MICROBIAL HAZARDS ASSOCIATED WITH FOOD PREPARATION IN CENTRAL SOUTH AFRICAN HIV/AIDS HOSPICES

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DECLARATION OF INDEPENDENT WORK

I, Jane Sebolelo Nkhebenyane, do hereby declare that this research project submitted to the Central University of Technology, Free State for the degree MAGISTER TECHNOLOGIAE: ENVIRONMENTAL HEALTH is my own work and has not been submitted before to any institution by myself or any other person in fulfillment of the requirements for the attainment of any qualification

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SIGNATURE OF STUDENT DATE

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If one dream should fall and break into a thousand pieces, never be afraid to pick up one of those pieces up and begin again.

(Flavia Weedn)

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SUMMARY

South Africa currently faces one of the highest HIV prevalence rates in the world. As this prevalence rises, the strain placed on its hospitals is likely to increase due to the shortage of beds. The devastating effects of HIV/AIDS initiated the establishment of a hospice which is a non-governmental organisation whose goal is the provision of care for terminally ill patients, either in their homes, in hospitals or in a hospice's own in-patients wards. Part of the hospice's mission is to offer palliative care without charge to anyone who requires it. The basic elements of hospice care include pain and symptom management, provision of support to the bereaving family and promoting a peaceful and dignified death. This also includes the provision of cooked foods to the patients using the kitchen facilities of the hospices for this activity. It is well known that the kitchen is particularly important in the spread of infectious disease in the domestic environment due to many activities that occur in this particular setting.

Food and water safety is especially important to the persons infected with the human immunodeficiency virus (HIV) or with immunodeficiency syndrome (AIDS).It is estimated that food-borne pathogens (disease–causing agents) are responsible for 76 million illnesses, some resulting in death, in the United States alone every year. In one study of patients with AIDS, two-thirds had diarrhoeal

disease and in two-thirds of these, the following enteric pathogens were identified: *Salmonella*, *Shigella, Listeria, Yersnia, Cryptosporidium, Entamoeba histolylica* and *Campylobacter sp.* In an epidemiological study of patients with HIV infection a close association was found between consumption of raw or partially cooked fish and antimicrobial-resistant *Mycobacterium avium* complex. Antibiotic resistance in food-borne pathogens has become a reality and this poses a serious threat to the medical fraternity since it diminishes the effectiveness of treatment. This study was undertaken to determine the prevalence of foodborne pathogens including bio aerosols isolated from the kitchen surfaces and food handler's before and after cooking. The antibiotic resistance of the isolated pathogens was further determined to assess their impact on treatment.

The following microbiota were isolated: Total viable counts (TVC), Coliforms, *Escherichia coli*, *Staphylococcus aureus, Pseudomonas* and presumptive *Salmonella*. The hospices had high counts of *E*.*coli* and *S*.*aureus* on the cutting boards for the breakfast session compared to the traditional home based kitchens. It was speculated that this could have originated from cross-contamination via the foodhandler's hands and the food served. It is evident from the results that hospices lack a management system regarding the prevalence of *E*. *coli* as it was present on the cutting boards throughout the food preparation sessions. Gram negative organisms (coliform and *P. aeruginosa*) were in particular both resistant to oxacillin and this pose a great challenge in this

particular setting. This can be addressed by putting emphasis on hygiene as a strategy per se for reducing antibiotic resistance.

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INTRODUCTION

1.1 HISTORY AND ORIGIN OF HIV

The acquired immunodeficiency syndrome (AIDS) was first described in 1981 and has since become a global pandemic (UNAIDS, 2000). Two years later the causative agent, the human immunodeficiency virus (HIV), was isolated and later typed into two groups with slightly different genome structures (Barre-Sinnousi, 1983). The two strains, HIV-1 and HIV-2, are lentiviruses that most likely evolved from simian immunodeficiency virus (SIV), crossing from its predominant hosts (chimpanzees) to humans sometime during the second half of the 20th century (Gao *et al*., 1999).

