic strain. It can be observed from the graph that clearly the potency of 9CH displayed the highest inhibitory effect against Enteropathogenic strain. There is some increase in inhibitory effect the potency increases to 200CH but not as high as the 9CH. The same trend was observed for the Uropathogenic strain. It is interesting to note that the greatest inhibitory effect of *Coli-b_E* was against the enteropathogenic strain of *E. Coli.*




**F**igure **4. 2** : Differences in the mean inhibitory effects by potency prepared from Enteropathogenic strain

### 4.3.3 Overview of the pilot study

This section summarises the findings of the pilot work.

- No statistical differences were observed amongst all the potencies prepared from Uropathogenic strain. In spite of this, 200CH *(Coli-b_U 200CH)* had the overall best inhibitory effect against Uropathogenic *E. coli*. Hence, this potency was selected for the main study.
- Similarly, no significant differences were found amongst all the potencies prepared from Enteropathogenic strain. This notwithstanding, 9CH (*Coli-b_E 9CH)* potency showed the best inhibitory effect against Enteropathogenic strain. The 9CH potency was thus selected for the main study.

## 4.4 Main study

### 4.4.1 Comparison of means for *Coli-b_U 200CH*

To determine whether parametric tests could be used, a One-Sample Kolmogorov-Smirnov Test was done. The normal distribution of the inhibitory effect is presented in the table below. The One-Sample Kolmogorov-Smirnov test for the normality revealed no significant differences against the normality of the variables ($p > 0.05$). Hence it can be inferred that the distributions are normal and that ANOVA can be used to analyse the respective data set.

**Table 4. 9: Normality test**

| | | Inhibitory effect |
|---|---|---|
| N | | 9 |
| Normal Parameters[a,b] | Mean | 19.2222 |
| | Std. Deviation | 18.38327 |
| Most Extreme Differences | Absolute | 0.236 |
| | Positive | 0.236 |
| | Negative | -0.226 |
| Test Statistic | | 0.236 |
| Asymp. Sig. (2-tailed) | | .158[c] |

a. Test distribution is Normal.

b. Calculated from data.

c. Lilliefors Significance Correction.

### 4.4.2: Uropathogenic inhibitory analysis

The one-way ANOVA, mean, standard deviation and standard error results are shown in Table 4-10. As indicated by the levels of significance, the one-way ANOVA tests revealed that the antibacterial inhibitory effect against Uropathogenic *E. coli* exhibited statistically significant differences amongst all the antibacterial agents ($p < 0.001$). The control group had the highest inhibitory effect (42.3±0.58), while the negative control (43% ethanol) had the lowest inhibitory effect (0.67±1.15).

**Table 4. 10: ANOVA results for the inhibitory effect against Uropathogenic *E.Coli***

| Antibacterial agents | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | p-value | Sig |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| *Coli-b_U* 200CH | 3 | 14.6667 | 1.15470 | 0.66667 | 11.7982 | 17.5351 | 0.000 | Significant |
| 43% ethanol (negative) | 3 | 0.6667 | 1.15470 | 0.66667 | -2.2018 | 3.5351 | | |
| Ciprofloxacin (control) | 3 | 42.3333 | 0.57735 | 0.33333 | 40.8991 | 43.7676 | | |
| Total | 9 | 19.2222 | 18.38327 | 6.12776 | 5.0916 | 33.3529 | | |

Table 4-11 lists the multiple comparison of the antibacterial against Uropathogenic *E. Coli*. The mean inhibitory values of 200CH prepared from Uropathogenic strain was significantly higher than the negative control ($p<0.001$). More so, the mean inhibitory effect values of 200CH was significantly lower than the control ($p<0.001$). Significant difference was also observed for the control and negative control ($p<0.001$).

**Table 4. 11: Multiple comparison Tests**

| Antibacterial agents | Bonferroni | |
|---|---|---|
| | P | Sig. |
| 200CH<br>43% ethanol | 0.000 | Significant |
| 200CH<br>Ciprofloxacin | 0.000 | Significant |
| Ciprofloxacin<br>43% ethanol | 0.000 | Significant |

### 4.4.3 Comparison of means for *Coli-b_E 9CH*

To determine whether parametric tests could be used, a One-Sample Kolmogorov-Smirnov Test was done. The normal distribution of the inhibitory effect is presented in the table below. The One-Sample Kolmogorov-Smirnov test for the normality revealed no significant differences against the normality of the variables (p> 0.05). Hence it can be inferred that the distributions are normal and that ANOVA can be used to analyse the respective data set.

**Table 4. 12: Normality test**

| | | Inhibitory effect |
|---|---|---|
| N | | 9 |
| Normal Parameters[a,b] | Mean | 20.7778 |
| | Std. Deviation | 18.20562 |
| Most Extreme Differences | Absolute | 0.211 |
| | Positive | 0.182 |
| | Negative | -0.211 |
| Test Statistic | | 0.211 |
| Asymp. Sig. (2-tailed) | | .200[c,d] |

a. Test distribution is Normal.

b. Calculated from data

c. Lilliefors Significance Correction.

d. This is a lower bound of the true significance.

