AN EVALUATION OF THE RELATIVE EFFECTIVENESS OF MOTHER TINCTURE, 3X AND 8X HOMEOPATHIC GARLIC (*ALLIUM SATIVUM*) DILUTIONS ON FIVE STRAINS OF NOSOCOMIAL MULTIDRUG-RESISTANT MICRO-ORGANISMS IN TERMS OF BACTERIOSTATIC AND BACTERICIDAL EFFECTS IN ORDER TO VALIDATE THE CLINICAL USE OF GARLIC IN PATIENTS INFECTED BY THESE ORGANISMS.

by
Johannes Willem de Smidt

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Dedication

This dissertation is dedicated to the improvement of treatment for suffering humanity.

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Abstract

The purpose of this study was to test the relative effectiveness of extract, 3X and 8X homeopathic garlic (*Allium sativum*) dilutions on *Candida albicans* and nosocomial multi-drug-resistant strains of Methicillin-resistant *Staphylococcus aureus* (MRSA), *Eschericia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* in terms of bacteriostatic and bactericidal effects in order to validate the clinical use of garlic in patients infected by these organisms.

The test organisms were provided by the Department of Microbiology at the University of Stellenbosch Medical School. A 1:2 aqueous extract was prepared from fresh garlic bulbs. From the extract the 3X and 8X dilutions were made in distilled water according to the method of the German Homeopathic Pharmacopoeia. In the experimental group, these three garlic preparations and standardised solutions of each of the five test organisms were serially added, in equal quantities, to nine samples of nutrient broth, incubated and optically evaluated for turbidity against McFarlane standards. Samples showing garlic activity were plated out, incubated and the Colony Forming Units (CFU's) counted. Similarly, in the control group, solutions of each of the five test organisms were added to nine samples of nutrient broth, incubated and optically evaluated for turbidity against McFarlane standards. The CFUs of the experimental and control groups were statistically compared by means of the Kruskal-Wallis test.

Those strains against which garlic exhibited a bactericidal effect (kill = >99,9%), were subjected to a time/kill experiment to determine the time required for the garlic to kill the

organisms. Samples were removed from the broth dilutions at two hourly intervals, plated out, incubated and examined for growth.

Garlic in extract form exhibited a bactericidal effect against *E. coli* and *C. albicans*. There was a statistically significant difference between the number of CFUs in the experimental group and the control group ($p=0.001$ for 100μℓ garlic extract/*E. coli* and $p=0.0003$ for 200μℓ garlic extract/*E. coli*). A time/kill experiment demonstrated total kill of *E. coli* was achieved within 14 hours after addition of 100μℓ garlic extract and within 10 hours after addition of 200μℓ garlic extract. There was a statistically significant difference between the time taken to kill all *E. coli* with the 100μℓ compared to the 200μℓ ($p=0.0025$). All *C. albicans* were killed six hours after the addition of 100μℓ garlic extract.

Garlic extract had no bacteriostatic effect on MRSA, *K. pneumoniae* or *P. aeruginosa*. There was however a statistically significant difference between the experimental and control groups when MRSA and *K. pneumoniae* were tested.

The 3X and 8X dilutions demonstrated no bacteriostatic effect against any of the five test organisms.

These results confirm the body of research indicating the *in vitro* bacteriostatic and bactericidal effect of garlic in low dilutions, and extends these results to include a multidrug-resistant nosocomial strain of *E. coli*.

As *in vitro* experiments probably require a pharmacological effect, this research model might be unsuitable for the use of higher dilutions such as the 3X and 8X. The efficacy of these dilutions used as drainage remedies may be successfully demonstrated in clinical trials. Since the *in vitro* bactericidal effect of garlic has been demonstrated against a number of bacteria, fungi and parasites by numerous previous studies, clinical trials are the next step in ascertaining clinical efficacy of garlic.

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Table of Abbreviations and Definition of Terms

BSI - blood stream infection

CFU's - Colony forming units

ESBL - Extended-spectrum beta-lactamase

GNBM - Gram-negative bacillary meningitis

MBC - minimum bactericidal concentration

MIC - minimum inhibitory concentration

MP - metalloporphyrin

MRSA - Methicillin-resistant *Staphylococcus aureus*

NCCLS - National Committee for Clinical Laboratory Standards

SLPI - Secretory leukoprotease inhibitor

succussion - The vigorous shaking of dilutions during serial dilution.

TMP-SMX - Trimethoprim-sulphamethoxazole

UTI - urinary tract infection

X - Denoting a potency in a serial range of 1:10 dilutions, e.g. 3X is the third potency in such a range, representing a dilution of 1:1000 which has been succussed.

1. Chapter 1 - Introduction

Following the introduction of antibiotics into clinical use, multidrug-resistant bacteria have been increasingly causing problems world-wide (Carmeli *et al.*, 1999; Krcmery, 1999). These problems are especially evident within hospitals, where they frequently present as nosocomial epidemics (Dennesen *et al.*, 1998). The clinical significance of the problem of nosocomial multidrug-resistant micro-organisms, is that hospital personnel increasingly have to deal with micro-organisms which have caused a dramatic increase in infection-related morbidity (Colsky *et al.*, 1998; Dennesen *et al.*, 1998; Linden, 1998), which have a higher associated mortality rate than non-resistant strains (Stephenson, 1998; Krcmery *et al.*, 1998; Holmes *et al.*, 1996) and which exhibit increasing resistance to antimicrobial agents (Kristensen *et al.*, 1999; File, 1999; Linden, 1998). This, in turn, leads to a prolongation of hospital stays and an escalation of healthcare costs (Linden, 1998).

The dramatic increase in nosocomial infections is illustrated by figures quoted by Crowe *et al.* (1998) which show that the incidence of bacteraemia and fungaemia in the adult intensive care unit of a teaching hospital in Nottingham, UK, increased from 17,7 per 1000 admissions in 1985 to 80,3 in 1996. Of a total of 315 episodes of bacteraemia and fungaemia documented over a 12-year period, 82% were hospital-acquired.

Hospital-acquired infections add an estimated $4.5 billion per year to health care costs in the United States, according to Stephenson (1998). About one third could be prevented by infection control measures and more prudent use of antimicrobial agents. A matched cohort analysis by Carmeli *et al.* (1999) demonstrated a trend toward increased total charges in patients demonstrating emergence of resistance (difference, $7340).

Antibiotic resistance is "the inevitable consequence of the selective pressure of antimicrobial drug use and the adaptive plasticity of the micro-organisms" (Liu *et al.*, 1999). "We're in an era of Darwinopoly, where survival of the fittest bacterial pathogen means being resistant to antibiotics", according to dr. Fred Tenover, laboratory chief of the Centers for Disease Control and Prevention, Atlanta (cited in Stephenson, 1998).

Misuse and overuse of antimicrobials, especially extended-spectrum cephalosporins (File, 1999) and broad-spectrum oral antibiotics (Wiener *et al.*, 1999), cause selective pressure favouring resistant strains. Excessive and irrational use of antimicrobial drugs is a problem in all countries. It is particularly troublesome in developing countries where there is a heavy burden of infectious diseases (Liu *et al.*, 1999).

The three micro-organisms which historically have caused most problems, especially regarding acquired antimicrobial resistance, according to Weinstein (1998), are the "so-called nosocomial infection troika" - staphylococci, pseudomonads and *E. coli.*

Of these three, Weinstein (1998) singles out vancomycin-resistant *Staphylococcus aureus* as "the pathogen of greatest concern". Dennesen *et al.* (1998) agree, adding vancomycin-resistant enterococci and Enterobacteriaceae with extended-spectrum beta-lactamases (ESBLs) as causing "the most important nosocomial resistance problems on a global scale". An obvious reason for this concern is provided by Wichelhaus *et al.* (1998) who in their study found that the mortality rate of Methicillin-resistant *S. aureus* (MRSA) -infected patients was higher (28,6%) than the mortality rate of all patients (6,5%). (Strains of staphylococci resistant to penicillinase-resistant beta-lactamase inhibitors are termed methicillin resistant.)

With antibiotic options for patients with MRSA infections "already severely limited" (Santos *et al.*, 1999), infections with *S. aureus* with reduced susceptibility to vancomycin (Ehlert, 1999; Ge *et al.*, 1999; Rotun *et al.*, 1999; Santos *et al.*, 1999; Smith *et al.*, 1999) and teicoplanin have been recently reported for the first time (Paterson, 1999).

Dennesen *et al.* (1998) describe vancomycin-resistant enterococci and Enterobacteriaceae, with MRSA, as currently causing the most important nosocomial resistance problems on a global scale.

Eschericia coli resistance has been demonstrated to ampicillin, fluoroquinolones, gentamicin and TMP-SMX (Allen *et al.*, 1999; Osterlund and Olsson-Liljequist, 1999).

Pseudomonas aeruginosa resistance has been reported to amikacin, aztreonam, ceftazidime, cefepime, ciprofloxacin, gentamicin, imipenem, meropenem, ofloxacin, piperacillin/tazobactam, ticarcillin, and tobramycin (Bouza *et al.*, 1999; Doern *et al.*, 1999; Hanberger *et al.*, 1999; Lee *et al.*, 1999; Mokaddas and Sanyal, 1999).

With Pseudomonas, Klebsiella species represent the second major Gram-negative non fermenting organisms causing problems with multidrug-resistance (Bryan, 1989). Klebsiella spp. have been identified as frequent causes of human nosocomial infections by Hanberger *et al.* (1999) and Podschun and Ullmann (1998).

Outbreaks of infection by multidrug-resistant ESBL Klebsiella spp, reached crisis proportions at the Tygerberg Hospital neonatal ward in 1997. Of three premature babies thus infected, two recovered after nasogastric treatment with a garlic dose of 1 ml/kg. Garlic was only used for one day on the third baby, who died on the same day. (Wasserman, 1998).

K. pneumoniae resistance has been identified to ceftazidime, ciprofloxacin hydrochloride, gentamicin, tobramycin, TMP-SMX (Wiener *et al.*, 1999).

A high incidence of *Candida albicans* infections is reported by Pfaller *et al.* (1998) and Milan *et al.* (1999). *C. albicans* drug resistance to fluconazole, itraconazole, ketoconazole and miconazole has been reported by Boschman *et al.* (1998) and Milan *et al.* (1999).

Allium sativum and its extracts allicin and ajoene have been shown to be effective bacteriostatic and bactericidal agents *in vitro* against organisms which include gram positive bacteria such as staphylococcus spp. (Adetumbi & Lau, 1983; Rode *et al.*, 1989), gram negative bacteria such as *E. coli* (Adetumbi & Lau, 1983; Rode *et al.*, 1989), Klebsiella spp. (Adetumbi & Lau, 1983; Rode *et al.*, 1989), and fungi such as *C. albicans* (Adetumbi & Lau, 1983; Rode *et al.*, 1989; San-Blas *et al.*, 1989). Its efficacy against nosocomial multidrug-resistant organisms, however, remains to be evaluated.

The present study is the first to test the *in vitro* effect of higher, dynamised dilutions on these organisms.

In cases of infection by the above organisms, garlic in homeopathic form may be one of the remedies prescribed as the similimum, i.e. on the basis of the similarity between the symptomatology expressed by the patient and that of the materia medica drug picture. Thus an infection by *K. pneumonia*, a frequent cause of hospital-acquired pneumonia, may cause sputum like currant jelly (Berkow, 1992). Garlic is listed in Kent (1993) under "Expectoration: jelly-like" (Kent, *ibid.*) which refers to the rubric "gelatinous" (Kent, *ibid.*) and under the rubric "Expectoration: Bloody" (Kent, *ibid.*).

Similarly the diarrhoeal symptoms caused by *S. aureus, E. coli* or antibiotics (*Berkow, ibid.*), may correspond to the materia medica picture of garlic (Allen, 1975; Clarke, *ibid.*).

The use of dilutions such as 3X has been practised in drainage therapy or detoxification, an extension of homeopathy defined by dr. Leon Vannier (Maury, *ibid.*). Drainage remedies are described as any homeopathic remedy given in low potency (1x or 3x) (Maury, *ibid.*).

Drainage is defined as an organic cleansing process brought about by means of homeopathic remedies indicated according to the law of similars in relation to the sick organism and preparing the ground for the more effective action of constitutional remedies. (Maury, *ibid.*).

Applying the above principles to garlic, the primary effect of which is on the intestinal mucous membranes (Boericke, *ibid.*), it may be prescribed as a drainage remedy in cases of e.g. haemorrhagic colitis caused by *E. coli*.

The present study is significant, firstly because there is currently no scientific basis for the prescription of garlic in cases of infection by nosocomial multidrug-resistant organisms, as garlic has not been tested on multidrug-resistant nosocomial strains of e.g. Pseudomonas spp., Klebsiella spp., *S. aureus* and *C. albicans*. Secondly, because the *in vitro* bacteriostatic and bactericidal effects of homeopathic garlic dilutions against multidrug-resistant micro-organisms have not been established.

The implications of the current study is that it strengthens the body of evidence proving that garlic has bacteriostatic and bactericidal effects in vivo against bacteria and fungi which are drug-resistant. This provides a scientific basis for the motivation of a clinical trial, testing the efficacy of garlic on hospital patients. Seriously ill patients for whom no

effective management of intermittent outbreaks of multidrug-resistant nosocomial infections currently exists, as well as patients who cannot afford expensive drugs, would therefore ultimately benefit by the positive outcome of this study.

Hospital authorities would benefit financially if a scientific basis for the widespread clinical application of garlic, which is relatively cheap and readily available, could be provided.

Chapter 2: Review of the Related Literature

2.1. Nosocomial multidrug-resistant micro-organisms

The emergence of antibiotic-resistant organisms is increasing the potential for poor outcomes of common infectious diseases and is a serious problem world-wide (Carmeli *et al.*, 1999; Holmes *et al.*, 1996).

2.1.1. Incidence

The likelihood of acquiring a nosocomial infection in an American hospital rose by 36% in the past 20 years in terms of patient-days in the hospital, even as the number of patients admitted to US hospitals and their lengths of stay have dwindled, according to Stephenson (1998). In a survey it was found that in 1995 about 35.9 million patients were admitted to US hospitals for an average stay of 5.3 days, compared with 37.7 million patients who spent an average of 7.9 days in the hospital in 1975. However, the rate of infections per 1000 patient-days increased from 7.18 in 1975 to 9.77 in 1995. Of further concern, according to Stephenson, is the proportion of nosocomial infections — as many as 70% — that are resistant to 1 or more antibiotics. "A large proportion of nosocomial infections and deaths are caused by antibiotic-resistant pathogens," according to Jarvis (in Stephenson, 1998).