It is estimated that HIV-1, the most common cause of AIDS, has infected more than 50 million individuals worldwide at a rate of new infections at nearly 6 million per year (UNAIDS, 2006). Feinberg (1995) has reported that infection with HIV-1 initiates a process that leads to progressive destruction of CD4 T lymphocytes, the target cell preference for HIV-1 infection. It has been found that most of the AIDS-defining symptoms occur when the CD4 T lymphocytes are below 200 cells/cubic millimetre of blood.

1.1.1 HIV pandemic

The extent of the pandemic is portrayed in Table 1.1, which depicts the verified number of global infections and deaths reported for 2008. In 2008, an estimated 1.9 million people living in Sub-Saharan Africa became newly infected with HIV, bringing the total number of people living with HIV to 22.4 million (UNAIDS, 2008). Table 1.2 and 1.3 also depict HIV prevalence respectively among antenatal clinic attendees and the population in general in all South African provinces. The provinces that recorded the highest HIV rates were KwaZulu-Natal, Mpumalanga and Free State. South Africa remains the area most heavily affected by the epidemic and it is home to the world's largest population of people living with HIV (5.7 million) (UNAIDS, 2008). During the early 2000s studies revealed the prevalence of HIV in the South African population to be 11.4% (15-19 year age group) and among the age group 15-49 years, 15.6% (HSRC, 2002). A census conducted by the WHO in 2005 estimated the total number of people living with HIV in South Africa at *circa* five and half million, resulting in an 18-year decrease in the average life expectancy of the general population (UN, 2005; UNAIDS/WHO, 2006).

Table 1.1: Reported cases of HIV infections and deaths (global) 2008.

People living with HIV	
Total	**33.4 million (33.1 – 35.8 million)**
Adults	31.3 million (29.2-33.7 million)
Women	31.3 million (14.2-17.2 million)
Children under 15 years	2.1 million (1.2-2.9 million)
People newly infected with HIV	
Total	**2.7million (2.4-3.0million)**
Adults	2.3million (2.0- 2.5 million)
Children under 15 years	430 000 (240 000-610 000)
AIDS related deaths	
Total	**2.0 million (1.7-2.4 million)**
Adults	1.7 million (1.4-2.1 million)
Children under 15 years	280 000 (150 000-410 000)

Table 1.2: Estimated HIV prevalence (%) among antenatal clinic attendees, by Province.

Province	2001	2002	2003	2004	2005	2006	2007
KwaZulu-Natal	33.5	36.5	37.5	40.7	39.1	39.1	37.4
Mpumalanga	29.2	28.6	32.6	30.8	34.8	32.1	32
Free State	30.1	28.8	30.1	29.5	30.3	31.1	33.5
Gauteng	29.8	31.6	29.6	33.1	32.4	30.8	30.3
North West	25.2	26.2	29.9	26.7	31.8	29	29
Eastern Cape	21.7	23.6	27.1	28	29.5	28.6	26
Limpopo	14.5	15.6	17.5	19.3	21.5	20.6	18.5
Northern Cape	15.9	15.1	16.7	17.6	18.5	15.6	16.1
Western Cape	8.6	12.4	13.1	15.4	15.7	15.1	12.6
National	**24.8**	**26.5**	**27.9**	**29.5**	**30.2**	**29.1**	**28**

Source: National Department of Health, 2002-2007

Table 1.3: HIV prevalence (%) by province (total population) 2002-2007.