### 4.4.4 Enteropathogenic inhibitory analysis

The one-way ANOVA, mean, standard deviation and standard error results are shown in Table 4-13. As indicated by the levels of significance, the one-way ANOVA tests revealed that the antibacterial inhibitory effect against Enteropathogenic *E. coli* exhibited statistically significant differences amongst all the antibacterial agents (p <0.001). The control group had the highest inhibitory effect (42.3±0.58), while the negative control (43% ethanol) had the lowest inhibitory effect (0.67±1.15).

**Table 4. 13: ANOVA results for the inhibitory effect against Enteropathogenic *E.coli***

| Antibacterial agents | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | p-value | Sig |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| *Coli-b_E* 9CH | 3 | 19.3333 | 4.16333 | 2.40370 | 8.9910 | 29.6756 | 0.000 | Significant |
| 43% ethanol (negative) | 3 | 0.6667 | 1.15470 | 0.66667 | -2.2018 | 3.5351 | | |
| Ciprofloxacin (control) | 3 | 42.3333 | 0.57735 | 0.33333 | 40.8991 | 43.7676 | | |
| Total | 9 | 20.7778 | 18.20562 | 6.06854 | 6.7837 | 34.7719 | | |

Table 4-14 lists the multiple comparison of the antibacterial against Enteropathogenic *E.coli.* The mean inhibitory values of 9CH prepared from Enteropathogenic strain was

significantly higher than the negative control ($p<0.001$). More so, the mean inhibitory effect values of 9CH was significantly lower than the control ($p<0.001$). Significant difference was also observed for the control and negative control ($p<0.001$).

**Table 4. 14: Multiple comparison Tests**

| Antibacterial agents | Bonferroni | |
|---|---|---|
| | P | Sig. |
| 9CH<br>43% ethanol | 0.000 | Significant |
| 9CH<br>Ciprofloxacin | 0.000 | Significant |
| Ciprofloxacin<br>43% ethanol | 0.000 | Significant |

# CHAPTER 5

## DISCUSSION

### 5.1 Uropathogenic Colibacillinum (Coli-b_U)

The results of this study demonstrated that the higher the potency, the greater the inhibitory effect against Uropathogenic strain of *E-coli*, while the lower the potency the greater the inhibitory effect against Enteropathogenic strain of *E-coli*. Fe-or example, *Coli-b_U* 200CH potency had the greatest inhibitory effect (14.67±1.15mm) against Uropathogenic *E-coli*, but was less effective against Enteropathogenic strain (1.0±1.73mm). In contrast, *Coli-b_U* 9CH potency had the best inhibitory effect (13.00±7.55mm) against Enteropathogenic *E-coli* and also showed an inhibitory effect against Uropathogenic strain (8.00±13.86mm). See table 4-2

The one-way ANOVA, mean, standard deviation and standard error results are shown in Table 4-10. As indicated by the levels of significance, the one-way ANOVA tests revealed that the antibacterial inhibitory effect against Uropathogenic *E. coli* exhibited statistically significant differences amongst all the antibacterial agents ($p < 0.001$). The positive control group (ciprofloxacin) had the highest inhibitory effect (42.3±0.58mm), while the negative control (43% ethanol) had the lowest inhibitory effect (0.67±1.15mm).

The mean inhibitory values of *Coli-b_U* 200CH was significantly higher than the negative control (43% ethanol) ($p<0.001$); since the 200CH was contained within a 43% ethanol vehicle (same batch as negative control) the biological activity (beyond that of 43% ethanol) of this ultra-high dilution was confirmed. Despite the significant inhibitory effect of *Coli-b_U* 200CH the inhibitory effect of ciprofloxacin was superior ($P<0.001$).

It is pertinent to emphasise that the level of dilution and succusion of the 200CH is $1:10^{400}$; a deconcentration well in excess of Avogadro's number which is exceeded beyond the 12CH; the biological effect displayed by this potency can thus not be explained in terms of chemistry since at such levels of deconcentration not a single molecule of the original starting substance remains and thus all that is officially chemically present in this potency is 43% ethanol.

It is interesting to note that that the most significant inhibitory effect of *Coli-b_U* was demonstrated against uropathogenic *E-coli* the similar trend was noted with *Coli-b_E* demonstrating a greater inhibitory effect against enteropathogenic *E-coli* i.e. the respective homoeopathic forms of *Colibacillinum* are most biologically active against the very strains of bacteria from which they were manufactured. This finding greatly supports the homoeopathic “Law of Similars”, SIMILIA SIMILIBUS CURENTUR which means “let like be cured by likes”, every substance which is capable of producing a set of symptoms in healthy human being has the capacity to remove them when administered to a sick person with similar set of symptoms (Vithoulkas, 2002).