Kristensen *et al.* (1999), in a study of antibiotic resistance patterns among blood culture isolates in a Danish county from 1981-1995, report a 14% increase in resistance to penicillin among coagulase-negative staphylococci. The frequency of resistance to methicillin, gentamicin and erythromycin increased by respectively 38%, 26% and 32%, whereas a 14% decrease in resistance to streptomycin was recorded. A 20% increase of coagulase-negative staphylococci resistant to three or more antibiotics was observed. Results of a sur-

veillance of antibiotic resistance in hospitalised dermatology patients show "an alarming trend toward antibiotic resistance", Colsky *et al.* (1998) report. This study demonstrates the "rapid emergence of antibiotic-resistant bacteria as a problem of growing significance in hospital dermatology". Linden (1998), in a study on the clinical implications of nosocomial gram-positive bacteremia and superimposed antimicrobial resistance, states the coexistence of a "pathogen population with an ever-increasing resistance to many antibiotics and a patient population characterised by increasingly complex clinical problems" has contributed to an increase in the bloodstream infections associated with gram-positive bacteria. "This serious therapeutic challenge has already been associated with an increase in infection-related morbidity and mortality, a prolongation of hospital stays, and an escalation of health care costs."

Increasingly, microbes are becoming resistant to a substantial proportion of drugs considered first-line treatments, often necessitating the use of more costly antimicrobial agents. Resistance is having an impact not only on the therapy of individual patients but also on infection control in the hospital (Stephenson, 1998). "Anytime a lab reports a vancomycin-resistant Enterococcus, it triggers a cascade of infection control activities that are key to controlling resistance —and all of those activities are expensive," according to Tenover (in Stephenson, 1998).

2.1.2. Causes

Other factors, besides antimicrobial pressure, that cause a rise in resistant strains, are increased numbers of immunocompromised hosts, lapses in infection control (Wiener *et al.*, 1999), increased use of invasive procedures and devices (File, 1999), clonal spread of genes of resistance among and intra species, local epidemiology, nosocomial transmission

(Krcmery, 1999), and the widespread use of antibiotics in agriculture and animal husbandry (File, 1999).

On the subject of veterinary antibiotic use, Al-Ghamdi *et al.* (1999) call for the banning of antibiotics in the poultry industry as growth promoters and recommend that their use be restricted to treating infections, as chicken could possibly be a source of a resistance pool for humans. This followed on their study on the antibiotic resistance of *E. coli* isolated from poultry workers, patients and chicken in the eastern province of Saudi Arabia. Sero-typing of *E. coli* isolates showed that 27% of the organisms isolated from patients were overlapping with 10.9% of the chicken isolates. Resistance to spectinomycin reached 96% in *E. coli* chicken isolates and 71% in organisms isolated from humans. Use of this drug in Saudi Arabia is mostly limited to veterinary purposes.

2.2. Main nosocomial drug-resistant organisms causing treatment failure and successes achieved

2.2.1. GRAM-POSITIVE BACTERIA - *STAPHYLOCOCCUS AUREUS*

Support for the predominance of *S. aureus* in hospital infections, is provided by Doern *et al.* (1999), and Ako-Nai (1999). Support for the increased incidence and predominance of *S. aureus* in the field of acquired antimicrobial resistance, is given by Colsky *et al.* (1998), Crowe *et al.* (1998), Korting *et al.* (1998), Linden (1998), Mikasa *et al.* (1998) and Morgan *et al.* (1999).

A high morbidity rate in intensive care units is attributed to methicillin-resistance in *S. aureus* by Ehlert (1999) who found that MRSA is the major cause of nosocomial bac-teraemias. File (1999), who refers to recent reports indicating the appearance of outpatient

MRSA infections, regards MRSA and oxacillin-resistant *S. aureus* as "a significant problem in the hospital".

2.2.1.1. INCIDENCE

S. aureus was the bacterial organism most frequently isolated from patients with skin and soft tissue infections in 1997, as reported by the SENTRY Antimicrobial Surveillance Program in the United States and Canada (Doern *et al.*, 1999). Out of 1562 bacterial isolates recovered from hospitalised patients with skin and soft tissue infections in 30 U.S. and 8 Canadian medical centres between October and December 1997, the overall rank order of recovery of the six most common pathogens was *S. aureus* (42.6%) > *P. aeruginosa* (11.3%) > Enterococcus spp. (8.1%) > *E. coli* (7.2%) > Enterobacter spp. (5.2%) > beta-haemolytic streptococci (5.1%).

Ako-Nai *et al.* (1999), reporting on the bacteriology of neonatal septicaemia in Ile-Ife, Nigeria, found gram-positive organisms, specifically *S. aureus*, were predominant (33.8%) among bacteria cultured from proven cases of septicaemia.. The bacterial isolates were "relatively resistant" to antibiotics traditionally employed to treat cases of septicaemia.

2.2.1.2. Current clinical problems caused by MRSA

Skin and soft tissue were the frequent sites of infection and colonisation by MRSA (Swanston, 1999) while difficulties in treatment of MRSA have been reported in cases of septicaemia in neonates (Ako-Nai *et al.*, 1999) and neonatal bloodstream infections (Cordero *et al.*, 1999).

2.2.1.3. Susceptibility and prevalence of resistance

Since the emergence of MRSA, the glycopeptide. vancomycin. has been the only "uniformly effective treatment for staphylococcal infections". With antibiotic options for patients with MRSA infections "already severely limited" (Santos *et al.*, 1999), infections with *S. aureus* with reduced susceptibility to vancomycin (Ehlert, 1999; Ge *et al.*, 1999; Rotun *et al.*, 1999; Santos *et al.*, 1999; Smith *et al.*, 1999) and teicoplanin have been recently reported for the first time (Paterson, 1999). "Current antibiotic therapy is therefore difficult and expensive. Often a combination of several antibiotics has to be used" (Ehlert, 1999).

In 1997, two infections due to *S. aureus* with reduced susceptibility to vancomycin were identified in the United States (Smith *et al.*, 1999). *S. aureus* with reduced susceptibility to vancomycin isolated from a patient with fatal bacteremia is reported on by Rotun *et al.* (1999).

This follows after MRSA sensitivity of all isolates to vancomycin at the General Hospital, Port-of-Spain, between June 1995 and May 1996 was reported by Swanston (1999). However, all but one were resistant to gentamicin.

Other antibiotics to which *S. aureus* has exhibited resistance, include clindamycin, erythromycin, fluoroquinolones, gentamicin, imipenem and mupirocin (Harbarth *et al.*, 1999; Ogiwara *et al.*, 1999; Swanston, 1999).

Ogiwara *et al.* (1999), in a study on susceptibilities to various antimicrobial agents, found the sensitive strains of *S. aureus* to imipenem and clindamycin that were isolated from pa-

tients with urinary tract infections (UTIs) in 9 hospitals during June 1997 to May 1998, had increased during the period of 1996-1997, but had decreased again during the latest period.

In a surveillance of MRSA in Wales, Morgan *et al.* (1999) report the majority of isolates were resistant to at least two antibiotics in addition to methicillin, most frequently erythromycin and the fluoroquinolones. Very little resistance to fusidic acid, mupirocin or rifampicin was reported.

Harbarth *et al.* (1999) in a randomised, placebo-controlled, double-blind trial on the efficacy of mupirocin for eradicating carriage of MRSA carriage during outbreaks in a 1500-bed teaching hospital with endemic MRSA, found nasal mupirocin is only marginally effective in the eradication of multisite MRSA carriage in a setting where MRSA is endemic.

The uniform activity of vancomycin is reported on by Cordero *et al.* (1999), Doern *et al.* (1999), Mikasa *et al.* (1998), and Yurdakok (1998). Mikasa *et al.* found the sensitivity of MRSA to vancomycin was 100%, while Doern *et al.* in the findings of the SENTRY Antimicrobial Surveillance Program in the United States and Canada for 1997, report although 24.0% of *S. aureus* isolates of bacterial pathogens from patients with skin and soft tissue infections, were oxacillin resistant; vancomycin was uniformly active.

All strains of methicillin- resistant coagulase-negative Staphylococcus remained vancomycin-susceptible, Cordero *et al.* (1999) report in a study over 12 years of bloodstream infections (BSIs) in a neonatal intensive-care unit. Out of 363 infants born from 1986 to 1991 and 1992 to 1997 who developed 433 BSIs, all 17 new-born BSI cases were due to methicillin-sensitive strains of *S. aureus*.

Susceptibility has also been demonstrated with fusidic acid, mupirocin, penicillinase-resistant penicillins (e.g. oxacillin, nafcillin and methicillin), quinupristin/dalfopristin and rifampicin (Berner *et al.*, 1998; Chang *et al.*, 1999; Morgan *et al.*, 1999; Yurdakok, 1998). Yurdakok (1998), reporting on antibiotic use in neonatal sepsis, found that staphylococci were susceptible to penicillinase-resistant penicillins (e.g. oxacillin, nafcillin and methicillin). Resistant strains were uniformly sensitive to vancomycin. Likewise, none of the *S. aureus* isolates from a group of paediatric oncology patients reported in a study by Berner *et al.* (1998), were resistant to oxacillin.

Kimura *et al.* (1998), in a prospective, randomised, placebo-controlled study on TMP-SMX (TMP-SMX) for the prevention of MRSA pneumonia in severely burned patients who required ventilator support, found that prophylactic treatment with TMP-SMX can prevent MRSA pneumonia in severely burned patients. The incidence of MRSA pneumonia was 4,8% in the TMP-SMX group and 36,8% in the placebo group, showing a significant difference ($p = 0.017$).

Kristensen *et al.* (1999), in an analysis of all episodes of bacteraemia during a 15-year period (1981-1995) in the County of Northern Jutland, Denmark, with regard to antibiotic resistance, found acquired antibiotic resistance in Denmark was maintained at a low level compared with most other European countries and regions during the 15-year period studied.

Quinupristin/dalfopristin was active *in vitro* against clinical isolates of MRSA and methicillin-susceptible S. aureus in Taiwan, Chang *et al.* (1999) report.

2.2.2. GRAM-NEGATIVE BACILLI: ENTEROBACTERIACEAE - *ESCHERICIA COLI*

2.2.2.1. Prevalence of infection by E. coli

E. coli is acknowledged as an important cause of nosocomial infections by Gupta *et al.* (1999) and Doern *et al.* (1999). Authors who identified this resistant species as a common cause of nosocomial infections as well as being characterised by increased antimicrobial resistance, include Bryan (1989), Colsky *et al.* (1998), Crowe *et al.* (1998), Doern *et al.* (1999), Hanberger *et al.* (1999), Korting *et al.* (1998) Mikasa *et al.* (1998) and Quinn (1998).

Dennesen *et al.* (1998) describe vancomycin-resistant enterococci and Enterobacteriaceae, with MRSA, as currently causing the most important nosocomial resistance problems on a global scale.

The predominance of *E. coli* as uropathogen in acute uncomplicated cystitis in a large population of women is reported on by Gupta *et al.* (1999). With *Staphylococcus saprophyticus* it accounted for 90% of the 4342 urine isolates studied.

E. coli (7.2%) was fourth in the overall rank order of recovery of the six most common bacterial pathogens isolated from patients with skin and soft tissue infections, reported by the SENTRY Antimicrobial Surveillance Program in the United States and Canada in 1997, after *S. aureus* (42.6%), *P. aeruginosa* (11.3%) and Enterococcus spp. (8.1%), report Doern *et al.* (1999).

Crowe *et al.* (1998) identified *E. coli* (6%) as one of the predominant gram-negative bacteria isolated in their study.

Enterobacteriaceae were the most frequently isolated organisms (59%) in eighteen hospitals in Belgium, 40 in France, 20 in Portugal, 30 in Spain, and 10 in Sweden, report Hanberger *et al.* (1999) in their study on antibiotic susceptibility among aerobic gram-negative bacilli in intensive care units in these 5 European countries. The second highest incidence of resistance was seen in all countries among Enterobacter species.

A high prevalence of Enterobacter sp. (4%) is reported on in a study on UTI and antibiotic sensitivity in the south of Albacete, Spain, by Morales *et al.* (1999).

Between November 1990 and October 1992, 55 hospital patients infected or colonised with ceftazidime-resistant *E. coli, K. pneumoniae*, or both, were identified in a 400-bed tertiary care hospital and a community nursing home, Wiener *et al.* (1999) report.

2.2.2.2. Current clinical problems

Drug-resistant strains of *E. coli* are currently causing clinical problems such as UTIs (Ortiz *et al.*, 1999), acute uncomplicated cystitis in women (Gupta, 1999), spontaneous bacterial peritonitis (Ortiz *et al.*, 1999), spontaneous bacteremia (Ortiz *et al.*, 1999) and bacterascites (Ortiz *et al.*, 1999).

2.2.2.3. Susceptibility and prevalence of resistance

In a study on more than 4000 female patients with acute cystitis between 1992 and 1996, there was a statistically significant increasing linear trend in the prevalence of resistance from 1992 to 1996 among *E. coli* and all isolates combined to ampicillin, and to cephalothin, trimethoprim, and TMP-SMX, reports Gupta *et al.* (1999). In contrast, the prevalence of resistance to nitrofurantoin, gentamicin, and ciprofloxacin hydrochloride was 0% to 2% among *E. coli* and less than 10% among all isolates combined, and did not change signifi-

cantly during the 5-year period. The high prevalence of resistance to the 2 -lactams studied, ampicillin and cephalothin, precludes their use in empirical treatment. Of "considerably greater concern" is the increasing prevalence of resistance to trimethoprim and TMP-SMX, which are commonly used as first-line regimens in the treatment of acute cystitis. Similar trends have also been reported among urinary isolates from women attending outpatient clinics. Trimethoprim and TMP-SMX, may therefore soon be unacceptable choices for empirical therapy.

In their study on the antibiotic resistance patterns among blood culture isolates in a Danish county from 1981-1995, Kristensen *et al.* (1999) report although the frequency of ampicillin resistance increased by 9% among *E. coli* and by 10% in all Enterobacteriaceae, among Enterobacteriaceae the level of resistance to third-generation cephalosporins, carbapenems, aminoglycosides and fluoroquinolones remained low (<1%). The frequency of resistance to three or more antibiotics remained fairly stable among Enterobacteriaceae, although a slight increase of 5% was noted among *E. coli.*

Antibiotic resistance to ampicillin was found in 37.9% of *E. coli* as reported in a study on Beta-lactam-resistance patterns of some gram-negative rods by Janicka *et al.* (1999).

Of 1636 *E. coli* isolates in Canada, 736 (45.0%) were resistant to ampicillin, 514 (31.4%) were resistant to TMP-SMX, 363 (22.2%) were resistant to both ampicillin and TMP-SMX, and 27 (1.7%) were resistant to both ampicillin and gentamicin, Allen *et al.* (1999) report in a surveillance study conducted from December 1992 to December 1994.

Despite warnings about the risk of resistance development due to excessive fluoroquinolone prescription, fluoroquinolone-resistance, a growing international problem, is

now developing among *E. coli* strains isolated from primary care patients with UTIs in Sweden, according to Osterlund and Olsson-Liljequist (1999).

"Considerable resistance" to quinolones in the south of Albacete, Spain, is reported by Morales *et al.* (1999). *E. coli* present in 69% of the positive urocultures, "presented with widespread resistance to quinolones, pipemidic acid and nitrofuration, while it had high sensitivity to fosfomicin, cephuroxim and amoxicilin-clavulanic acid".

The susceptibilities to quinolones of *E. coli* isolated from complicated UTIs, had decreased during the period of 1995-1997, but those have recovered during the latest period, report Ogiwara *et al.* (1999). The susceptibilities of *E. coli* to MINO have been better in the latest period with the MIC90 ranging from 2 to 4 micrograms/ml.