Province	2002	2005	2008
KwaZulu-Natal	11.7	16.5	15.8
Mpumalanga	14.1	15.2	15.4
Free State	14.9	12.6	12.6
North West	10.3	10.9	11.3
Gauteng	14.7	10.8	10.3
Eastern Cape	6.6	8.9	9
Limpopo	9.8	8	8.8
Northern Cape	8.4	5.4	5.9
Western Cape	10.7	1.9	3.8
National	**11.4**	**10.8**	**10.9**

Source: National Department of Health, 2002-2007

1.1.2 Transmission of HIV

HIV-1 is blood-borne and mainly a sexually transmitted disease (STD). Selik *et al.* (1995) noted that the mentioned virus is primarily transmitted through insertive or receptive sexual intercourse, vertical transmission from mother to child as the virus can pass to the baby during pregnancy, during birth of the baby or through breast-feeding. Also transmission can occur through exposure to contaminated blood, blood products or bodily fluids.

According to the Center for Disease Control and Prevention, persons who are at risk include people with infected sexual partners; infants born to HIV infected mothers (statistics reveal that only about one in three babies born infected mothers get HIV), intravenous drug users who share HIV contaminated unsterilized needles and persons who receive inadequately screened blood products (CDC, 2003). Heterosexual transmission is the route by which most people with AIDS have become infected with HIV worldwide. The risk of infection from the infected mother to the child is found high in the absence of treatment. In the health-care setting workers have been infected with HIV after being stuck with needles containing HIV infected blood or after the infected blood has made contact with the worker's open cut.

1.1.3 Pathogenesis of HIV

There appears to be a correlation between the HIV-1 life-cycle and the level of activation of immune cells supporting viral replication. Infection with HIV-1 initiates a process that leads to progressive destruction of the population of CD4 T-lymphocytes with roles in the generation and maintenance of host immune responses (Feinberg, 1995). The target cell preference for HIV-1 infection and depletion is determined by the identity of the cell surface molecule CD4 that is recognised by the HIV-1 envelope *(env)* glycoprotein as the virus binds to and enters the host cells to initiate the virus replication cycle (Fig. 1.1). Lawn *et al.* (2001) describe this replication cycle as having three distinct stages: a) viral cellular entry, b) reverse transcription, and c) pro-viral transcription.

1.2 STAGES OF HIV INFECTION

The illness caused by HIV has a chronic progression. It starts with an acute syndrome and serological conversion followed by long term phase of clinical latency (asymptomatic phase) (Soria *et al.,* 2009). Fauci *et al.* (1991) further

states that during this period there is little, if any, detectable vireamia, the numbers of infected cells in the blood are low, and it is extremely difficult to demonstrate virus expression in these cells. The different clinical stages of HIV are outlined in table 1.4 according to the world health organisation (UNAIDS/WHO. 2000).