Furthermore, in the case of *Coli-b_U* in the current study the anti-microbial effect was seen to be greatest at the 200c potency; a finding which supports the Law of Infinitesimals which states that the serial dilution and succussion process *increases* the curative power of homeopathic medications (as the degree of dilution and succusion increases). This means that a 1-part-per-million solution of a substance is more medicinally powerful than a 1-part-per-thousand solution, which in turn has more curative power than a 1-part-per-hundred solution (Sagar, 2007).

De Waard (1995) tested *Staphylococcinum 5CH, 9CH, 15CH* against *S Aureus* and found that the only potency which had inhibitory effect was the 15CH (the highest potency tested) this trend was further confirmed by Joshi *et al*. (2016), Passeti *et al*. (2015) and once more in the present study on *Coli-b_U* i.e. the higher the potency the greater it anti-microbial efficacy.

### 5.2 Enteropathogenic Colibacillinum (Coli-b_E)

9CH potency had the best inhibitory effect against both Uropathogenic (12.33±1.54mm) and Enteropathogenic (22.67±6.11mm) *E. coli*. It is interesting to note that all respective potencies of *Coli-b_E* had consistently greater antimicrobial effects against Enteropathogenic *E. coli* than it had against Uropathogenic *E. coli* – this phenomenon was also seen in the data on *Coli-b_U* See table 4-6 and supports the Homoeopathic Law of Similars, which means “let like be cured by likes” (Vithoulkas, 2002).

The mean inhibitory values of *Coli-b_E* 9CH was significantly higher than the negative control (43% ethanol) ($p<0.001$) since the 9CH was contained within a 43% ethanol vehicle (same batch as negative control) the biological activity (beyond that of 43% ethanol) of this dilution was confirmed. Despite the significant inhibitory effect of *Coli-b_E* 9CH the inhibitory effect of ciprofloxacin was superior ($P<0.001$). See table 4-14

It is interesting to note that although peak anti-microbial activity of *Coli-b_E* was determined to be at the 9CH potency the deconcentration of a 9CH is still high ($1:10^{18}$) yet the antimicrobial effect was significantly superior to the 43% ethanol.

### 5.3 General discussion

The difference in zones of inhibition in 43% ethanol (negative control), was due to the use of dry discs in preference to wet discs. This technique effectively nullified the superfluous anti-microbial effect of the ethanol on the bacteria, as all the ethanol present had been evaporated off before the placement of the discs on the inoculated media. This resulted in minimal or no zones of inhibition being formed around the ethanol discs. The same was done to the homoeopathic potencies; the discs were soaked in *Coli-b* remedies and allowed to dry in the incubator thus discs treated with the respective homoeopathic potencies were too void of ethanol.

The findings for *Coli-b_U* in the current study support those found in the *in vitro* experimentation with homoeopathic Mycobacterium nosodes. Mycobacterium was potentised and sucuccused applying techniques described in the Indian Homoeopathic Pharmacopeia, 30 potencies were prepared from 1Ch to 30Ch using a centesimal scale (Ch), the study confirmed that higher the potency the greater the antimicrobial effect i.e. anti-microbial effect was directly proportionate to potency level (Joshi *et al.* 2016).

Passeti *et al.* (2015) too concluded that the *in vitro* anti-microbial effects of homoeopathic potencies of *Belladonna* and the *S. aureus* nosode against methicillin-resistant Staphylococcus aureus were greater as potency increased, cultures treated with Belladonna 6Ch and the antibiotic in the dilution 4 µg/mL showed a decrease of 40% of the growth, while in the 30Ch the drop was of 75%. Cultures treated with the

*S. aureus* nosode 30Ch and the antibiotic at 4 μg/mL dilution, showed a decrease of 60% in bacterial growth in vitro (Passeti *et al*. 2015). De Waard (1995) tested *Staphylococcinum 5CH, 9CH, 15CH* against S Aureus and found that the only potency which had inhibitory effect was the 15CH. This is another study which used the homoeopathic Law of Similars to investigate the anti-microbial efficacy of homoeopathic remedies and from the results it can be concluded that the higher the potency the greater it anti-microbial efficacy.

Although the potency dependent antimicrobial effect for *Coli-b_U* are supported by related literature this trend was not noted for *Coli-b_E* in which the potency with the greatest anti-microbial effect was the 9CH. Colibacillinum prepared from Enteropathogenic strain (*Coli-b_E)* did not conform with hypothesies one, two and four that were proposed in chapter one. Colibacillinum prepared from Uropathogenic strain *(Coli-b_U)* however conformed to all the respective hypotheses proposed in chapter one.