The emergence of infections caused by *E. coli* resistant to quinolones has recently been observed in cirrhotic patients undergoing prophylactic norfloxacin, report Ortiz *et al.* (1999).

Vancomycin resistance among Enterococcus spp. (16.5%) was observed only in the U.S. and not in Canada, according to Doern *et al.* (1999). Numerous beta-lactams, aminoglycosides and fluoroquinolones were "broadly active" against *E. coli* SSTI isolates (i.e. < 5% resistance). ESBL production was uncommon both with *E. coli* and Klebsiella spp. in both nations. Cefepime, imipenem, and meropenem; the aminoglycosides; and fluoroquinolones were "conspicuously more active" against Enterobacter spp. than other agents tested. Cefepime generally retained activity against ceftazidime-resistant organisms.

Nitrofurantoin remains highly active against *E. coli* and most non–*E. coli* isolates and is a "reasonable option for empirical therapy", reports Gupta *et al.* (1999). The two oral agents with the best *in vitro* susceptibility profiles if TMP-SMX cannot be used, were nitrofuran-

toin and ciprofloxacin. Ciprofloxacin, a representative of the fluoroquinolone class of antimicrobials, "remains highly active against both *E. coli* and non–*E. coli* isolates in vitro". Although other studies have reported increasing fluoroquinolone resistance among *E. coli*, these authors did not identify any such isolates in this patient group. Although several factors influence the choice of an antimicrobial agent for empirical therapy of acute cystitis, the *in vitro* susceptibilities of common uropathogens clearly are an important consideration, they conclude.

All strains in 55 hospital patients who were admitted from nursing homes between November 1990 and July 1992 infected or colonised with ceftazidime-resistant *E. coli, K pneumoniae*, were resistant to ceftazidime, gentamicin, and tobramycin; 96% were resistant to TMP-SMX and 41% to ciprofloxacin hydrochloride, report Wiener *et al.* (1999). Infections caused by ceftazidime sodium-resistant gram-negative bacteria that harbour ESBLs are increasing in frequency in hospitals in the United States.

Reporting on the beta-lactam-resistance patterns of some gram-negative rods, Janicka *et al.* (1999) found 37.9% of 290 *E. coli* strains were resistant to ampicillin.

The susceptibility of *E. coli* has been reported to amoxicillin-clavulanic acid, cefotaxime, ceftazidime, ciprofloxacin, gentamicin, imipenem, moxifloxacin and other fluoroquinolones (Berner *et al.*, 1998; Cordero *et al.*, 1999; Korting *et al.*, 1998; Ortiz *et al.*, 1999; Tankovic *et al.*, 1999).

Over a period of twelve years, *E. coli* isolated from a new-born intensivecare unit, unlike those strains from other hospital units, remained fully susceptible to ceftazidime and gentamicin, report Cordero *et al.* (1999).

Out of 106 infections caused by *E. coli* in 99 hospitalised cirrhotic patients, none of the *E. coli* resistant to norfloxacin were also resistant to cefotaxime and only one of them was resistant to amoxicillin-clavulanic acid, reports Ortiz *et al.* (1999). Selective intestinal decontamination with norfloxacin is useful to prevent bacterial infections in several groups of cirrhotic patients at high risk of infection.

Korting *et al.* (1998) found gentamicin to be active against a large majority of Enterobacteriaceae strains, while Berner *et al.* (1998) found the antibiotic susceptibility profile was quite favourable in Enterobacteriaceae. All gram-negative bacteria were fully susceptible to ceftazidime, imipenem and ciprofloxacin.

Cross-resistance against aerobic gram-negative bacilli and enterococci is exhibited by moxifloxacin and other fluoroquinolones, report Tankovic *et al.* (1999). The in-vitro activity of moxifloxacin was greater than that of ofloxacin and slightly less than that of ciprofloxacin and sparfloxacin against Enterobacteriaceae .

2.2.3. GRAM-NEGATIVE BACTERIA: PSEUDOMONAS AERUGINOSA

2.2.3.1. Incidence and prevalence of infection

P. aeruginosa is a major nosocomial problem as a multiresistant nosocomial pathogen, especially in burns and other immunocompromised patients in their hospital, Hanberger *et al.* (1999) report. These authors also found *P. aeruginosa* (24%) was the most frequently isolated organism after Enterobacteriaceae (59%) taken from 18 hospitals in Belgium, 40 in France, 20 in Portugal, 30 in Spain, and 10 in Sweden.

P. aeruginosa (11.3%) was second in the overall rank order of recovery of the six most common bacterial pathogens isolated from hospitalised patients with skin and soft tissue

infections in 30 United States (U.S.) and 8 Canadian medical centres between October and December 1997, according to Doern *et al.* (1999).

Results from 1991-1998 demonstrate an increased incidence of superficial pus infection or colonisation with O12 *P. aeruginosa* and a endemic clonal spread of O12 *P. aeruginosa* in a general hospital in Rodez, France, and in neighbouring extended-care facilities, report Watine *et al.* (1999).

A high prevalence of Pseudomonas spp. (4%), which disagrees with other studies, was observed by Morales *et al.* (1999) in their study on UTI and antibiotic sensitivity in the south of Albacete, Spain, during 1997.

P. aeruginosa accompanied MRSA infections in 64,7% of cases, Mikasa *et al.* (1998) report. Crowe *et al.* (1998) identified *P. aeruginosa* as one of the predominant gram-negative bacteria, implicated in 5,1% cases of bacteraemia in the adult intensive care unit of a teaching hospital in Nottingham, UK, 1985-1996. Other authors who identified this resistant species as a common cause of nosocomial infections and as being characterised by increased antimicrobial resistance, include Bryan (1989), Colsky *et al.* (1998), Korting *et al.* (1998) and Quinn (1998).

2.2.3.2. Current clinical problems

P. aeruginosa endobronchial infection causes significant morbidity and mortality among cystic fibrosis patients (Burns *et al.*, 1999) while the organism has also been identified as the source of Severe Community-acquired Pneumonia (Ruiz *et al.*, 1999).

2.2.3.3. Susceptibility and prevalence of resistance

According to Hanberger *et al.* (1999), the highest incidence of resistance was seen in all

countries among *P. aeruginosa* (up to 37% resistant to ciprofloxacin in Portuguese ICUs and 46% resistant to gentamicin in French ICUs).

Out of 489 inpatients with positive clinical cultures for *P. aeruginosa*, 144 had a resistant baseline *P. aeruginosa* isolate and 30 had resistance emerge during follow-up, report Carmeli *et al.* (1999). The overall in-hospital mortality rate was 7.6%, 7.7% in patients with a resistant isolate at baseline and 27% in patients in whom resistance emerged. Secondary bacteremia developed in 1.4% of patients in whom resistance did not emerge and in 14% of those in whom resistance emerged.

Of 97 *P. aeruginosa* isolates from 97 patients, 35 were resistant to ceftazidime, report Lee *et al.* (1999) in a prospective study from 1994 to 1995.

The best susceptibility profiles have been demonstrated with amikacin, azlocillin, aztreonam, cefepime, cefoperazone, ceftazidime, gentamicin, imipenem, isepamicin, levofloxacin, meropenem, tobramycin, and piperacillin with or without tazobactam (Bert and Lambert-Zechovsky, 1999; Cappelletty, 1999; Cordero *et al.*, 1999; Doern *et al.*, 1999; Mokaddas and Sanyal, 1999; Segatore *et al.*, 1999; Yurdakok, 1998).

Amikacin and imipenem had the best susceptibility profiles in an Italian survey on comparative levofloxacin susceptibility in 334 clinical isolates of *P. aeruginosa* , report Segatore *et al.* (1999). In this national survey on susceptibility patterns of *P. aeruginosa* isolates from intensive care units and haematology and oncology wards from 13 Italian hospitals, the activity of levofloxacin, an injectable oral fluoroquinolone, was found to be superior to those of the other quinolones and was comparable to that of ceftazidime.

Among aminoglycosides, amikacin and isepamicin are the most frequently active drugs

against *P. aeruginosa*, according to Bert and Lambert-Zechovsky (1999). The use of fluoroquinolones is limited by a high incidence of acquired resistance.

Several antimicrobial agents which remained broadly active for SSTI isolates of *P. aeruginosa*, include meropenem, amikacin, tobramycin, and piperacillin with or without tazobactam (Doern *et al.*, 1999). Imipenem resistance was observed in 11.9% of isolates of *P. aeruginosa.* Ceftazidime, and cefepime were equally active (85.2% and 85.8% susceptible, respectively).

P. aeruginosa is least resistant to meropenem followed by imipenem and piperacillin/tazobactam, according to Mokaddas and Sanyal (1999). Cross resistance between the carbapenems and between carbapenems and piperacillin/tazobactam was found in their prospective study, conducted between June 1996 and December 1997. Of the 357 *P. aeruginosa* isolates tested from 188 patients 37 (10.4%) were resistant to imipenem, 21 (5.9%) to meropenem and 50 (14%) to piperacillin/tazobactam. Cross resistance between the two carbapenems was observed in 5.9% of the isolates. Sixteen (43%) of the imipenem-resistant isolates were susceptible to meropenem but the reverse was observed in none. Amongst the 50 piperacillin/tazobactam-resistant isolates, cross resistance with the two carbapenems was observed in 18 (36%) and in 9 (18%) only with imipenem; 23 (46%) were susceptible to both.

Cefepime is effective both as monotherapy and in combination therapy against a nonmucoid strain of *P. aeruginosa*, report Cappelletty (1999). All cefepime and ceftazidime monotherapy simulations resulted in 99.9% killing of the nonmucoid isolate within 4 to 8h and within 4 to 6h, respectively. Against the mucoid isolate, 99.9% killing was achieved only with combination therapy. Combination therapy with tobramycin and either cefepime

or ceftazidime enhanced the killing of both the mucoid and nonmucoid *P. aeruginosa* isolates.

Over a period of twelve years *P. aeruginosa* isolated from the new-born intensivecare unit, unlike those strains from other hospital units, remained fully susceptible to ceftazidime and gentamicin, report Cordero *et al.* (1999).

Data on 1014 isolates from a survey by Bouza *et al.* (1999) of *P. aeruginosa* resistance in 136 hospitals in Spain 1999 included resistance to the following antimicrobials:

Antimicrobial agent	Percentage of resistant isolates
piperacillin-tazobactam	7%
meropenem	8%
amikacin	9%
tobramycin	10%
piperacillin	10%
ticarcillin	13%
imipenem	14%
ceftazidime	15%
cefepime	17%
ciprofloxacin	23%
aztreonam	23%
ofloxacin	30%
gentamicin	31%

Table 2.1 - *P. aeruginosa* antimicrobial resistance in Spanish hospitals

The susceptibilities to most agents of *P. aeruginosa* from patients with UTIs in 1997 stayed constant, report Ogiwara *et al.* (1999). Decreased susceptibilities to cefozopran, carbapenems and monobactams of *P. aeruginosa* observed in 1996 appeared to have been retrieved in 1997.

P. aeruginosa is least resistant to meropenem followed by imipenem and piperacillin/tazobactam. Cross resistance between the carbapenems and between carbapenems and piperacillin/tazobactam was found by Mokaddas and Sanyal (1999).

Piperacillin and azlocillin was found to be the most active of extended-spectrum penicillins against *P. aeruginosa,* reports Yurdakok (1998) in a study on neonatal sepsis. Among the third-generation cephalosporins, cefoperazone and ceftazidime possess anti-Pseudomonas activity. New antibiotics for gram-negative bacteria resistant to other agents are carbapenems, aztreonam, quinolones and isepamicin.

2.2.4. GRAM-NEGATIVE BACTERIA - *KLEBSIELLA PNEUMONIAE*

With Pseudomonas, Klebsiella species represent the second major Gram-negative non fermenting organisms causing problems with multidrug-resistance (Bryan, 1989).

2.2.4.1. Incidence of infections

In an epidemiological study on Klebsiella spp. as nosocomial pathogens, Podschun and Ullmann (1998) identify bacteria of the genus Klebsiella as frequent causes of human nosocomial infections. The medically most important Klebsiella species, *K. pneumoniae*, accounts for "a significant proportion of hospital-acquired UTIs, pneumonia, septicaemia's, and soft tissue infections".

Because of their ability to spread rapidly in the hospital environment, these bacteria tend to cause nosocomial outbreaks (Podschun and Ullmann, 1998; Royle *et al.,* 1999; Shannon *et al.,* 1998; Wasserman, 1998; Wiener *et al.,* 1999). Hospital outbreaks of multidrug-resistant Klebsiella spp., especially those in neonatal wards, are often caused by new types

of strains, the ESBL producers. The incidence of ESBL-producing strains among clinical Klebsiella isolates has been "steadily increasing over the past years" (Podschun and Ullmann, 1998).

Outbreaks of infection by multidrug-resistant ESBL Klebsiella spp reached crisis proportions at the Tygerberg Hospital neonatal ward in 1997. Of three premature babies thus infected, two recovered after nasogastric treatment with a garlic dose of 1 ml/kg. Garlic was only used for one day on the third baby, who died on the same day. (Wasserman, 1998).

An hospital outbreak of ESBL-producing *K. pneumoniae* is reported by Shannon *et al.* (1998). Between July and September 1997 a ceftazidime- and aminoglycoside- resistant strain of *K. pneumoniae* infected seven patients on paediatric wards at Guy's Hospital in London.

Infections caused by ceftazidime sodium-resistant gram-negative bacteria that harbour ESBLs are increasing in frequency in hospitals in the United States, report Wiener *et al.* (1999). They report on a city-wide nursing home-centred outbreak of infections caused by ESBL-producing gram-negative bacilli occurred between November 1990 and October 1992. A total of 55 hospital patients infected or colonised with ceftazidime-resistant *E. coli, K pneumoniae*, or both were identified. Of the 35 admitted from 8 nursing homes, 31 harboured the resistant strain on admission. An outbreak of ESBL-producing *K. pneumoniae* in a neonatal unit is reported by Royle *et al.* (1999). Seven cases of septicaemia resulted in two deaths. No further episodes of sepsis occurred.

K. pneumoniae accounted for 14% of cases of septicaemia among neonates in Ile-Ife, Nigeria, report Ako-Nai *et al.* (1999). The bacterial isolates were "relatively resistant to antibiotics traditionally employed to treat cases of septicaemia".

2.2.4.2. *Current clinical problems*

Drug-resistant strains of *K. pneumoniae* have been identified as the aetiological agents of hospital-acquired UTIs (Podschun and Ullmann, 1998), nosocomial pneumonia (Podschun and Ullmann, 1998); nosocomial septicaemia's (Podschun and Ullmann, 1998); nosocomial soft tissue infections (Podschun and Ullmann, 1998); and neonatal septicaemia (Ako-Nai *et al.*, 1999).