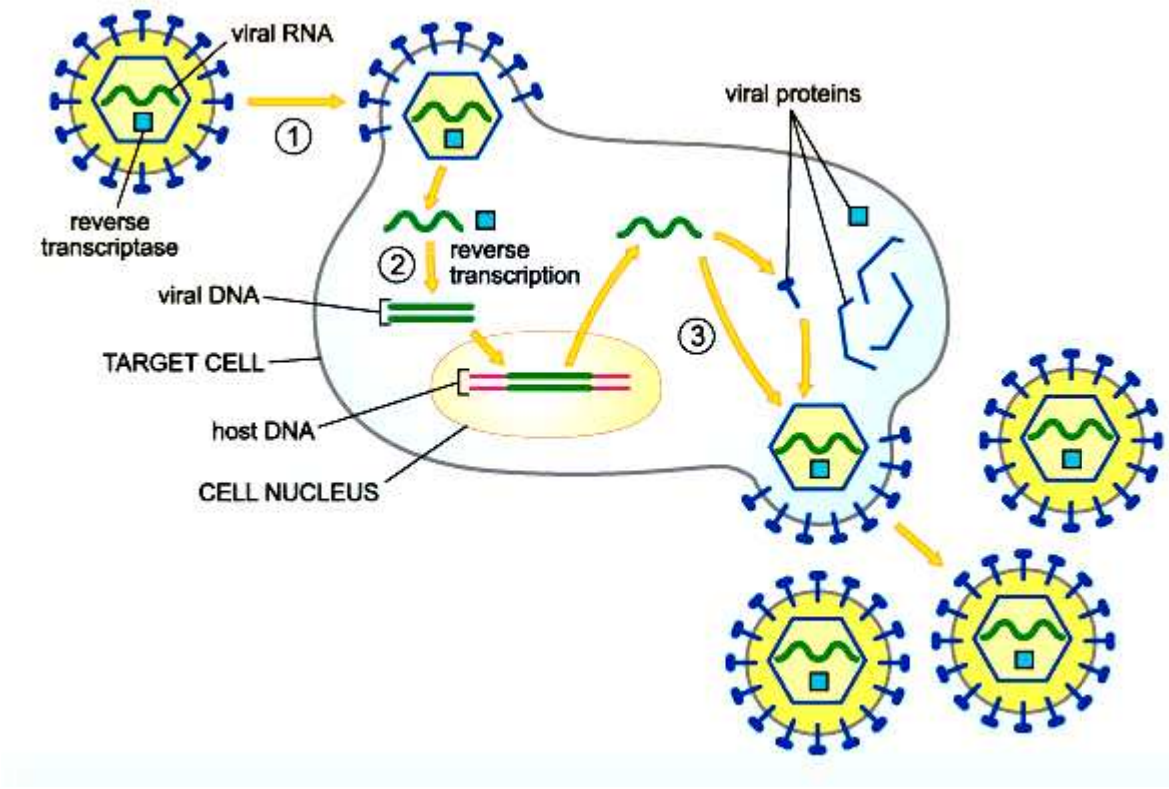

Figure 1.1: Three stages of the HIV life cycle.

Table 1.4: Clinical stages of HIV infection (UNAIDS/WHO, 2000)

Clinical stage	Symptoms
Stage 1	Asymptomatic and generalised lymphadenopathy
Stage II	Weight loss, <10% of body weight and recurrent upper respiratory tract infections
Stage III	Weight loss, > of the body weight, unexplained chronic diarrhoea and prolonged fever for more than one month
Stage IV	HIV wasting syndrome, *Pneumocystis carinni* pneumonia and cytomegalovirus disease

1.3 HIV PREVENTION AND TREATMENT

Antibiotic drugs have for more than half a century ensured that potentially life threatening bacterial infections is treatable. Antibiotic resistance poses a threat to everyone but people living with HIV/AIDS are more at risk than healthy individuals. HIV is a viral rather than a bacterial disease therefore antibiotics cannot be used to directly treat this disease. However, because the virus disrupts the body's own disease-fighting immune system, antibiotics are critical for treating patients infected with HIV to treat opportunistic infections (Travers, 2002).

Because no vaccine or cure for HIV is currently available, the only way to prevent infection by the virus is to avoid behaviour that puts a person at risk of infection, such as sharing needles and having unprotected sex. Education therefore remains the primary methodology for HIV prevention programmes (Kirby *et al.,* 2007). Once contracted, the best treatment for HIV-1 infections is the application of an antiretroviral therapy that targets multiple steps in the viral life cycle (Liu *et al.,* 2005). Therefore a Highly Active Antiretroviral Therapy (HAART) is the recommended treatment for HIV infections which combines three or more anti-HIV medications on a daily regimen (Table 1.5).

Table 1.5: Four classes of approved anti-HIV medications.

Class	Function
Nucleoside reverse transcriptase inhibitors (NRTIs) e.g. zidovudine (AZT or ZVD)	NRTIs block HIV's ability to copy a cell's DNA, which the virus needs to make copies of itself.
Non-nucleoside reverse transcriptase inhibitors (NNRTIs) e.g. nevirapine (NVP)	NNRTIs block the same protein as the NRTI, but are chemically different. Resistance to this class of medications develops quickly if not used in combination with an NRTI.
Protease inhibitors e.g. indinavir	PIs block protease, an enzyme that the HIV virus needs to make copies of itself. As a group, PIs are very potent and relatively well tolerated
Fusion Inhibitors (FIs) e.g. enfuvirtide	FIs block HIV from entering the body's healthy cells. This medication must be administered by injection.