The respective homoeopathic remedies did show an effect on the growth parameters of the organism (if only in the 9Ch *Coli-b_E* and 200Ch *Coli-b_U*), and the effect of the remedies on the organism were subtler than that of the antibiotic positive control.

# CHAPTER 6

## 6.1 CONCLUSION

The aim of this controlled *in vitro* study was to determine the antimicrobial effectiveness of parallel potencies of the homoeopathic remedy *Colibacillinum* (manufactured from a uropathogenic strain and enteropathogenic strain of *E-coli* respectively) against uropathogenic and enteropathogenic cultures of E. coli *in vitro* by means of the disc diffusion assay method.

*Colibacillinun* 200CH prepared from a Uropathogenic strain of *E-coli (Coli-b_U 200CH)* displayed statistically significant antimicrobial effects against uropathogenic *E.coli;* such antimicrobial effects were significantly greater than 43% ethanol (negative control); the antimicrobial effect was however inferior to Ciprofloxacin (positive control). *Colibacillinum* 9CH prepared from Enteropathogenic strain of *E-coli (Coli-b_E 9CH)* also displayed statistically significant antimicrobial effects against enteropathogenic *E.coli* which were significantly greater than 43% ethanol (negative control) but inferior to Ciprofloxacin.

The study confirmed the biological (anti-microbial) activity of an ultra-high homoeopathic dilution (*Coli-b_U* 200CH) (1:$10^{400}$) and in the case of *Coli-b_U* the findings support existing literature which suggests that the anti-microbial properties of homeopathic nosodes increase with potency; all hypotheses for this remedy were thus accepted.

This trend was not noted for *Coli-b_E* in which the potency with the greatest anti-microbial effect was the 9CH, thus *Colibacillinum* prepared from Enteropathogenic strain (*Coli-b_E)* did not conform with hypothesis one which stated *Colibacillinum* 3Ch and 9Ch (low potency) prepared from enteropathogenic and uropathogenic strains will have limited antibacterial effects on the *in vitro* growth of enteropathogenic and uropathogenic *E. coli.*Hypothesis two which stated *Colibacillinum* 30Ch and 200Ch (high potency) prepared from enteropathogenic and uropathogenic strains will have significant antibacterial effects on the *in vitro* growth of enteropathogenic and uropathogenic *E. coli.*

Hypothesis four which stated *Colibacillinum* prepared from uropathogenic and enteropathogenic strains above 30CH will have significant antibacterial effects compared to positive control (Ciprofloxacin).

The results of this study comform with Hypothesis five which stated that parallel potencies of enteropathogenic and uropathogenic *Colibacillinum* will have significant antibacterial effects compared to the negative control (43% ethanol) on the growth of uropathogenic and enteropathogenic strains of *E.Coli.* The results also comform with hypothesis six which stated that antibacterial effects of respective forms (enteropathogenic and uropathogenic) of homoeopathic *Colibacillinum* will be greater against corresponding *E. Coli* strains.

That were proposed in chapter one, this might because the 30CH,and 200CH potency discs were not well prepared during the process of impregnating them with the test substance. Despite this the confirmation of significant antimicrobial effects of a substance at this level of deconcentration ($1{:}10^{18}$) is noteworthy. Since both forms of *Colibacillinum* are most biologically active against the very strains of bacteria from which they were manufactured these findings further support the homoeopathic “Law of Similars”, (SIMILIA SIMILIBUS CURENTUR). Further more the study results also support the Isopathic principle of ‘ Same treating Same”, it also demonstrated the homoepathic potencies are not inert or just a placebo effect.

## 6.2 RECOMMENDATIONS

The researcher, based on his experiences of the present study and his knowledge of the related literature in this field makes the following recommendations for future studies accordingly:

1. The use of distilled water instead of 40% ethanol in the preparation of potencies to investigate if similar results will be observed.

2. Use of the Well diffusion method to determine the antimicrobial efficacy of potencies to investigate if this method would yield similar results.
3. Bioautographic method direct (chromatogram layer) to determine the antimicrobial efficacy of potencies by determining the zone of inhibition (Mean Inhibition Concentration).
4. Bioautographic method indirect (agar diffusion) to determine the antimicrobial efficacy of potencies.
5. Potencization of 43% ethanol (negative control) into same potencies as the test substance.

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# APPENDICES

## Appendix A: Preparation of Colibacillinum by German Pharmacopeia Homoeopathic Method

**Method 43: Mother tinctures and liquid dilutions**

Mother tinctures made by method 43 are made from animal or human organs or parts of organs that are subject to pathological changes. Disperse 1 part of minced starting material (raw comminuted material), which must comply with the 'Test for sterility' in the German Pharmacopoeia, in 10parts glycerol 85%, store in a dark place with 25°C temperature. Leave to stand for not less than 5days, with maceration every morning before filtering using a muslin cloth (2grams of E. coli strain into 20ml of 85% glycerol in a 50ml amber glass bottle, cap seal label and leave for 5days with frequent agitation every morning. After 5days filter using muslin cloth)

**Potentization**

The first centesimal dilution (1CH) is made with

- 10 parts of mother tincture and
- 90 parts of ethanol 30%,
- Succuss 10 times than label as 1CH.