2.2.4.3. *Susceptibility and prevalence of resistance*

The highest incidence of resistance in Portugal and France was seen among Klebsiella species (Hanberger *et al.*, 1999). A total of 85.7% of 56 Klebsiella sp. were resistant to the Beta-lactam ampicillin, report Janicka *et al.* (1999). *K. pneumoniae* was one of eight resistant pathogens identified in 93 patients with gram-negative bacillary meningitis (GNBM) at Kaohsiung Chang Gung Memorial Hospital, over a period of 12 years (Lu *et al.*, 1999). Resistance was demonstrated to third-generation cephalosporins, accounting for 9% of the total GNBM cases and 29% of the postneurosurgical GNBM cases. These eight patients, six males and two females aged 18-61 years, all had nosocomially acquired meningitis associated with head trauma and/or postneurosurgical states. Six patients received imipenem/cilastatin treatment; five survived and one died. The other two died because they did not receive appropriate antibiotic treatment.

Among Klebsiella spp. strains isolated from uncomplicated UTIs in 1997 by Ogiwara *et al.* (1999), those with low susceptibilities to most cephems have increased in the latest period. To other antimicrobial agents, the susceptibilities of Klebsiella spp. did not show any changes during the latest period.

All strains of *K. pneumoniae* identified by Wiener *et al.* (1999) in a nursing home-centred outbreak of infections caused by ESBL-producing gram-negative bacilli between November 1990 and July 1992 were resistant to ceftazidime, gentamicin, and tobramycin; 96% were resistant to TMP-SMX and 41% to ciprofloxacin hydrochloride.

The successful treatment of *K. pneumoniae* infections is reported with ceftazidime and gentamicin (Cordero *et al.*, 1999). Over a period of twelve years *K. pneumoniae* isolated from the new-born intensivecare unit remained fully susceptible to ceftazidime and gentamicin, report Cordero *et al.* (1999).

ESBL-production was uncommon among Klebsiella spp. in the United States of America and Canada in 1997, found Doern *et al.* (1999) in their SENTRY Antimicrobial Surveillance Program in the US and Canada on bacterial pathogens isolated from patients with skin and soft tissue infections.

2.2.5. FUNGI - CANDIDA ALBICANS

Candida species, which are normal intestinal flora, can invade tissue and produce life-threatening pathology in patients whose immune systems have been altered by disease or iatrogenic intervention (Murray *et al.*, 1995). The incidence of Candida fungaemia represents a serious patient management problem.

2.2.5.1. Incidence of infection

Candida was the fourth leading cause of nosocomial blood stream infection BSI in the USA between April 1995 and June 1996, accounting for 8% of all infections, report Pfaller *et al.* (1998) in a SCOPE Program national surveillance of nosocomial BSIs due to *C. albi-*

cans. Surveillance of nosocomial BSI revealed that 52% of 379 episodes of candidaemia were due to *C. albicans*.

Milan *et al.* (1999) found 82% of oral yeast carriage, 22% of them harbouring non-albicans species, among 132 AIDS patients admitted at a teaching tertiary care after swabbing oral cavities.

2.2.5.2. Susceptibility and prevalence of resistance

Drug resistance is emerging in many important microbial pathogens, including *C. albicans*, report Boschman *et al.* (1998) who conclude that although increased resistance to newer antifungal agents has occurred at their medical centre, it is not focal to any high-risk patient population. Fungal susceptibility tests with isolates obtained from 1984 - 1993 and fresh clinical isolates carried by cancer patients at a large medical centre obtained from 1994 - 1997 were tested for susceptibilities to fluconazole, ketoconazole, and miconazole and compared to the results of the rate of fluconazole use. All isolates recovered prior to 1993 were susceptible to fluconazole. Within 3 years of widespread azole use, resistance to all agents in this class was detected. A total of 101 yeasts were recovered from 97 patients and were tested for their susceptibilities to amphotericin B, fluconazole, flucytosine, ketoconazole, and miconazole. The yeasts from this surveillance study were at least as susceptible as the overall hospital strains. There did not appear to be a direct linkage between prior receipt of antifungal agent therapy and carriage of a new, drug- resistant isolate.

Overall rates of susceptibility dose dependent/resistance to azoles was 16% for itraconazole, 13% for ketoconazole, and 10% for fluconazole with a high agreement rate among the susceptibility profiles of all isolates tested against the triazoles, report Milan *et al.* (1999) in their study on azole resistance among oral Candida species isolates from AIDS

patients under ketoconazole exposure. Samples were obtained by swabbing the oral cavities of 132 AIDS patients admitted at a teaching tertiary care.

Treatment successes have been reported against *C. albicans* infections with 5-fluorocytosine, D0870 (a new triazole antifungal), amphotericin B, fluconazole, flucytosine, intraconazole, itraconazole and miconazole (Cartledge *et al.*, 1998; Cordero *et al.*, 1999; Pfaller *et al.*, 1998; and Yamazumi *et al.*, 1998).

Oral candidiasis, considered a defining illness for Aids (Murray *et al.*, 1995), has been successfully treated with garlic in four Tygerberg Hospital Aids patients. Patients who could not afford expensive antibiotics such as fluconazole, were cured of the oral candidiasis within 24 hours after having chewed a clove of garlic, twice a day for 15 -20 minutes. (Bouic, 1997).

In vitro susceptibility studies demonstrated that 92% of *C. albicans* isolates were susceptible to 5-fluorocytosine and 90% were susceptible to fluconazole and itraconazole, report Pfaller *et al.* (1998) in their national USA surveillance of nosocomial blood stream infection due to *C. albicans* in the SCOPE Program.

Most isolates of 285 strains of *C. albicans* isolates tested at Kinki University Hospital from March 1995 to December 1996, showed a relatively low MIC value, Yamazumi *et al.* (1998) report. The antifungal agents tested were fluconazole, miconazole, intraconazole, amphotericin B and flucytosine. Only one strain showed high resistance against fluconazole and it showed cross-resistance against miconazole and itraconazole. There were two flucytosine resistant strains. No strains were resistant to amphotericin B.

All fungi recovered from 48 patients over twelve years by Cordero *et al.* (1999) were susceptible to amphotericin.

D0870, a new triazole antifungal, shows promise in the treatment of fluconazole-resistant oro-oesophageal candidosis and was well tolerated, reports Cartledge *et al.* (1998) in their study on the treatment of HIV-related fluconazole-resistant oral candidosis. Of 26 evaluable HIV-seropositive patients with oro-oesophageal candidosis despite at least 7 days of treatment with fluconazole at doses of 100 mg per day or more, 16 showed partial improvement, nine showed no improvement, and only one had full clearance of thrush by day 7. *In vitro* testing of the cleared patient's isolate suggested that it was susceptible to fluconazole.

2.3. Risk factors

The following risk factors for infection or colonisation with multidrug-resistant microorganisms have been identified:

- **Selective antimicrobial pressure** (Allen *et al.*, 1999; Krcmery *et al.*, 1998; Lee *et al.*, 1999; Santos *et al.*, 1999; Swanston, 1999; Weber *et al.*, 1999; Wiener *et al.*, 1999). Continuous antibiotic use is one of the risk factors for resistance to "first-line" antimicrobials among urinary tract isolates of *E. coli* in children, conclude Allen *et al.* (1999). Subjects who had received antimicrobials for more than 4 weeks in the previous 6 months were about 23 times more likely to have isolates resistant to TMP-SMX than were subjects without this risk factor. Previous cephalosporin or piperacillin use are independent risk factors for nosocomial ceftazidime-resistant *P. aeruginosa* infection, report Lee *et al.* (1999). Recent antimicrobial therapy (79%) was the fourth greatest risk factor associated with MRSA infection or colonisation, found Santos *et al.* (1999). A

total of 17 of the 18 patients with MRSA had received antibiotics previously, including 13 who had received multiple antibiotics, according to Swanston (1999). Patients infected with resistant strains of bacteria are more likely than control patients to have received prior antimicrobials, and hospital areas that have the highest prevalence of resistance also have the highest rates of antibiotic use, report Weber *et al.* (1999). Prior receipt of ciprofloxacin or TMP-SMX was a predictor of resistance, report Wiener *et al.* (1999).

- **Antimicrobial prophylaxis** (Krcmery *et al.*, 1998; Ortiz *et al.*, 1999). Continuous long-term prophylaxis with norfloxacin favours the development of infections caused by norfloxacin-resistant *E. coli* (Krcmery, 1999; Ortiz *et al.*, 1999). As cancer departments, mainly in centres treating haematologic malignancies and organising bone marrow transplantation (BMT), are known to have extensive consumption of either prophylactically or therapeutically administered antibiotics and antifungals, it is significant that the first strains of quinolone resistant *E. coli*, vancomycin resistant enterococci and staphylococci and fluconazol-resistant *C. albicans* appeared in the patients treated for cancer with antineoplastic chemotherapy, resulting in profound granulocytopenia (Krcmery, 1999).

- **Therapy with several classes of antimicrobials** (Krcmery *et al.*, 1998) or with Beta-lactamase-inhibitors or cephalosporins (Bryan, 1989);

- **Multiple admissions to hospital** (Allen *et al.*, 1999). Compared with children who had no hospital admissions in the previous year, those with 1 admission in that period were more likely to have resistant isolates, as were those with 2 or more admissions in that period.

- **Severe disease, trauma** (File, 1999; Hanberger *et al.*, 1999; Kimura *et al.*, 1998; Weber *et al.*, 1999; Wichelhaus *et al.*, 1998). Owing to the high rate of antibiotic use and other risk factors, a person is more likely to acquire an antibiotic-resistant infection in the ICU than anywhere else, either inside or outside the hospital according to File (*ibid.*). Responsible antibiotic use and stringent infection-control policies are needed to discourage the development of resistant strains. Weber *et al.* (1999) found patients hospitalised in ICUs are 5 to 10 times more likely to acquire nosocomial infections than other hospital patients. As the infection rates are much higher in ICUs than in other hospital wards and most epidemics with multiresistant bacteria originate in ICUs, surveillance of antibiotic resistance is especially important in ICUs (Hanberger *et al.*, 1999).

- **Prolonged hospitalisation** (Bryan, 1989; Carmeli *et al.*, 1999; Wichelhaus *et al.*, 1998) Emergence of resistance, but not baseline resistance, was significantly associated with a longer hospital stay, according to Carmeli *et al.* (1999). The greatest risk factor associated with MRSA infection or colonisation, according to Santos *et al.* (1999) is >7 days hospitalisation (95%). A study by Mokaddas and Sanyal (1999) suggests that burns, cardiac-neuro-pediatric surgical, cancer and transplant patients are more susceptible to acquiring infection due to multiresistant *P. aeruginosa* than other types of patients.

- **Invasive procedures** (Santos *et al.*, 1999) such as the presence of a gastrostomy tube (Wiener *et al.*, 1999) and wound infection after surgery (Bryan, 1989);

- **Poor functional level** (Wiener *et al.*, 1999);

- **Decubitus ulcers** (Wiener *et al.*, 1999); and

- **Very dependent patients** (Santos *et al.*, 1999) was one of the main risk factors associated with MRSA infection or colonisation.

2.4. Management recommendations

Dennesen *et al.* (1998) ascribe the existence of multiresistant micro-organisms and their "concurrent spread to lapses in compliance with infection control measures". The following recommendations have been made to contain multidrug-resistant micro-organisms:

- **Rationing of antibiotics** (Al-Ghamdi *et al.*, 1999; Dennesen *et al.*, 1998; File, 1999; Linden, 1998; Ortiz *et al.*, 1999; Smith *et al.*, 1999; Weber *et al.*, 1999; Wiener *et al.*, 1999). As prophylaxis with norfloxacin favours the development of infections caused by norfloxacin-resistant *E. coli,* according to Ortiz *et al.* (1999), long-term antibiotic prophylaxis should be restricted to highly selected groups of patients at high-risk of infection. Due to the risk of high-level resistance developing among *E. coli* strains further exposed to fluoroquinolones, the prescribing of these drugs, according to Osterlund and Olsson-Liljequist (1999), should be limited. Programs to prevent or control the development of resistant organisms, report Weber *et al.* (1999), often focus on the overuse or inappropriate use of antibiotics, for example, by restriction of widely-used broad-spectrum antibiotics (e.g., third-generation cephalosporins) and vancomycin. Other approaches are to rotate antibiotics used for empirical therapy and use combinations of drugs from different classes. The importance of the prudent use of antibiotics, according to Smith *et al.* (1999) is highlighted by the emergence of *S. aureus* with intermediate resistance to glycopeptides. The Joint Commission on Accreditation of Healthcare Organizations says that a system should be in place to monitor and evaluate prophylactic, therapeutic, and empirical use of drugs. Other possible strategies include mandatory

auditing of antimicrobial drugs, pharmacy or formulary controls, clinical guidelines, and "antibiotic stop orders," whereby a pharmacy computer would automatically limit a prescription after a specified period of time (Stephenson, 1998). Nursing homes should monitor and control antibiotic use and regularly survey antibiotic resistance patterns among pathogens, conclude Wiener *et al.* (1999). Assessment of risks of antibiotic prophylaxis among cancer patients with quinolones and azoles and extensive use of empirical therapy with glycopeptides and polyenes needs to be considered, according to Krcmery (1999). Intensive prophylactic strategies should be limited to group of high risk, leukaemic patients or bone marrow transplantation recipients.

- **Stringent infection-control policies** (File, 1999; Smith *et al.*, 1999). Strict attention to asepsis is propagated by Dennesen *et al.* (1998), Hughes (1999), Mikasa *et al.* (1998), Royle *et al.* (1999), and Wichelhaus (1999). Drug resistant pathogens are becoming "an increasing problem affecting paediatric patients, requiring strict adherence to infection control hygiene measures to prevent transmission to susceptible patients," concludes Hughes (1999). The hospital outbreak of ESBL-producing *K. pneumoniae* reported by Shannon *et al.* (1998) was controlled by patient isolation and attention to hand washing, and there were no fatalities. Control of the spread of nosocomial MRSA must include the reinforcement of hand washing and appropriate isolation of patients in the surgical and intensive care wards, suggests Swanston (1999). Frequent hand washing, one of the simplest infection control measures, (Stephenson, 1998), has, however, been "dismayingly difficult to implement". Studies have documented that health care workers often fail to wash their hands after using the toilet or examining patients. "The 'Holy Grail' for infection control is finding a way to improve hand washing." (Dr. Robert Weinstein, head of the infection control program at Cook Country Hospital, Chicago, Illinois, in

Stephenson, 1998). Weinstein suggested that hospitals may follow the fast-food industry, which is experimenting with microchips on employee badges that light up or signal with a buzzing noise when workers fail to wash their hands after using the restroom. An outbreak of ESBL-producing *K. pneumoniae* in a neonatal unit, as reported by Royle *et al.* (1999), was controlled using simple measures such as prevention of cross infection by strict attention to hand washing. Proper handwashing, according to Weber *et al.* (1999), should be part of effective infection control programs, which should also include appropriate patient isolation, prompt evaluation and intervention when an outbreak occurs, adherence to standard guidelines on disinfection and sterilisation, and an occupational health program for health-care providers.