1.4 HIV HOSPICES

1.4.1 Background

The high prevalence of the HIV in South Africa has led to the establishment of hospices to assist in the care of HIV/AIDS patients. A hospice is a non-governmental organisation that provides care to the terminally ill patients, either in their homes, in hospitals or in a hospice's own in-patients wards. Part of the hospice's mission is to offer palliative care without charge to anyone who requires it. The basic elements of hospice care include pain and symptom management, provision of support to the bereaving family and promoting a peaceful and dignified death (Johnson and Slaninka, 1999). At the core of a hospice's work is the concept of "palliative care" which is defined by the (WHO, 2000) as the active total care of patients whose disease is not responsive to curative treatment and whose goal is the achievement of the best quality of life for patients and their families. This service is provided without government funding and relies mainly on donations from families, charity organisations and fundraising campaigns. Palliative care is delivered by a professional interdisciplinary team comprising professional nurses, social workers, doctors, spiritual counsellors and professional volunteers. There are currently more than fifty hospices throughout South Africa. This study concentrated on nine hospices

in the Free State Province, namely the Bethlehem, Ladybrand, Viljoenskroon, Smithfield, Ons Plek, Sunflower, Naledi, Tshepo and St Thomas hospice. The tenth hospice, namely St Bernard, is situated in East London in the Eastern Cape and provides palliative care to the terminally ill cancer and AIDS patients. This hospice also has an in-patient unit as part of a home care programme. Sunflower hospice, which is situated in Bloemfontein in the Free State Province, provides palliative care for children only. The tradition at this hospice is that after having made a donation, they send a card imprinted with the hands of the youngest patient to the donor (see appendix a).The hospice has constructed a wall of remembrance on which sunflowers have been painted - each bearing the name of a deceased child patient. Annually, on 6 December (St Nicholas' Day), a special remembrance service is held for the families of the deceased children, as well as the hospice staff.

1.4.2 Donation of office space

Some hospitals have set up direct links with a hospice's home-based care programmes, offering office or ward space in their facilities. This can be in the form of a 'step-down facility', which offers a similar service to hospice's in-patient care, but is attached to the hospital itself. For instance, both the Naledi and Sunflower hospices in Bloemfontein occupy under-utilised wards of local

hospitals in their region. Patients are under the care of community caregivers and volunteer doctors, and their families that are encouraged to learn palliative care skills during this time, including good hygiene and nutrition in order to strengthen the patients' resistance in the ongoing battle against opportunistic infections.

1.5 NUTRITION AND HIV

Adequate nutrition cannot cure an HIV infection or AIDS but remains essential to maintain a person's immune system; thus allowing him or her to maintain a healthy level of physical activity and ensure optimal quality of life (WHO, 2005). The relationship between infection, nutritional status and immune function has been described as a complex triad (Figure 1.2). Individuals with HIV/AIDS have an increased risk of food poisoning and food-borne illness, which usually results from improper food handling, storage and preparation methods (Scott, 1996). In addition, HIV-infected patients are also at an increased risk of malabsorption of nutrients and systemic opportunistic infections can contribute to malnutrition at any point in their illness (Johann-Liang *et al.,* 2000). Decreased nutrient intake and absorption together with metabolic derangement in the face of chronic HIV infection are the leading causes of malnutrition. It is generally known that malnutrition, a complication of AIDS, affects the quality of life, immune function and survival. HIV infection and subsequent development of AIDS has a profound

effect on nutritional status, and malnutrition itself is related to mortality in AIDS both via depletion of body cell mass (Kotler *et al.,* 1989) and by alteration of the immune function in the immuno-compromised host (Beach *et al.*, 1989).