The 2nd centesimal dilution (2CH) with

- 1 part of the 1st centesimal dilution(1CH) and
- 99 parts of ethanol 43%.
- Succuss 10 times than label as 2CH.
- Subsequent dilutions will be made this way until the desired potencies are reached, which are 6CH, 12CH, 30CH AND 200CH.

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## Appendix B: Preparation of media

The medium of choice will be the Mueller-Hilton agar due to its pH of 7.2 to 7.4. It will be prepared as follows:

- 38g of Mueller-Hinton agar powder will be weighed out.
- The Mueller-Hinton agar powder will be added to 1 liter of distilled water in a screw top flask.
- A magnetic stirrer will be added to aid dissolution.
- Then the mixture will be shaken until well mixed.
- Then the autoclaved at 121$^{0}$C for 15 minutes.
- The flask will then be allowed to cool whilst placed on a magnetic stirrer machine. This ensures adequate mixing and prevents the mixture from solidifying.
- Once the flask has been cooled enough to hold, the agar will be poured into the agar plates as follows:
  - The top of the flask will be flamed with a burner before pouring each plate to prevent contamination.
  - Each plate will then be poured to a depth of approximately 4 millimeters.
  - A total of 24 plates will be prepared per strain.
  - The plates will then be stacked and allowed to solidify.
- They will then be checked for contamination.

## Appendix C: Preparation of inoculums and saline cultures

**Preparation of the inoculums**

- Single colonies obtained from the University of Kwazulu Natal Medical School microbiology department, cultures of each bacterial strain will be tested and used to inoculate separate Mueller-Hinton agar plates, and allowed to incubate for 24 hours at $37^{0}$C.
- The bacteria will be;
  - Escherichia coli: - Enteropathogenic strain and Uropathogenic strain.

**Controls**

Positive control- Ciprofloxacin (purchased from sigma Aldrich)

Negative control- Ethanol 43% (purchased from Parceval manufacturers)

. **Preparation of saline test cultures**

- A few colonies from the overnight Mueller- Hinton agar cultures of the selected bacterial strains will be suspended in 10ml sterile solution (8.5 g/l) and the solution adjusted to 0.5 McFarland Equivalence Turbidity Standard

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## Appendix D: Preparation of medicated discs and ethanol only dry discs

**Preparation of medicated discs**

**Preparation of *Colibacillinum* in 43% ethanol base dry discs**

- Sterile, 5mm, Whatman® filter paper number 4 discs will be evenly placed upon the bottom of a sterile dish using a pair of sterile forceps, so that each petri dish contained 6 discs.
- 10 microliters of *Colibacillinum* potency will be pipetted onto each disc using a calibrated micropipette.
- The petri dishes will then be placed in a dark incubator at $37^0$C, and the discs allowed to dry.
- The dry discs will then be labelled sterile jars until used.

**Preparation of the 43% ethanol only discs**

- Sterile 5mm, Whatman® filter paper number 4, discs will be evenly placed upon the bottom of a sterile petri dish using a sterile forceps, so that each petri dish contained 6 discs.
- 10 microliters of 62% ethanol will be pipetted onto each disc using a calibrated micropipette.
- The petri dishes will then be placed in a dark incubator at $37^0$, and discs allowed to dry.
- The dry discs will then be stored in labelled sterile jars until used.

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## Appendix E: Disc diffusion method

- A marker pen will be used to label the side of the agar plate with a number to denote which bacteria were streaked on the plate.
- the marker pen will then be used on the under surface of the agar plates, referring to a particular disc, namely:

  Group A

  4) *Colibacillinum* Enteropathogenic strain potency 43% ethanol vehicle
  5) 43% ethanol
  6) Ciprofloxacin

  Group B

  4) *Colibacillinum* Uropathogenic strain potency in 43% ethanol vehicle
  5) 43% ethanol
  6) Ciprofloxacin
- A sterile cotton swab is then dipped into a well- mixed saline test culture and excess inoculum removed by pressing the saturated swab against the inner wall of the culture tube.
- Using the swab, the entire agar surface of the plate will be streaked 1$^{st}$ in a horizontal direction and then vertically to ensure a heavy growth over the entire surface.
- With sterile forceps filtered paper discs will be distributed over the agar surface.
- All the plate cultures will be inoculated in an inverted position for 24 hours at 37®C.