- **Increased surveillance measures** (Boschman *et al.*, 1998; Carmeli *et al.*, 1999; Colsky *et al.*, 1998; Cordero *et al.*, 1999; Crowe *et al.*, 1998; Hanberger *et al.*, 1999; Weber *et al.*, 1999; Webster *et al.*, 1998). Efforts should be directed toward early detection and prevention of emergence of antibiotic resistance, as the emergence of antibiotic resistance in *P. aeruginosa* results in severe adverse outcomes, concludes Carmeli *et al.* (1999). Hanberger *et al.* (1999) conclude that surveillance of antibiotic resistance is especially important in ICUs because of the much higher infection rates there than in other hospital wards and because most epidemics with multiresistant bacteria originate in ICUs. Continuous total population surveillance has provided "a minimum incidence of MRSA in Wales and has allowed a simple and intelligible picture of the problem to be determined, which has been fed back to hospitals to assist decisions on control," report Morgan *et al.* (1999). Surveillance obviously includes the laboratory capacity to identify resistant strains (Smith *et al.*, 1999). Cordero *et al.* (1999) recommend controlled antibiotic programs and periodic evaluations based on individual unit, and not on hospital-

wide antibiograms, are advisable. Continued surveillance for infections caused by *C. albicans* and other species of Candida among hospitalised patients is recommended by Pfaller *et al.* (1998). Monitoring of susceptibility to antifungal agents appears to be necessary for optimising clinical therapeutic decision making, according to Boschman *et al.* (1998).

- **Screening of patients** (Shannon *et al., 1998*) by the increased use of typing methods (Podschun and Ullmann, 1998; Santos *et al.,* 1999). Control and prevention of MRSA infections should include early identification of patients at higher risk of MRSA acquisition and analysis of isolates by discriminatory bacterial DNA typing methods, conclude Santos *et al.* (1999).

- **Institution of new measures**, e.g. vaccination (Podschun and Ullmann, 1998). Such a new genetically engineered vaccine, the *E. coli* O157 vaccine, tested in a small group of volunteers appears safe and stimulates the production of antibodies against the potentially fatal pathogen. A vaccine against *E. coli* O157 would be a "valuable weapon" against the pathogen, because the infection doesn't respond well to antibiotics. Moreover, some researchers who believe that antibiotics may increase the incidence of a sometimes fatal complication of the infection, haemolytic uraemic syndrome, by promoting the release of a bacterial toxin into the bloodstream, tested the vaccine on a group of 87 volunteers, nearly all of whom developed antibodies within one week, with no adverse effects "other than a mild irritation at the site of the injection".

- **Definition of strategy against multidrug-resistant micro-organisms**

- **Rapid development of new antimicrobial agents** (Dennesen *et al.*, 1998; Linden, 1998). The development of such a new agent, Secretory leukoprotease inhibitor (SLPI), a native antimicrobial protein, is reported on by Tomee *et al.* (1998) who conclude that, given the "unpropitious development of drug resistance to infectious micro-organisms in the human population, the need for therapeutic alternatives in the treatment of infectious diseases has become clear". SLPI may prove valuable in the prophylaxis and future treatment of infectious diseases, yet the clinical efficacy of SLPI remains largely to be elucidated. Scientific evidence suggests that SLPI may have a broad spectrum antibiotic activity that includes antiretroviral, bactericidal, and antifungal activity. Ge *et al.* (1999) suggest new strategies for designing glycopeptide antibiotics that overcome bacterial resistance in the form of Vancomycin analogues containing modified carbohydrates which are very active against resistant micro-organisms. A new group of potent antibacterial compounds, non-iron metalloporphyrins (MPs), is described by Stojiljkovic *et al.* (1999). Non-iron metalloporphyrins exploit haem/Hb uptake systems of pathogenic bacteria. MPs possess a strong antibacterial activity against Gram-positive bacteria, Gram-negative bacteria and mycobacteria. Anaerobically grown bacteria and micro-organisms that do not respire and/or express haem uptake systems were resistant to MPs. Antibacterial activity of MPs was not affected by known antibiotic resistance mechanisms operating in bacteria. The most potent MP against *Y. enterocolitica*, MRSA and *M. smegmatis* was gallium protoporphyrin IX (Ga-PPIX). When tested alone, Ga ions and metal-free porphyrins had approximately 100- fold higher minimum inhibitory concentration (MIC) values for these organisms. Ketolides represent a new generation of macrolide antibiotics, reports Xiong *et al.* (1999). Novel antibiotics to combat staphylococcal bacteraemias, which prevent further spread of resistance are urgently needed, reports Ehlert (1999). One approach might be the investigation of the mechanism of methicillin resistance, which is mediated by PBP2a, an

additional penicillin- binding protein present in resistant strains with low affinity to ss-lactams. These proteins might serve as attractive novel anti-staphylococcal targets for a small-range antibiotic.

2.5 Garlic, Homeopathy and Drainage

2.5.1. GARLIC (*ALLIUM SATIVUM*)

A. sativum, and its extracts allicin and ajoene, have been shown to be effective bacteriostatic or bactericidal agents *in vitro* against organisms which include gram positive bacteria such as staphylococcus spp. (Adetumbi & Lau, 1983; Rode *et al.,* 1989), gram negative bacteria such as *E. coli* (Adetumbi & Lau, 1983; Rode *et al.,* 1989) and Klebsiella spp. (Adetumbi & Lau, 1983; Rode *et al.,* 1989), and fungi such as *C. albicans* (Adetumbi & Lau, 1983; Rode *et al.,* 1989; San-Blas *et al.,* 1989). Its efficacy against nosocomial multi-drug-resistant organisms, however, remains to be evaluated.

The MIC of standard garlic dilutions on a range of gram positive and gram negative organisms and fungi determined in the Rode study (1989) ranged from 1/4 for *P. aeruginosa* to 1/32 for *S. aureus* and *C. albicans*. Higher homeopathic garlic dilutions, e.g. 1:1000, as tested in this study, have not been tested against these organisms.

2.5.1.1. Efficacy of garlic against specific organisms

2.5.1.1.1. STAPHYLOCOCCUS AUREUS

Garlic in crude form has a bacteriostatic effect *in vitro* on *S. aureus* (Adetumbi and Lau, 1983; Anesini and Perez, 1993; Cavallito and Bailey, 1944; Chen *et al.,* 1985; Dankert *et al.,* 1979; Gonzalez-Fandos *et al.,* 1994; Huddleson *et al.,* 1944; Jezpwa *et al.,* 1966;

Kumar and Berwal, 1998; Naganawa *et al.*, 1996; Rhode *et al.*, 1989; Sharma *et al.*, 1977) as well as a bactericidal effect (Rhode *et al.*, 1989).

Dankert *et al.* (1979) in a study on the antimicrobial activity of crude juices of *A. sativum*, found *S. aureus*. had a high D-value, whilst the bacteriostatic concentration was low. Crude juices of garlic showed strong antibiotic properties and the complete absence of development of resistance when tested in an agar diffusion test for their growth inhibitory effect on five gram negative and three gram positive bacterial species and two yeast species. All test organisms were inhibited by garlic juice.

Jezpwa *et al.* (1966), using serial dilution and filter paper disk techniques, found fresh garlic and vacuum dried, powdered garlic preparations were effective against organisms including *S. aureus*. At dilutions up to 1/32, garlic has a bacteriostatic effect against drug-resistant *S. aureus* (Jezpwa *et al.*, 1966). A high response toward the vacuum-dried garlic preparation was shown by bacteria resistant to two antibiotics tested.

Kumar and Berwal (1998) found garlic has an inhibitory activity against *S. aureus* as measured by the "turbidity" method. The MIC of garlic at 80% inhibition level was calculated for these bacteria.

Gonzalez-Fandos *et al.* (1994) in their study on staphylococcal growth and enterotoxins and thermonuclease synthesis in the presence of dehydrated garlic, found the growth of *S. aureus* was inhibited by dehydrated garlic at levels of 1.5% (w/v) and over. Enterotoxins A, B and C1 were only detectable in broth containing <1% of garlic while enterotoxin D was produced at a level of 2%.

Anesini and Perez (1993), who screened plants used in Argentine folk medicine for antimicrobial activity, found a boiling water extract of *A. sativum* bulbs was among the 12 plant species active against a penicillin G resistant strain of *S. aureus*.

Rhode *et al.* (1989) determined MIC dilutions of 1/32 for *S. aureus* ATCC 25923. No bactericidal effect was obtained. For MRSA, MIC and MBC values of 1/16 and 1/8 respectively were determined. Kill was achieved within ten hours.

Sharma *et al.* (1977), using the crude extract of garlic against 12 species of bacteria, found all spp. except *P. aeruginosa* produced a zone of inhibition in the presence of garlic. Garlic inhibited the growth of some bacterial cultures which were resistant to some of the 12 antibiotics tested.

Ajoene, a garlic-derived sulphur-containing compound that prevents platelet aggregation, exhibited broad-spectrum antimicrobial activity. *S. aureus* were inhibited below 20 micrograms of ajoene per ml, according to Naganawa *et al.* (1996). The microbicidal effect of ajoene on growing cells was observed at slightly higher concentrations than the corresponding MICs. However, the minimal microbicidal concentrations for resting cells were at 10 to 100 times higher concentrations than the corresponding MICs.

S. aureus was not susceptible to garlic extract up to the maximum thiosulfinate concentration tested (160 micrograms/ml), report Sivam *et al.* (1997).

Staphylococcus spp. were susceptible to allicin, an active ingredient from garlic, at a low concentration of 1:125 000, according to Cavallito and Bailey (1944).

2.5.1.1.2. ESCHERICHIA COLI

Garlic up to a concentration of 1/16 has bacteriostatic activity against *E. coli* (Adetumbi and Lau, 1983; Anesini and Perez, 1993; Chen *et al.*, 1985; Chowdhury *et al.*, 1991; Jezpwa *et al.*, 1966; Kumar and Berwal, 1998; Rhode *et al.*, 1989; Sharma *et al.*, 1977) as well as a bactericidal effect within at least ten hours at concentrations up to 1/4 (Johnson and Vaughn, 1969; Rhode *et al.*, 1989).

Boiling water extracts of *A. sativum* bulbs was among the 12 species plant materials active against *E. coli,* reports Anesini and Perez (1993).

E. coli was most sensitive to garlic, which has an inhibitory activity against *E. coli* as measured by the "turbidity" method, according to Kumar and Berwal (1998).

Freshly-reconstituted, dehydrated garlic had a lethal effect on *E. coli*, according to Johnson and Vaughn (1969), which became non-viable in 2-6 hours in the presence of 10% garlic extract.

E. coli ATCC 25922 was inhibited at a MIC dilution of 1/16 and killed at an MBC value of 1/4, found Rhode *et al.* (1989). Kill was achieved with in ten hours.

For gram-negative bacteria, such as *E. coli*, ajoene MICs were between 100 and 160 micrograms/ml, report Naganawa *et al.* (1996).

2.5.1.1.3. KLEBSIELLA PNEUMONIAE

Garlic has an inhibitory effect on *K. pneumoniae* (Adetumbi and Lau, 1983; Jezpwa *et al.*, 1966; Rhode *et al.*, 1989; Sharma *et al.*, 1977; Vijaya, 1997) as well as a bactericidal effect within ten hours at concentrations up to 1/16 (Rhode *et al.*, 1989).

K. pneumoniae ATCC 13883 was killed at a MBC dilution of 1/16 (Rhode *et al.*, 1989). Kill was achieved within ten hours.

Outbreaks of infection by multidrug-resistant ESBL Klebsiella spp, reached crisis proportions at the Tygerberg Hospital neonatal ward in 1997. Of three premature babies thus infected, two recovered after nasogastric treatment with a garlic dose of 1 ml/kg. Garlic was only used for one day on the third baby, who died on the same day. (Wasserman, 1998).

Ajoene MICs for gram-negative bacteria, such as *K. pneumoniae* were between 100 and 160 micrograms/ml, found Naganawa *et al.* (1996).

2.5.1.1.4. PSEUDOMONAS AERUGINOSA

Garlic has an inhibitory effect on *P. aeruginosa* (Chen *et al.*, 1985; Dankert *et al.*, 1979; Rhode *et al.*, 1989) as well as a bactericidal effect at concentrations up to 1/4 within fourteen hours (Rhode *et al.*, 1989).

Dankert *et al.* (1979) found *P. aeruginosa* had a very low D-value, whilst the bacteriostatic concentration was high. This indicates a large concentration exponent of crude garlic juice for this organism. Crude juice of garlic was tested in an agar diffusion test for their growth inhibitory effect on five gram negative and three gram positive bacterial species and two yeast species. All test organisms were inhibited by garlic juice.

P. aeruginosa ATCC 27853 and *P. aeruginosa* NTCC 10662 were both killed at MBC dilutions of 1/4, reports Rhode *et al.* (1989). Kill was achieved within 14 hours.

Sharma *et al.* (1977), using the crude extract of garlic against 12 species of bacteria, found all spp. except *P. aeruginosa* produced a zone of inhibition in the presence of garlic.

Outbreaks of infection by multidrug-resistant *P. aeruginosa* reached crisis proportions at Tygerberg Hospital, Cape Town, in 1997. A terminal Guillain-Barré patient, who developed nosocomial pneumonia due to multidrug- resistant *P. aeruginosa*, recovered from pneumonia after treatment with garlic. (Wasserman, 1998). Due to the lack of scientific studies on the effect of garlic on nosocomial multidrug-resistant micro-organisms, the substance however cannot be applied widely. The present study adds to the body of evidence indicating the *in vitro* efficacy of garlic.

2.5.1.1.5. CANDIDA ALBICANS

Garlic up to concentrations of $5x10^{-3}$ has a fungistatic effect on *C. albicans* (Adetumbi and Lau, 1983; Appleton and Tansey, 1975; Dankert *et al.*, 1979; Ghannoum, 1988; Jezpwa *et al.*, 1966; Kabelik, 1970; Tansey and Appleton, 1975; Moore and Atkins, 1977; Rhode *et al.*, 1989; Sandhu *et al.*, 1980; Yoshida *et al.*, 1999; Yoshida, 1987) as well as a fungicidal effect within six hours up to concentrations of 1:128 (Arora and Kaur, 1999; Rhode *et al.*, 1989).

C. albicans was completely inhibited by garlic extract tested in liquid medium with a final concentration of $5x10^{-3}$ fresh garlic extract, Appleton and Tansey (1975) report. After the 21 day incubation period, growth was checked and viability tested in tubes that showed no growth.

Dankert *et al.* (1979) found MICs in a dilution test were low for both yeast species tested.

Garlic extract was more effective than nystatin against pathogenic yeasts especially *C. albicans*, reports Arora and Kaur (1999) and Kabelik (1970).

Several species of yeast, including *C. albicans*, were inhibited in the presence of aqueous extract of fresh garlic, reports Moore and Atkins (1977). For *C. albicans* the MIC and MLC was 1:512 and 1:128 respectively.

C. albicans was killed at an MBC dilution of 1/32, reports Rhode *et al.* (1989). Kill was achieved within six hours. Ghannoum (1988) calculated the MIC of aqueous garlic extract in six clinical yeast isolates, including *C. albicans,* to be between 0.8 and 1.6 mg ml-1.

Sandhu *et al.* (1980) report all except two of 16 strains of *C. albicans* isolated from vaginal, cervical and buccal swabs of vaginitis patients and from pollinating bees were sensitive to an aqueous extract of fresh garlic.