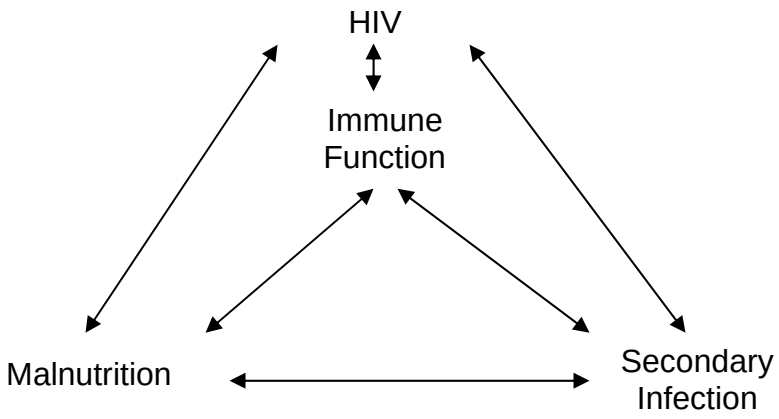

Figure 1.2: Model of interaction of (HIV) infection, nutritional state and immune function.

1.6 FOOD-BORNE PATHOGENS

One area that is often overlooked in preventative health care and HIV care in general is the importance of food safety. Malnourished people and those living with HIV/AIDS are less able to fight food-borne diseases due to their weakened immune system. A study of campylobacteriosis in developing countries, by Coker *et al.* (2002) gave some insight into the prevalence of *Campylobacter* species, which is the most commonly isolated bacterial pathogen from children under two years of age suffering from diarrhoea. Coker *et al*. (2002) reported that this disease is projected to remain one of the top ten isolated bacterial pathogens globally until 2020.Campylobacteriosis is considered to be a greater burden in the developing world, partly because Campylobacter species-associated diarrhoea and bacteraemia occur in HIV/AIDS patients (Scott, 2003). It is estimated that food-borne pathogens (disease–causing agents) are responsible for 76 million cases of illness, some resulting in death, in the United States alone every year (CDC, 2006). A food-borne illness is generally caused by micro-organisms consumed by eating any type of food. The aetiologic agents of food-borne illness are bacteria, viruses, parasites and food toxins with effects ranging from relatively minor discomfort to more serious symptoms and manifestations such as fever, diarrhoea, dehydration and even death (CDD, 2004).

Within the home, there can be a chain of events that result in the transmission of infection from its source to a new recipient (Figure1.3). Certain sectors of the population are especially vulnerable after contracting a food-borne illness, i.e. the elderly, pregnant women, young children and those with a compromised immune system (Mootsikapun, 2007). Meer and Misner (2000) have demonstrated that food-borne illness is associated with improper storage or reheating, food stored inappropriately and cross-contamination in the home. Good hygiene practices, including proper hand washing and food handling practices, are essential in the reduction and prevention of the spread of infectious disease in the home. Although good hygiene dictate disinfectants should be used to clean food particles from surfaces, any bacteria remaining on these surfaces are not visible to the naked eye and may therefore be left behind. The significance of contaminated surfaces in relation to pathogen transmission to food is apparent in the food-processing, catering and the domestic environment. Pathogen exposure on surfaces may occur either by direct contact with contaminated objects or indirectly through airborne particles. Lack of food hygiene awareness and implementation are also contributing factors in this regard.