Following incubation, the plates will be examined for the presence of growth inhibition, indicated by a clear zone surrounding each disc. The susceptibility of an organism will be determined by the size of this

## Appendix F: Permission Application Letter to use Microbiological Laboratory

HOD: Food and Technology Department

Z1640 Mzwakhe Nene

Cycle

Umlazi township

4066

Faculty of Applied Sciences

Department of Biotechnology and Food technology

Head of Department

P.O. BOX 1334

Durban

4000

Dear Prof K Permaul

**Permission Application Letter to use the laboratory**

Thank you for reading this letter. My name is Nkululeko Xaba (21018336). I am currently registered for M. Tech. Homoeopathy and I am requesting to use the laboratory at Steve Biko, S10 level 0. My Co-supervisor is Dr S.K.K Pillai, a senior lecturer in your department. The tittle of my study is: A controlled invitro study of the antimicrobial effectiveness of *Colibacillinum* agains*t E. coli* using disc diffusion assay

**Outline of the Procedures:** The experimental study will take approximately a week to be conducted of which only a few hours will be used each day. Namely for make

pure cultures of bacterial strains; medicated discs and to perform diffusion tests (disc). Everything will be done under the supervision of the lab technician or Co-supervisor.

Yours faithfully.

Mr N. Xaba (210108336)-Researcher 072 5478 262

Dr. D. Naude (Supervisor) – 031 373 2514 (David@dut.ac.za)

Dr. S. K.K Pillai (Co-Supervisor)- 031 373 5329 (Santhoshk@dut.ac.za)

DUT DURBAN UNIVERSITY OF TECHNOLOGY

## Appendix G: Permission Application Letter to use Laminar flow room

HOD: Homoeopathy Department

Z1640 Mzwakhe Nene

Cycle

Umlazi township

4066

Faculty of Health Sciences

Department of Homoeopathy

Head of Department

P.O. BOX 1334

Durban

4000

Dear Dr.  C. Hall

**Permission Application Letter to use the Laminar Flow room**

Thank you for reading this letter. My name is Mr N. Xaba (21018336). I am currently registered for M. Tech. Homoeopathy and I am requesting to use the Lamina Flow room at Ritson. My Supervisor is Dr. David F. Naude, a senior lecturer in your department. The tittle of my study is: Antimicrobial effectiveness of *Colibacillinum* against *E. coli* using disc diffusion assay

**Outline of the Procedures:** The experimental study will take approximately a week to be conducted of which only a few hours will be used each day. Namely to make potencies from mother tincture prepared from *E. coli,* the mother tincture will be

prepared from Biotechnology and Food Technology laboratory. Everything will be done under the supervision of the lab technician or Supervisor.

Yours faithfully.

Mr N. Xaba (21018336)-Researcher 072 5478 262

Dr. D. Naude (Supervisor) – 031 373 2514 (David@dut.ac.za)

Dr. S. K.K Pillai (Co-Supervisor)- 031 373 5329 (Santhoshk@dut.ac.za)

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## Appendix H: Application Letter for increase of research budget


Z1640 Mzwakhe Nene

Cycle

Umlazi Township

4066



Director: Research and Postgraduate Support

Tromso Annex, 1st Floor

Gate 1, Steve Biko Campus

P.O. BOX 1334

Durban


Dear Professor Moyo

**<u>Application Letter for increase of research budget</u>**

Thank you for reading this letter. My name is Mr Nkululeko Xaba (21018336). I am currently registered for M. Tech. Homoeopathy and I am requesting an increase of budget for my research study with an additional amount of **R5800**. Most of my research budget is for statistical analysis and consumables. The tittle of my study is: A controlled invitro study of the antimicrobial effectiveness of *Colibacillinum* against *E. coli* using disc diffusion assay

**Outline of the Procedures:** The experimental study will take place at the Durban University of Technology (DUT), Steve Biko S10 level 0. It will take approximately a week to be conducted of which only a few hours will be used each day. Namely for

make pure cultures of bacterial strains; medicated discs and to perform diffusion tests (disc). Everything will be done under the supervision of the lab technician or Co-supervisor.

Yours faithfully.

Mr N. Xaba (21018336)-Researcher 081 8414 731

Dr. D. Naude (Supervisor) – 031 373 2514 (David@dut.ac.za

Dr. S.K.K Pillai(Co-supervisor) - 031 373 5329 (santhoshk@dut.ac.za)

DURBAN UNIVERSITY OF TECHNOLOGY

## Appendix I: Data collection table

Table 1: Data collection tables for *Colibacillinum* 3CH (**prepared from Uropathogenic strain**) in 43% ethanol on the growth of Escherichia coli: - Uropathogenic and Enteropathogenic strains using disc diffusion assay.

| Organism- E.coli | | Colibacillinum 3CH (in 43% ethanol) | 43% ethanol( - control) | Ciprofloxacin(+ control) |
|---|---|---|---|---|
| Uropathogenic strain | Trial 1 | | | |
| | Trial 2 | | | |
| | Trial 3 | | | |
| | Average | | | |
| Enteropathogenic strain | Trial 1 | | | |
| | Trial 2 | | | |
| | Trial 3 | | | |
| | Average | | | |