Oral candidiasis, considered a defining illness for Aids (Murray *et al.*, 1995), has been successfully treated with garlic in four Tygerberg Hospital Aids patients. Patients who could not afford expensive antibiotics, were cured of the oral candidiasis within 24 hours after having chewed a clove of garlic, twice a day for 15 -20 minutes. (Bouic, 1997).

Arora and Kaur (1999) compared the sensitivity of some human pathogenic bacteria and yeasts to various spice extracts and commonly employed chemotherapeutic substances. Yeasts were totally killed in 1h by garlic extract. Greater anti-candidal activity was shown by garlic than by nystatin.

In vitro studies with garlic in experimental candidiasis done by Prasad and Sharma (1980) showed chicks infected by oral inoculation with *C. albicans* and subsequently fed 2-4 % garlic chips were cured after 10 days of therapy, while the untreated groups showed characteristic lesions at post-mortem examination.

Of six fractions derived from garlic investigated in an *in vitro* system by Yoshida *et al.* (1987), ajoene had the strongest antifungal activity. The growth of both *Aspergillus niger* and *C. albicans* was inhibited by ajoene at less than 20 micrograms/ml, reports Naganawa *et al.* (1996).

Allicin was effective *in vitro* against Candida, Cryptococcus and three genera of dermatophytes with MICs in the range of 1.57 to 6.25microg/ml, according to Yamada and Azuma (1977).

2.5.2. HOMEOPATHY

In cases of infection by the above organisms, garlic in homeopathic form may be one of the remedies prescribed as the similimum, i.e. on the basis of the similarity between the symptomatology expressed by the patient and that of the materia medica drug picture. Thus an infection by *K. pneumonia*, a frequent cause of hospital-acquired pneumonia, may cause sputum like currant jelly (Berkow, 1992). Garlic is listed in Kent (1993) under "Expectoration: jelly-like" (816) which refers to the rubric "gelatinous" (820) and under the rubric "Expectoration: Bloody" (813).

Similarly the diarrhoeal symptoms caused by *S. aureus* and *E. coli* or antibiotics (Berkow, 1992), may correspond to the materia medica picture of garlic (Allen, 1975; Clarke, 1947).

Clinically, garlic is homeopathically indicated in bronchitis (Clarke, *ibid.*), catarrhs (Allen, *ibid.*; Boericke, 1991; Clarke *ibid.*), coughs (Allen, *ibid.*; Clarke, *ibid.*); colitis (Boericke, *ibid.*) and diarrhoea (Allen, *ibid.*; Clarke, *ibid.*). In the materia medicas it's characteristics are - "Bronchial catarrh with gelatinous, difficult expectoration" (Clarke, *ibid.*). "Cough causing most fetid breath" (Allen, *ibid.*; Clarke, *ibid.*).

Some symptoms listed are: "Stools - diarrhoeic stools, 3 a.m. preceded , accompanied and followed by cuttings in abdomen and loins" (Allen, *ibid.*; Clarke, *ibid.*).

Respiratory Organs - "Difficult respiration as if sternum compressed" (Allen, *ibid.*; Clarke, *ibid.*). "Almost continuous mucous rales in bronchi" (Allen, *ibid.*; Boericke, *ibid.*; Clarke, *ibid.*); "Morning cough, after leaving his bedroom, with extremely copious mucous expectoration" (Allen, *ibid.*; Boericke, *ibid.*; Clarke, *ibid.*). "Great difficulty in expectorating a glutinous mucous" (Allen, *ibid.*; Boericke, *ibid.*; Clarke, *ibid.*). "Expectoration of thin, yellowish, purulent-looking, blood-streaked mucus of putrid odour" (Allen, *ibid.*; Clarke, *ibid.*); "haemoptysis" (Boericke, *ibid.*).

Regarding dosage of garlic, Boericke suggests using the third to sixth potency (3 X - 6 X, i.e. succussed dilutions of $1x10^{-3}$ to $1x10^{-6}$, respectively).

2.5.2.1. DRAINAGE

The use of even lower dilutions, such as 3X , has been practised in drainage therapy or detoxification, an extension of homeopathy defined by dr. Leon Vannier (Maury, 1965). Drainage remedies are described as any homeopathic remedy given in low potency (1X or 3X). (Maury, *ibid.*).

According to Maury, all people in the modern world have been subjected to the effects, of one or other of "toxins", including a range from alcohol and tobacco to the metabolic by-products of anxiety. These "toxins" act on the excretory organs (skin; mucous membranes of respiratory, digestive and genital systems; serous membranes and viscera such as the liver, spleen, pancreas, kidneys) and on the endocrine glands (e.g. adrenal gland, spleen,

pituitary, thyroid, sexual glands), impairing their function and ultimately causing a lesion, as in hepatic sclerosis. (Maury, *ibid.)*.

Drainage is defined as an organic cleansing process brought about by means of homeopathic remedies indicated according to the law of similars in relation to the sick organism and preparing the ground for the more effective action of constitutional remedies. (Maury, *ibid.*).

When accompanied by a disease with a different aetiology, the symptomatology of the intoxication obscures the pure pathogenic symptomatology of the other disease, thus preventing the practitioner from determining the similimum. The effects of drainage are fourfold: clearing the pathogenic picture (i.e. removing the symptomatology caused by the intoxication by performing organic stimulation and elimination of toxins); preparing the organism for the action of high potencies; safeguarding against temporary aggravation crises due to the effect of remedies administered; and rendering the action of the subsequent similimum more rapid and effective. (Maury, *ibid.*).

The higher potency homeopathic remedy plays the role of a catalyst, specific in regard to both the affection to be treated and the tissues or organ affected. In the process of catalysis by-products and toxins are formed which often cause an elimination crisis manifesting in the affected organ. Drainage, directed at the organ, prepares it to cope with the effects of this beneficial shock. (Maury, *ibid.*).

The drainage remedy is prescribed after determination of the causes of intoxication, which may indicate the organ/s involved, is strictly based on the Materia Medica and must complement the general action of the similimum. (Maury, *ibid.*).

Applying the above principles to garlic, the primary effect of which is on the intestinal mucous membranes (Boericke, *ibid.*), it may be prescribed as a drainage remedy in cases of e.g. haemorrhagic colitis caused by *E. coli.*

3. Materials and Methods

3.1. Preparation of garlic dilutions

Garlic extract and garlic in 3X and 8X potencies were prepared according to published methods. The detailed methodology is set out in Appendix A.

3.2. Setting up controls

3.2.1 Using the nutrient broth method to determine the sterility of each of the 3 garlic dilutions, 0.1ml of each dilution and 0.1ml of tryptone broth, were added to 2ml of tryptone broth. An optical evaluation was done for turbidity after 20h incubation at 35^0C . (The National Committee for Clinical Laboratory Standards (NCCLS), 1990: M7/82)

3.2.2 Using the nutrient broth method to obtain an indication of the viability of each of the 5 micro-organisms (NCCLS, 1990: M7/82), 0,1 ml of $1x10^6$ organisms/ml, determined optically according to McFarlane Standards (NCCLS, 1992: 5.2.1), and 0.1ml of tryptone broth, were added to 2ml of tryptone broth.

3.2.3 The solution was evaluated optically for turbidity after 20h incubation at 35^0C . Values were compared to McFarlane standards (NCCLS, 1992: 5.2.1).

3.2.4 All organisms were plated out in 5$\mu\ell$ quantities on agar plates (NCCLS, 1992: 5.16.1), incubating for 20 hours at 37^0C. Plates were examined for growth, counting the number of colony-forming units. For *C. albicans,* tryptone broth was substituted with RPMI 1640 with L-glutamine and a pH indicator and without sodium bicarbonate (NCCLS, 1992: M27-P in Murray *et al.,* 1995).

3.3. Determination of antimicrobial activity

3.3.1. Bacteriostatic effect

3.3.1.1 The nutrient broth method was used to obtain an indication of the bacteriostatic effect of each of the 3 garlic dilutions against four strains of antibiotic-resistant bacterial clinical isolates and one strain of fungus (NCCLS, 1990: M7/82). Samples of the strains were obtained from the Department of Microbiology at the University of Stellenbosch: Tygerberg Hospital. The following 5 strains were tested: Methicillin-resistant *Staphylococcus aureus; Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*, and *C. albicans.*

3.3.1.2 The *in vitro* bacteriostatic effects of the three garlic dilutions were determined against each of the five organisms by standard procedure, exposing 0,1 ml of $1x10^6$ organisms/ml, determined optically according to McFarlane Standards (NCCLS, 1992: 5.2.1) to 0,1ml of each of the dilutions in 2ml of tryptone broth. For *C. albicans,* tryptone broth was substituted with RPMI 1640 with L-glutamine and a pH indicator and without sodium bicarbonate. (NCCLS, 1992: M27-P in Murray *et al.*, 1995).

3.3.1.3 The procedure in 3.3.1.2 was repeated eight times, testing the three dilutions against all five organisms a total number of nine times to obtain a statistical average.

3.3.1.4 A range of nine controls for each of the five organisms were similarly set up as described in 3.3.1.2.

3.3.1.5 The dilutions were evaluated optically for turbidity after 20h incubation at $35^{o}C$.

3.3.1.6 Values were compared to McFarlane standards (NCCLS, 1992: 5.2.1).

3.3.2. Bactericidal effects

3.3.2.1 The *in vitro* bactericidal effects of the specific garlic dilutions thus exhibiting bacteriostatic effects, were determined against the various organisms by plating out 5μℓ of each of the nine samples of each of the indicated organisms, on agar plates (NCCLS, 1992: 5.16.1), incubating for 20 hours at 37^0C . For *C. albicans*, blood agar used above was substituted with Sabouraud's dextrose agar and incubated at 35^0C.

3.3.2.2 Likewise, the nine controls of each organism were plated out.

3.3.2.3 Plates were examined for growth, counting the number of colony-forming units (CFU's).

3.3.2.1. Time/Kill curve

3.3.2.1.1 For a time/kill curve, to demonstrate the time taken to obtain bactericidal effects brought about on the strains in 3.2.2, 0,1ml of the aqueous garlic dilutions was added to an inoculum of 0,1 ml of $1x10^6$ organisms/ml of these strains in 2ml tryptone broth.

3.3.2.1.2 Dilutions were incubated as in 3.3.1.5 above.

3.3.2.1.3 At 2-hourly intervals for a period of up to 14 hours, 5μℓ of the mixture was removed and transferred to a blood agar plate. It was spread over the agar surface and examined for growth after incubation for 20 hours at 37^0C to determine when bactericidal effects started occurring (Rode & De Wet, 1989).

3.3.2.1.4 This procedure was repeated five times, testing the selected strains a total number of six times.

3.4. Statistical analysis

Data was analysed with the Kruskal-Wallis ANOVA by Ranks test. This test is used in the case of two or more independent random samples to determine if they come from populations with identical distributions. A statistic called H is calculated that is approximately chi square distributed with $df = n' - 1$, where n' is the number of independent samples and no sample is smaller than 5. H is based upon the sum of the ranks of the values in each sample with the ranks being determined by first combining all samples and constructing an ordered array (Cangelosi, Taylor and Rice, 1983; Kotze, 2000).

The formula for computing H is: $$H = \frac{12}{n(n+1)} \sum \frac{R_i^2}{n_i} - 3(n+1)$$

where n = the size of all samples combined; R_i = the sum of the ranks of the ith sample; n_i = the size of the ith sample.

4. Chapter 4 - Results

4.1. Bacteriostatic effects

4.1.1. Escherichia coli

In 100μℓ test quantities, garlic in extract form had a bacteriostatic effect approaching a bactericidal effect (kill = 85.17%), on the multidrug-resistant strain of *E. coli*. In five of the nine samples tested, all organisms were killed after 20 hours incubation (c.f. table 4.1). In the remaining four samples the number of organisms surviving after incubation ranged from 200 organisms/ml to 6600 organisms/ml. The averages for the control and experimental group in terms of organisms/ml was 869000 and 1288.889 respectively. The difference in terms of CFU's/ml between the experimental and control groups was statistically significant (p=0.001).

Table 4.1 - No. of *E. coli* CFU's/ml with 100μℓ garlic extract

	Organisms/ml	
Tube nr	Control	Experiment
1	865500	6600
2	868500	0
3	906000	0
4	849000	3400
5	844500	0
6	865500	200
7	865500	0
8	870000	1400
9	886500	0
Average	869000	1288.889

4.2. Bactericidal effects

4.2.1. Eschericia coli

In 200μℓ test quantities, garlic in extract form had a bactericidal effect (kill = >99.9%) on the multidrug-resistant strain of *E. coli.* All organisms in all nine samples were killed after 20 hours incubation (c.f. Table 4.2). In the control group, the organisms replicated to an average of 869 000 organisms/ml after incubation. The difference in terms of CFU's between the experimental and control groups was statistically significant (p=0.0003).

Table 4.2 - No. of *E. Coli* CFU's/ml with 200μℓ garlic extract

	CFU's/ml	
Tube nr	*Control*	*Experiment*
1	865500	0
2	868500	0
3	906000	0
4	849000	0
5	844500	0
6	865500	0
7	865500	0
8	870000	0
9	886500	0
Average	**869000**	**0**

4.2.2. Candida albicans

In 100μℓ test quantities, garlic in extract form had a bactericidal effect (kill = >99.9% of organisms) on *C. albicans*. All organisms were killed after 20 hours incubation (c.f. Table 4.3). In the control group, the organisms multiplied to an average of 15169.444 organisms/ml after incubation. The difference between the experimental and control groups, in terms of CFU's, was statistically significant (p=0.0001).