1.7 DOMESTIC AND SMALL-SCALE KITCHENS

1.7.1 Transmission of food-borne pathogens

It is well known that the kitchen is particularly significant in the spread of

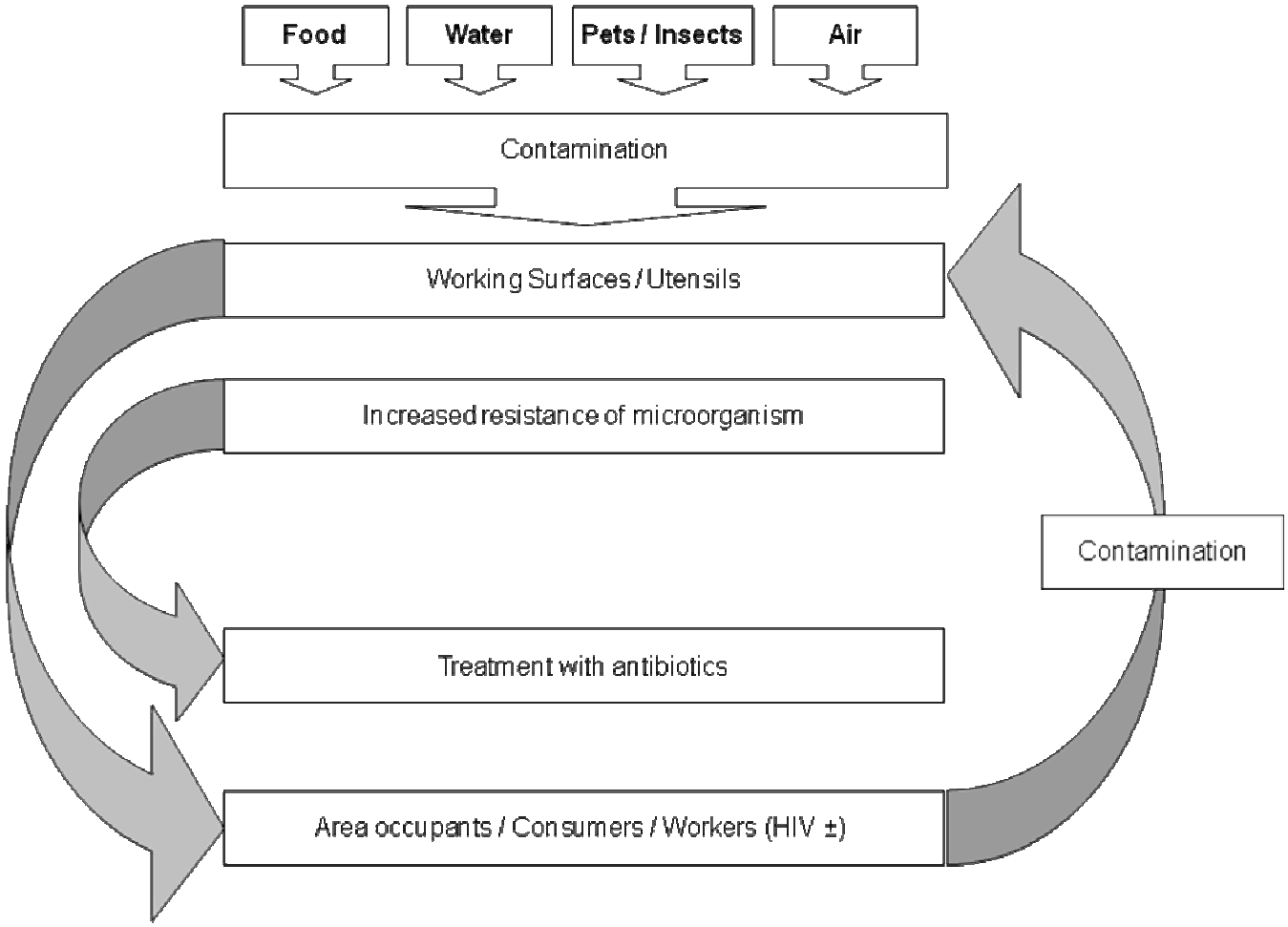

Figure 1.3: Chain of transmission of infection within a domestic home.