- Measurements are in millimeters (mm)

Table 2: Data collection tables for *Colibacillinum* 9CH (**prepared from Uropathogenic strain**) in 43% ethanol on the growth of Escherichia coli: - Uropathogenic and Enteropathogenic strains using disc diffusion assay.

| Organism- E.coli | | Colibacillinum 9CH (in 43% ethanol) | 43% ethanol( - control) | Ciprofloxacin(+ control) |
|---|---|---|---|---|
| Uropathogenic strain | Trial 1 | | | |
| | Trial 2 | | | |
| | Trial 3 | | | |
| | Average | | | |
| Enteropathogenic strain | Trial 1 | | | |
| | Trial 2 | | | |
| | Trial 3 | | | |
| | Average | | | |

- Measurements are in millimeters (mm)

Table 3: Data collection tables for *Colibacillinum* 30CH (**prepared from Uropathogenic strain**) in 43% ethanol on the growth of Escherichia coli: - Uropathogenic and Enteropathogenic strains using disc diffusion assay.

| Organism- E.coli | | Colibacillinum 30CH (in 43% ethanol) | 43% ethanol( - control) | Ciprofloxacin(+ control) |
|---|---|---|---|---|
| Uropathogenic strain | Trial 1 | | | |
| | Trial 2 | | | |
| | Trial 3 | | | |
| | Average | | | |
| Enteropathogenic strain | Trial 1 | | | |
| | Trial 2 | | | |
| | Trial 3 | | | |
| | Average | | | |

- Measurements are in millimeters (mm)

Table 4: Data collection tables for *Colibacillinum* 200CH (**prepared from Uropathogenic strain**) in 43% ethanol on the growth of Escherichia coli: - Uropathogenic and Enteropathogenic strains using disc diffusion assay.

| Organism- E.coli | | Colibacillinum 200CH (in 43% ethanol) | 43% ethanol( - control) | Ciprofloxacin(+ control) |
|---|---|---|---|---|
| Uropathogenic strain | Trial 1 | | | |
| | Trial 2 | | | |
| | Trial 3 | | | |
| | Average | | | |
| Enteropathogenic strain | Trial 1 | | | |
| | Trial 2 | | | |
| | Trial 3 | | | |
| | Average | | | |

- Measurements are in millimeters (mm)

Table 5: Data collection tables for *Colibacillinum* 3CH (**prepared from Enteropathogenic strain**) in 43% ethanol on the growth of Escherichia coli: - Uropathogenic and Enteropathogenic strains using disc diffusion assay.

| Organism- E.coli | | Colibacillinum 3CH (in 43% ethanol) | 43% ethanol( - control) | Ciprofloxacin(+ control) |
|---|---|---|---|---|
| Uropathogenic strain | Trial 1 | | | |
| | Trial 2 | | | |
| | Trial 3 | | | |
| | Average | | | |
| Enteropathogenic strain | Trial 1 | | | |
| | Trial 2 | | | |
| | Trial 3 | | | |
| | Average | | | |

- Measurements are in millimeters (mm)

Table6: Data collection tables for *Colibacillinum* 9CH (**prepared from Enteropathogenic strain**) in 43% ethanol on the growth of Escherichia coli: - Uropathogenic and Enteropathogenic strains using disc diffusion assay.

| Organism- E.coli | | Colibacillinum 9CH (in 43% ethanol) | 43% ethanol( - control) | Ciprofloxacin(+ control) |
|---|---|---|---|---|
| Uropathogenic strain | Trial 1 | | | |
| | Trial 2 | | | |
| | Trial 3 | | | |
| | Average | | | |
| Enteropathogenic strain | Trial 1 | | | |
| | Trial 2 | | | |
| | Trial 3 | | | |
| | Average | | | |

- Measurements are in millimeters (mm)

Table 7: Data collection tables for *Colibacillinum* 30CH (**prepared from Enteropathogenic strain**) in 43% ethanol on the growth of Escherichia coli: - Uropathogenic and Enteropathogenic strains using disc diffusion assay.

| Organism- E.coli | | Colibacillinum 30CH (in 43% ethanol) | 43% ethanol( - control) | Ciprofloxacin(+ control) |
|---|---|---|---|---|
| Uropathogenic strain | Trial 1 | | | |
| | Trial 2 | | | |
| | Trial 3 | | | |
| | Average | | | |
| Enteropathogenic strain | Trial 1 | | | |
| | Trial 2 | | | |
| | Trial 3 | | | |
| | Average | | | |

- Measurements are in millimeters (mm)

Table8: Data collection tables for *Colibacillinum* 200CH (**prepared from Enteropathogenic strain**) in 43% ethanol on the growth of Escherichia coli: - Uropathogenic and Enteropathogenic strains using disc diffusion assay.