Table 4.3 - No. of *C. albicans* CFU's/ml with 100μℓ garlic extract

	Organisms/ml	
Tube nr	Control	Experiment
1	14900	0
2	15200	0
3	15375	0
4	14925	0
5	15425	0
6	15650	0
7	14725	0
8	15300	0
9	15025	0
Average	15169.444	0

4.3. Time/Kill Curves

4.3.1. E. coli

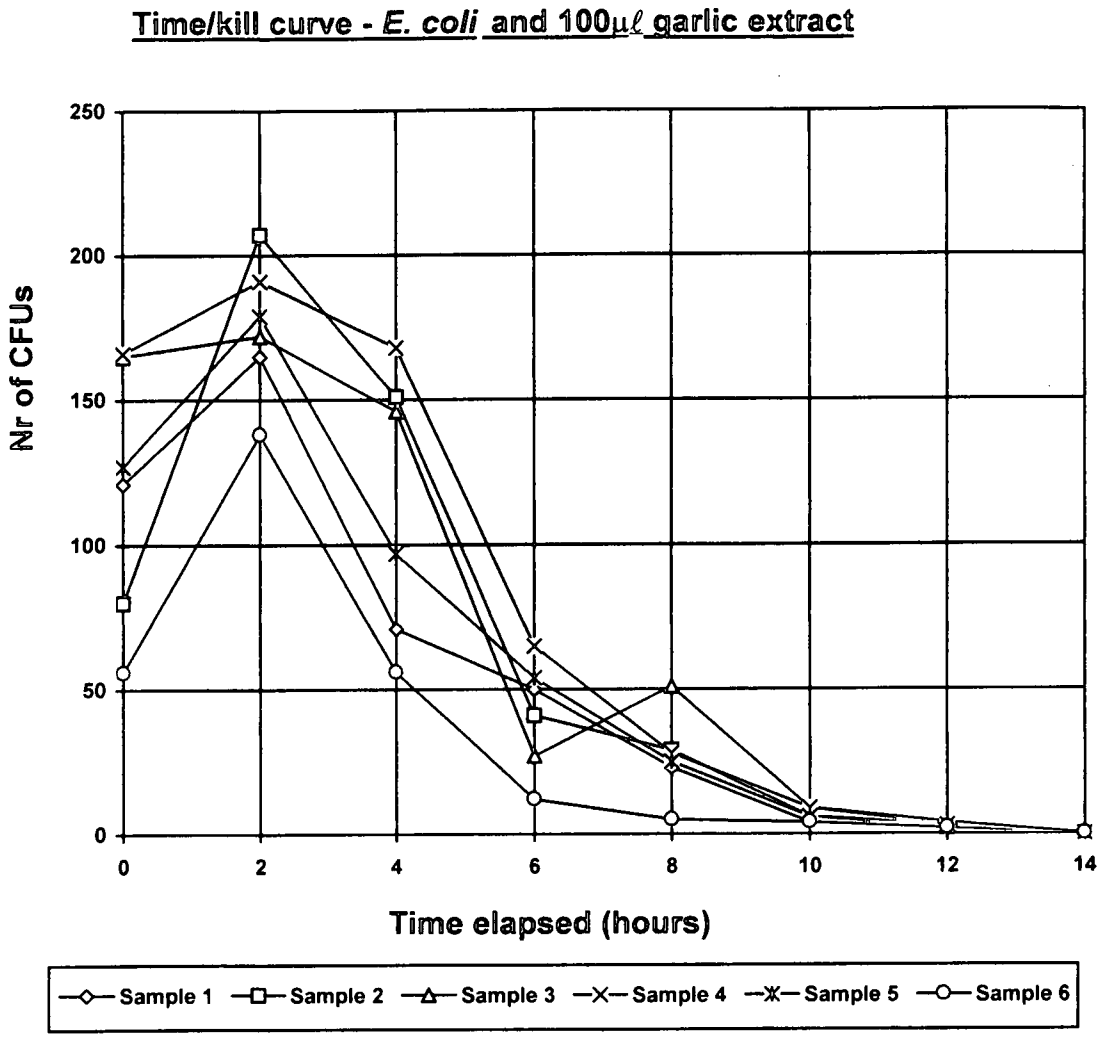

Figure 4.1

After an initial rise in the number of *E. coli* CFUs in the first two hours after addition of 100µℓ garlic extract, there was a sharp decrease in the number of CFUs within six hours (C.f. Figure 4.1). Fourteen hours after the addition of 100µℓ garlic extract, all *E. coli* had been killed.

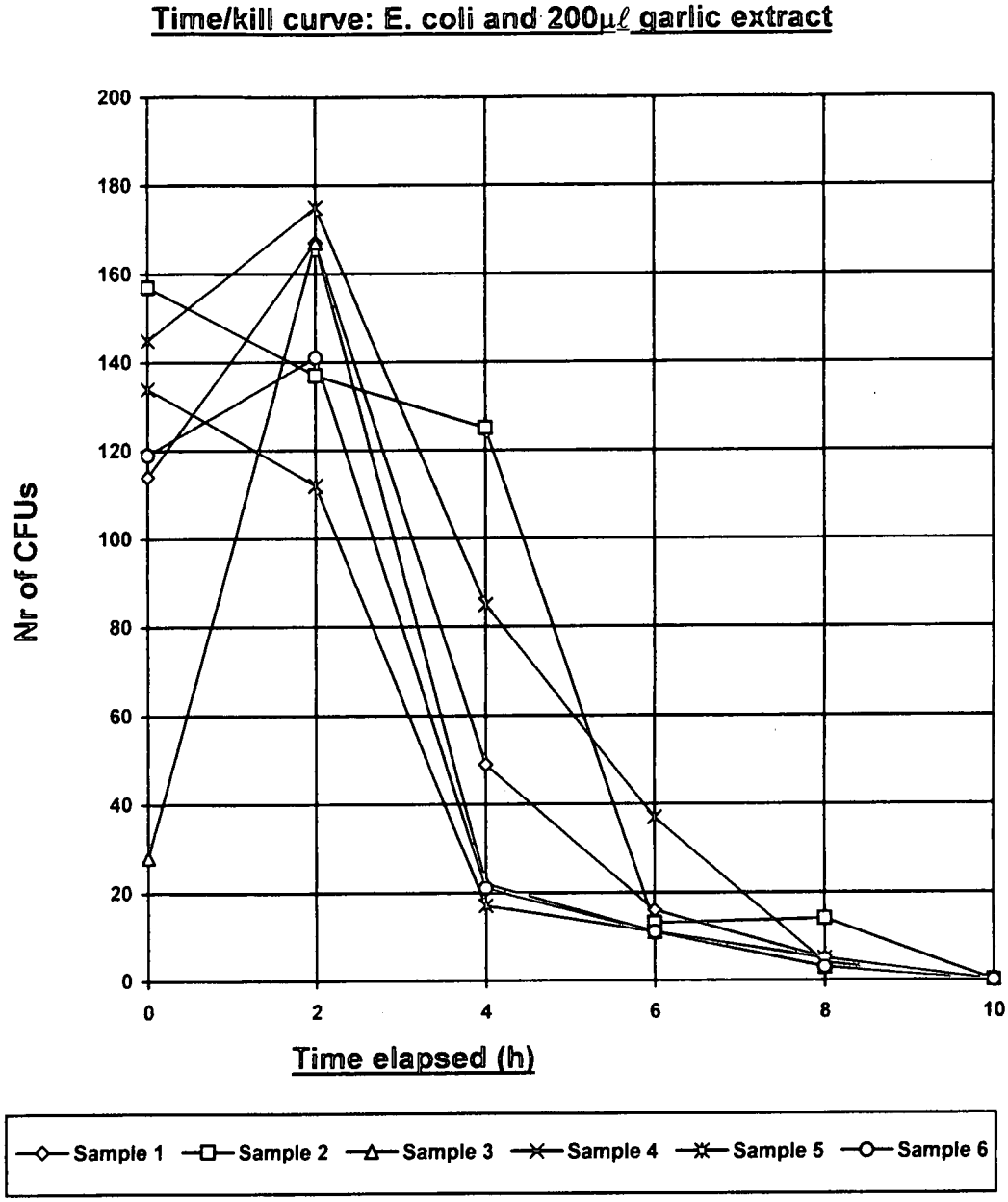

Figure 4.2

While the *E. coli* CFU's in only three of the six samples increased within the first two hours after the addition of 200µℓ garlic extract, the CFU's in all six samples decreased sharply within four to six hours (C.f. Figure 4.2). Within ten hours, all *E. coli* in all six test samples were killed. Garlic in 200µℓ quantities kills *E. coli* in a statistically significant shorter time than in 100µℓ quantities ($p=0.0025$).

4.3.2. *Candida albicans*

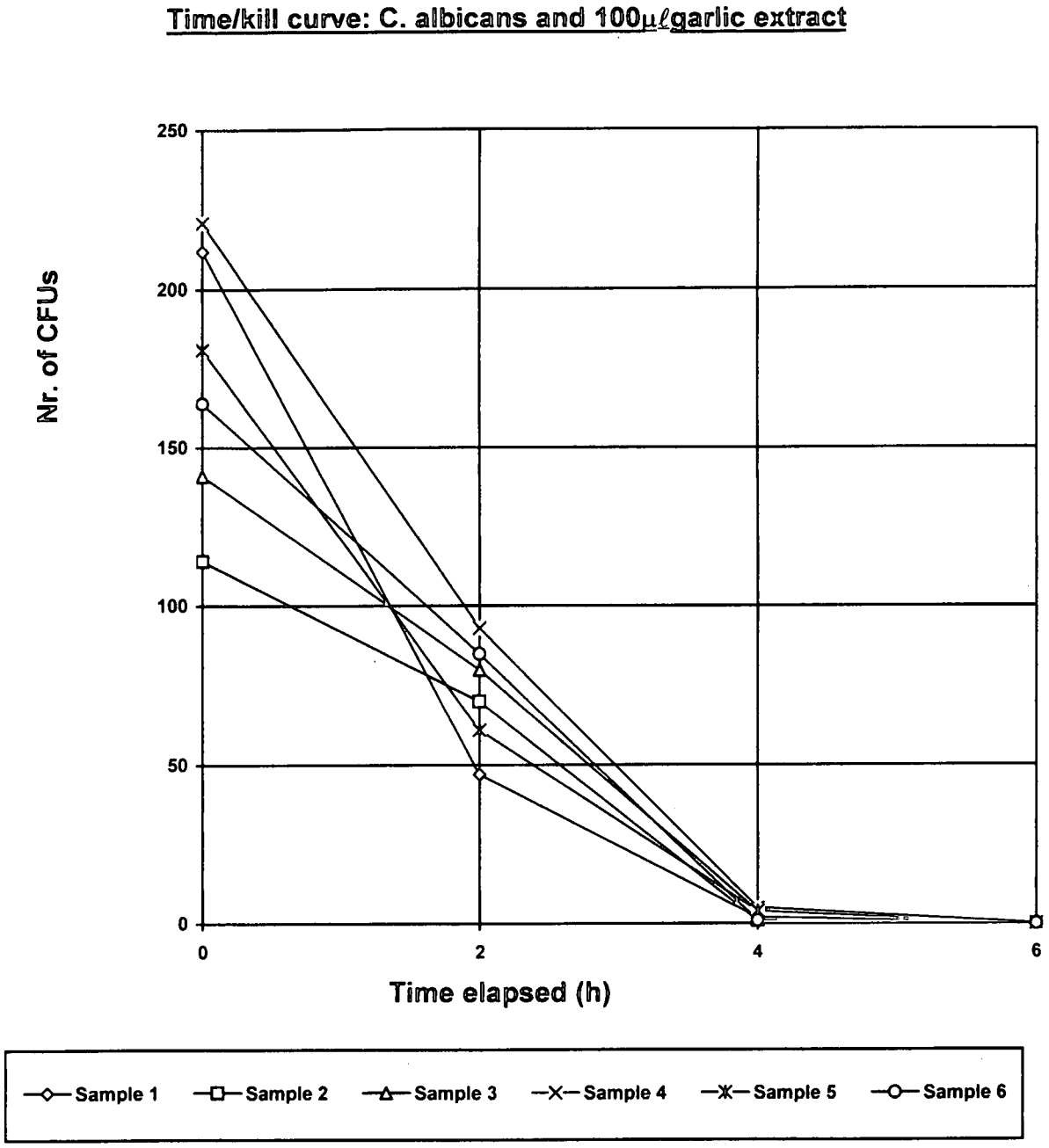

Figure 4.3

The *C. albicans* CFU's in all six test samples decreased sharply within the first two hours after the addition of 100µℓ garlic extract (C.f. Figure 3). Within four hours, almost all CFU's had been eliminated, with a complete kill achieved within six hours.

4.4. Negative results

4.4.1. Garlic extract

4.4.1.1. *P. aeruginosa*

Although garlic in extract form in 100μℓ test quantities, initially indicated a bactericidal effect (kill = >99.9%) on the multidrug-resistant strain of *P. aeruginosa.* (c.f. Table 4.4) and the difference in terms of CFU's between the experimental (average 785333.3 org/ml) and control groups (average 44.444 org/ml) was statistically significant (p=0.0005), this result was excluded from the positive results as a subsequent time/kill experiment and a confirmatory repeat of the broth count experiment, failed to confirm these observations. No killing was achieved and no bacteriostatic effect could be detected, as measured by McFarlane standards.

	Organisms/ml	
Tube nr	Control	Experiment
1	805500	0
2	868500	0
3	861000	400
4	777000	0
5	804000	0
6	784500	0
7	727500	0
8	741000	0
9	699000	0
Average	785333.3	44.444

Table 4.4 - No. of *P. aeruginosa* CFUs/ml with 100μℓ garlic extract

Sample nr	McFarlane value
1	>0,5
2	1
3	>0.5
4	1
5	1
6	1
7	1
8	1
9	>0.5

Table 4.5 - Final optical evaluation of repeat *P. aeruginosa* broth dilutions with 100μℓ garlic extract

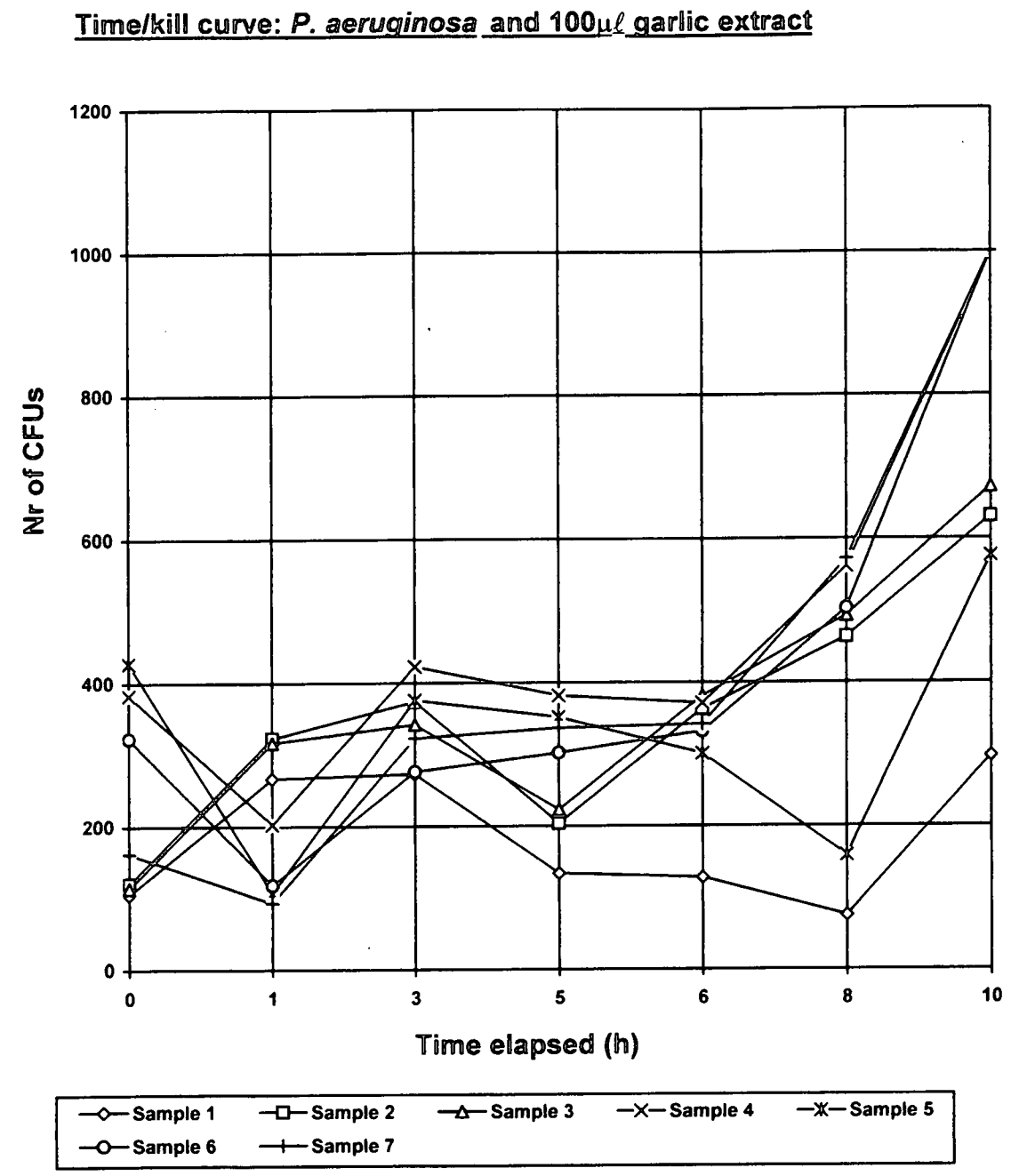

Figure 4.4

4.4.1.2. MRSA, K. PNEUMONIAE

A seemingly bacteriostatic effect of garlic in 100µℓ test quantities, on *MRSA* and *K. pneumoniae*, indicated by a McFarlane standard of 0.5 for all nine samples in both organisms, was negated during subsequent plating out, CFU's in both experimental groups having grown to more than that of the 0.5 McFarlane control.