| Organism- E.coli | | Colibacillinum 200CH (in 43% ethanol) | 43% ethanol( - control) | Ciprofloxacin(+ control) |
|---|---|---|---|---|
| Uropathogenic strain | Trial 1 | | | |
| | Trial 2 | | | |
| | Trial 3 | | | |
| | Average | | | |
| Enteropathogenic strain | Trial 1 | | | |
| | Trial 2 | | | |
| | Trial 3 | | | |
| | Average | | | |

- Measurements are in millimeters (mm)

## Appendix J: Quotation for statistics

Deepak Singh (DUT senior lecturer) M.sc

Faculty of Applied Sciences

Department of Mathematics, Physics & Statistics

Cell: 031 373 5266

Email: singhd@dut.ac.za

July 2016

The cost of your research would be R2500. It will be a complete chapter with tables and graphs, with all the necessary stats.

Deepak Singh

DUT DURBAN UNIVERSITY OF TECHNOLOGY

## Appendix K: Quotation for making up the remedy from DALGEN products.

**Bottles**

- 5ml clear glass bottle & cap is R3.60 each,
- BN101 25ml medical round amber & lid is R3.19 when you buy 1-5 box (133/tray).

**SHALOM laboratory suppliers**

- Ethanol absolute 99% 2.5L -R585.00
- Glycerin 500ml -R 85.00

February 2017

## Appendix L: Permission Application Letter to use National Health Laboratory at King Edward

HOD: Department of Medical Microbiology

Z1640 Mzwakhe Nene

Cycle

Umlazi township

4066

Department of Medical Microbiology

School of Laboratory Medicine & Medical Sciences

Inkosi Albert Luthuli Central Hospital Academic Complex

University of KwaZulu-Natal & National Health Laboratory Services

Durban, 4001

Dear Prof K. P. Mlisana

**Permission Application Letter to use the Microbiological Laboratory**

Thank you for reading this letter. My name is Nkululeko Xaba (21018336). I am currently registered for M. Tech. Homoeopathy and I am requesting to use the laboratory at National Health Laboratory in King Edward Hospital. My supervisor is Dr D.F Naude, senior lecturer, Homoeopathy and Iridologist practitioner, Co-supervisor is Dr S.K.K Pillai, a senior lecturer in the department of Biotechnology and Food

Technology. The tittle of my study is: A controlled invitro study of the antimicrobial effectiveness of *Colibacillinum* agains*t E. coli* using disc diffusion assay

**Outline of the Procedures:** The experimental study will take approximately a week to be conducted of which only a few hours will be used each day. Namely for make pure cultures of bacterial strains; medicated discs and to perform diffusion tests (disc). Everything will be done under the supervision of Dr Pratisha Mahabeer.

Yours faithfully.

Mr N. Xaba (210108336)-Researcher 072 5478 262

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Dr. D. Naude (Supervisor) – 031 373 2514 (David@dut.ac.za)

Dr. S. K.K Pillai (Co-Supervisor)- 031 373 5329 (Santhoshk@dut.ac.za)

## Appendix M: Permission letter to use National Health Laboratory

DEPARTMENT OF MEDICAL MICROBIOLOGY
NELSON R. MANDELA SCHOOL OF MEDICINE /
NATIONAL HEALTH LABORATORY SERVICE
LEVEL 4, Laboratory Building,
Inkosi Albert Luthuli Central Hospital
800 Bellair Road, Mayville, 4058

24 July 2017

Mr Nkululeko Xaba
C/o Dr David F. Naude
Senior Lecturer
Department of Homeopathy
Durban University of Technology
P O Box 1334
Durban
4000

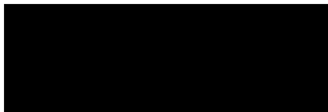
Via Email : david@dut.ac.za

***RE : Study Title: A controlled in vitro study of the antimicrobial effectiveness of Colibacillinum against E.coli***

This is to allow student Nkululeko Xaba the use of our laboratory space for his research study. The student will be under the supervision of Dr Mahabeer and he will clearly state the amount of time he needs to spend in the laboratory to complete his antimicrobial susceptibility testing.

We confirm that the student has committed to provide all the required consumables for his project. The lab will provide him bench space and use of laboratory equipment under supervision for his study.

Yours sincerely,



Prof Koleka P. Mlisana [MB ChB, MMedPath(Micro), PhD(Med Micro)]
Associate Professor & Head: Dept of Medical Microbiology
School of Laboratory Medicine & Medical Sciences
Inkosi Albert Luthuli Central Hospital Academic Complex
Univ of KwaZulu-Natal & National Health Laboratory Services
Durban, 4001
tel: +27 31 2402787; fax: +27 31 2402786
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*Prof Koleka P. Mlisana* *Head : Department of Medical Microbiology NHLS & UKZN*