In the case of *MRSA*, the average for the number of CFU's in the experimental group was 227888.889 org/ml, compared to the 412500 org/ml counted in the control group. There was however a significant difference between the control and experimental groups (p=0.0008). The average number of CFU's/ml in the *K. pneumoniae* control and experimental group was 923 500 and 180 155.556 respectively. There was a significant difference between the two groups (p=0.0008).

	Organisms/ml	
Tube nr	Control	Experiment
1	396000	144600
2	433500	231000
3	420000	196000
4	418500	210600
5	415500	276600
6	421500	259500
7	402000	164100
8	409500	314900
9	396000	253700
Average	412500	22788.889

Table 4.6 - Number of MRSA CFUs/ml with 100μℓ garlic extract

	Organisms/ml	
Tube nr	Control	Experiment
1	741000	134200
2	892500	249800
3	955500	150600
4	936000	210000
5	955500	38400
6	952500	234600
7	943500	155200
8	949500	211200
9	985500	237400
Average	923500	180155.556

Table 4.7 - Number of *K. pneumoniae* CFUs/ml with 100μℓ garlic extract

4.4.2. Garlic 3X, 8X

Garlic in 3X and 8X dilutions, measured by McFarlane standards and compared to the starting dilutions of 0.5 McFarlane, had no bacteriostatic effect on either of the five test organisms (See following tables).

SAMPLE	*MRSA*	*E. coli*	*P. aeruginosa*	*K. Pneumoniae*	*C. albicans*
1	1	2	1	2	1
2	1	2	1	2	1
3	1	2	1	2	1
4	1	2	1	2	1
5	1	2	1	2	1
6	1	2	1	2	1
7	1	2	1	2	1
8	1	2	1	2	1
9	1	2	1	2	1

Table 4.8 - McFarlane standard measurements resulting from addition of 3X garlic dilutions

SAMPLE NR	*MRSA*	*E. coli*	*P. aeruginosa*	*K. pneumoniae*	*C. albicans*
1	1	2	2	2	1
2	1	2	2	2	1
3	1	2	2	2	1
4	1	2	2	2	1
5	1	2	2	2	1
6	1	2	2	2	1
7	1	2	2	2	1
8	1	2	2	2	1
9	1	2	2	2	1

Table 4.9 - McFarlane standard measurement resulting from addition of 8X garlic dilutions

5. Chapter 5 - Discussion of Results

The results of this study are consistent with those obtained by previous researchers, in that raw garlic in relatively high doses, has a bacteriostatic as well as bactericidal effect on Gram-negative bacteria such as *E. coli* and fungi such as *C. albicans*.

5.1. Garlic extract

5.1.1. *E. coli*

That garlic has **bacteriostatic** activity against *E. coli* as demonstrated by this study, is supported by Adetumbi and Lau (1983), Anesini and Perez (1993), Chen *et al.* (1985), Jezpwa *et al.* (1966), Kumar and Berwal (1998), Rhode *et al.* (1989) and Sharma *et al.* (1977).

The observed **bactericidal** effect, furthermore concurs with the findings by Johnson and Vaughn (1969) and Rhode *et al.* (1989).

The dilutions at which *E. coli* was **killed** (1:44 and 1:23) in the present study, are both higher than that of the study by Rhode *et al.* (1989), who calculated the MIC dilution of 1:16 and MBC value of 1:4, as well as the dilution of 1:10 at which Johnson and Vaughn (1969) found *E. coli* to become non-viable.

That 0.1ml of garlic initially only had a bacteriostatic effect on *E. coli,* whereas it later showed a bactericidal effect, could be due to the fact that a fresh garlic extract sample was used for the latter part of the experiment.

The **time taken to kill** *E. coli* at a concentration of 1:23 was the same in the present study and in the study by Rhode *et al.*, i.e. ten hours, which was longer than the 2-6 hours found in the study by Johnson and Vaughn (1969). In the present study it was, however, achieved

at a higher dilution — 1:23 compared to 1:16 (Rhode *et al.,* 1989) and 1:10 (Johnson and Vaughn, 1969).

That *E. coli* in the present study was killed in a shorter time at the lower dilution of 1:23, compared to the fourteen hours at 1:44, is also consistent, considering to the lower dose.

5.1.2. Candida albicans

That garlic had a fungiostatic effect on *C. albicans,* is supported by Adetumbi and Lau (1983), Appleton and Tansey (1975), Dankert *et al.* (1979), Ghannoum (1988), Jezpwa *et al.* (1966), Kabelik (1970), Tansey and Appleton (1975), Moore and Atkins (1977), Rhode *et al.* (1989), Sandhu *et al.* (1980) and Yoshida *et al.* (1987).

The observed **fungicidal** effect, is supported by Arora and Kaur (1999) and Rhode *et al.* (1989).

That *C. albicans* in the present study was most sensitive to garlic, being killed within six hours, is consistent with the findings of Rode *et al.* (1989) who found an MBC of 1:32, a larger dose than the 1:44 used in this study, killed the fungus within six hours. The bacteriostatic effect is obtained at even smaller doses of 1:128 fresh garlic extract, as demonstrated by Moore and Atkins (1977). Moore and Atkins determined the MIC at 1:512.

Ghannoum (1988) calculated the MIC of aqueous garlic extract ranged between 0.8 and 1.6 mg ml-1. Arora and Kaur (1999) report a kill within 1 hour.

5.1.3. MRSA, K. pneumoniae, P. aeruginosa

That the present study shows no bacteriostatic activity of garlic against MRSA, *K. pneumoniae* and *P. aeruginosa* may be attributable to either the lower dose (1:44) used in the

present study (the successes in other studies followed the use of higher doses), or the presence of multidrug-resistant mechanisms present in organisms tested in this study (other studies have mostly been done on susceptible organisms, or on organisms resistant to single antimicrobials).

The absence of garlic activity up to 160 micrograms/ml thiosulfinate concentration against *S. aureus* was also shown by Sivam *et al.* (1997).

Among the authors who demonstrated garlic activity against *S. aureus*, Gonzalez-Fandos *et al.* (1994) found the growth of *S. aureus* was inhibited by dehydrated garlic at levels of 1.5% (w/v) and over. Rhode *et al.* (1989) determined MIC dilutions of 1/32 for *S. aureus* ATCC 25923. No bactericidal effect was obtained. For MRSA, MIC and MBC values of 1/16 and 1/8 respectively, were determined. Kill was achieved within ten hours.

K. pneumoniae ATCC 13883 was killed at a MBC dilution of 1/16 (Rhode *et al.*, 1989). Kill was achieved within ten hours.

Allicin, one of the main active principles of garlic, is extremely unstable (Krest & Keusgen, 1999). The apparent loss of bioactivity during the latter part of testing garlic extract against *P. aeruginosa* in the current project, may be attributable to the instability of allicin. The quantification of the active principles of garlic was however beyond the scope of this project.

P. aeruginosa had a very low D-value, found Dankert *et al.* (1979) whilst the bacteriostatic concentration was high. *P. aeruginosa* ATCC 27853 and *P. aeruginosa* NTCC 10662 were both killed at MBC dilutions of 1/4 (Rhode *et al.*, 1989). Kill was achieved within 14 hours.

5.2. Garlic 3X and 8X

That no garlic activity was found in the present *in vitro* study using the higher dilutions of garlic 3X ($1x10^{-3}$) and 8X ($1x10^{-8}$), is consistent with the literature reviewed. Results by the authors discussed (Rhode *et al.*, 1989; Johnson and Vaughn, 1969) suggest that garlic *in vitro* activity against micro-organisms fall in the lower range of dilutions.

This, however, does not preclude the clinical use of garlic as homeopathically indicated drainage remedies, as there is little evidence of the correlation between *in vitro* susceptibility results and clinical outcome (Acar and Goldstein, 1998; Murray, 1995).

Chapter 6 - Conclusions and Recommendations

6.1. CONCLUSIONS

Under antimicrobial pressure since the introduction of antibiotics in clinical use, micro-organisms have been acquiring resistance to these chemotherapeutic agents, to the point where the clinical scope of antibiotics are becoming increasingly restricted. This restriction is especially evident within hospitals, where multidrug-resistant micro-organisms frequently cause nosocomial epidemics.

The epidemics in hospitals such as in South Africa, have at times necessitated the emergency use of alternative antibiotics such as garlic.

Although the problem of multidrug-resistance has spread world-wide, it is more pronounced in developing countries, where infectious diseases are more prevalent.

Nosocomial infections have dramatically increased in the past decade, causing an increasing strain on health care budgets. Likewise the increasing incidence of multidrug-resistant strains of micro-organisms, especially vancomycin-resistant S. aureus, is cause for concern.

Risk factors for infection or colonisation with multidrug-resistant micro-organisms include selective antimicrobial pressure, multiple admissions to hospital, prolonged hospitalisation and severe disease or trauma.

Management recommendations such as rationing of antibiotics, stringent infection-control policies and increased surveillance measures, could, if applied stringently, have a limiting effect on the problem. As micro-organisms have demonstrated a remarkable ability of developing resistance to antimicrobials, it is questionable whether the problem can be con-

tained by management measures such as the rapid development of new antimicrobial agents.

Searching for alternative antibiotics, either natural or artificial, a natural antibiotic researchers have been investigating for a number of years, is garlic.

One of the effects of garlic in pharmacological doses, is a bacteriostatic and bactericidal *in vitro* efficacy on a large range of infections or infestations by Gram-positive bacteria such as staphylococcus spp., Gram-negative bacteria such as *E. coli*, Klebsiella spp, fungi such as *C. albicans* and parasites.

Its efficacy *in vivo* against some of these organisms has also been demonstrated. It has even been shown to be effective against micro-organisms showing resistance to single antibiotics.

The current study shows that the efficacy of garlic in low doses *in vitro* extends to certain multidrug- resistant micro-organisms, i.e. *E. coli* and nosocomial strains such as *C. albicans*.

The study confirms that, *in vitro*, the action of garlic is limited to these low dilutions.

As positive results have been attained through this *in vitro* experiment, this could serve as a motivation for clinical trials which would prove whether these *in vitro* results also extend to *in vivo* situations. This could provide a solution to the clinical problems currently being experienced in treating drug-resistant cases of urinary tract infections, acute uncomplicated cystitis in women, spontaneous bacterial peritonitis, spontaneous bacteremia and bacterascites, caused by *E. coli*, and candidiasis caused by *C. albicans*.

The failure of garlic against multidrug-resistant organisms such as MRSA, *P. aeruginosa* and *K. pneumoniae*, may be due to advanced mechanisms of resistance present in these organisms, through which they have become resistant to multiple antibiotics. An investigation into the mechanism of multidrug-resistance of these organisms, could explain the failure of garlic in this *in vitro* experiment. This was, however, beyond the scope of the present study.

Biochemical evidence of the mechanism of action of garlic extract on multidrug-resistant micro-organisms could provide an explanation for both the positive and negative results against these organisms. This was also beyond the scope of the study.

As bioactivity might have been lost at a certain stage of the experiment, a means of measuring bioactivity to quantify the active principle could provide an answer. As the present study was concerned with garlic as a whole substance, this was not attempted.

The lack of positive *in vitro* results with slightly higher dynamised garlic preparations, is in line with the body of evidence reviewed. The *in vitro* effect is possibly attained only through a pharmacological effect. Higher, dynamised dilutions, the general *in vivo* clinical efficacy of which has been demonstrated in homeopathy, might by unsuitable for testing in an *in vitro* microbiological model such as the one utilised in this study.

The lack of positive results with these higher, dynamised dilutions, however, does not preclude their clinical use as e.g. drainage remedies, as *in vitro* results have been shown to be unreliable indicators of clinical efficacy.

6.2. RECOMMENDATIONS

The following recommendations are made:

6.2.1 Clinical trials, testing the efficacy of garlic on nosocomial, multidrug-resistant micro-organisms, should be conducted, as the current study has also demonstrated the *in vitro* efficacy of garlic on these organisms.

6.2.2 *In vitro* studies with doubling dilutions at lower concentrations than 1:44 could be done on multidrug-resistant nosocomial strains of MRSA, *K. pneumonia* and *P. aeruginosa* to provide a basis for clinical trials also including these strains.

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Appendix A

Preparation of test substance

1. Twelve bulbs of garlic (*Allium sativum*) were divided into separate cloves, dehusked, fragmented and ground at 25^0C in a blender. A total of 150g of garlic pulp was agitated with 300ml of sterile distilled water for 60 min after which the preparation was allowed to stand for an hour. The paste was refrigerated for two hours and squeezed through gauze to remove larger tissue particles. Supernatant, containing only small particles, was collected and centrifuged for 15 minutes. The paste was decanted and microfiltrated with size 0.45µm filter paper to clear smaller particles. Supernatant, containing only small particles, was collected. The particle-free extract was kept frozen at -20^0C until used. (Rode & De Wet 1989:463).

2. From 20 ml of the garlic extract prepared in 1, a serially diluted and succussed (shaken) range of eight 20ml decimal potencies ranging from 1X to 8X was prepared. Using multiple vials or glass containers, the dilution was 1:10 and the dilutions were manually succussed for 2.5 minutes between each dilution in the following way:

3. To prepare 20 ml of the 1X dilution from the extract, 2ml of the extract was pipetted into 18ml of distilled water in a sterile container. Container was closed and manually shaken for 2.5 minutes. (Kayne:1997: 48-52).

4. To prepare 20ml of the 2 X from the 1X, the procedure in 3 was repeated, pipetting 2ml of the 1X into 18ml of distilled water in a sterile container. Container was closed and manually shaken for 2.5 minutes. (Kayne: 1997: 48-52).

5. The process was continued likewise till the 8 X dilution. (Kayne: 1997: 48-52).