infectious disease in the domestic environment due to various activities that occur in this particular setting. The food-preparation surfaces are a focal point in the kitchen (Figure 1.4). The layout of a hospice kitchen is similar to that of a domestic setting, where the retention of bacteria on food contact surfaces increases the risk of cross-contamination of these micro-organisms to food (as illustrated in figure 1.3 above). Exposure of surfaces to pathogens may occur either by direct contact with contaminated objects or indirectly through airborne particles. Zhao *et al.* (1998) found that during food handling and preparation, micro-organisms on raw foods can be transferred to various surfaces, such as cutting boards and water- tap spigots. The persistence of micro-organisms, the presence and density of pathogens and the potential spread of microbial contamination from contaminated food in the household kitchen have been extensively studied and examined. Several studies have indicated that various bacteria, including *Escherichia coli, Staphylococcus aureus and Salmonella spp*., can survive on human hands, sponges or cloths, utensils and currency for hours or days after initial contact (Scott and Bloomfield, 1990; Kusumaningrum, *et al*., 2002). Other studies have quantified the extent of bacterial survival and cross-contamination between hands and various food items and kitchen surfaces (Zhao *et al.,* 1998; Chen *et al*., 2001; Montville *et al*., 2001). It became evident that quantifying the cross-contamination risk associated with various steps in the food preparation process can provide a scientific basis for risk management efforts in

both the home and in food service. Hand-washing and effective cleaning of food-preparation surfaces have been recognised as the most effective measures to prevent cross-contamination and reduce the transfer of micro-organisms to ready-to-eat foods in modern homes and institutional kitchens (Fendler *et al.,* 2002).

Figure 1.4: Food handler preparing food in a hospice kitchen.

1.7.2 Measures for preventing cross-contamination

Many cases of food poisoning originate in the domestic environment and can be associated with improper cleaning and food handling. The primary sources whereby pathogenic micro-organisms are introduced into the home are people, food, pets, water, insects and air (Beumer *et al.,* 1999).The spread of infection can be interrupted by good hygiene practices, which include good hand hygiene and the cleaning and disinfecting of surfaces. Cross-contamination of bacterial and viral pathogens in the home and in food service establishments is a major contributing factor for sporadic and epidemic food-borne illness (Knabel, 1995). In addition, increasing numbers of people with reduced immunity to infection are now being cared for at home and the consequences of infection can be fatal for this specific group. Cross-contamination simulations by De Boer and Hanne (1990) demonstrated the ease with which *Campylobacter jejuni and Salmonella* are transferred from raw chicken products to chopping boards, plates and hands during food preparation. According to Kaplan (2005), the hands of food handler may also serve as a community reservoir for antimicrobial resistant strains of clinical importance, thus further emphasising the crucial role of the human hand as a vehicle for the transfer of food-borne pathogens. Simple personal hygiene coupled with soap utilisation has therefore been hailed as the most successful public health measure in the pre-disinfectant era (Greene, 2001) - hence the recommendation by International Forum on Home Hygiene (IFH, 2007) that

organisms be physically removed from hands and other surfaces by means of washing with soap or detergent-based cleansers, and that microbes be killed *in situ* by the application of a disinfectant or sanitizer.

It later emerged that bacterial populations can be reduced by ensuring that cloths and surfaces are kept dry and that disinfectants are used. However, it was demonstrated by Scott and Bloomfield (1990) that drying alone is insufficient to prevent the transfer of infectious micro-organisms between household surfaces and food handlers, and that cleaning with detergents is only a temporary measure when cloths are kept moist. Although heat is an effective form of disinfection, Beumer *et al.* (1999) conceded that it may not be a possibility when it comes to large surface areas and might be unreliable in unskilled hands. In order to reduce the risk of sponges and cloths being contaminated with micro-organisms, it is recommended that these items be soaked in a bleach solution or be heated for one minute in a microwave oven, or alternatively be immersed in boiling water for five minutes (IFH, 2000).

1.7.3 The influence of bio-aerosols

Indoor and outdoor air contains suspended biological particulate matter that can pose a threat to public health through infectious diseases. For HIV- infected persons, the effects are much more devastating due to their already compromised immune systems. A typical example of such a disease is tuberculosis (TB) which is caused by various strains of *Mycobacterium*, usually *M. tuberculosis*, which are spread from person to person through airborne particles released through a cough or sneeze by an infected person (Kumar *et al.*, 2007). According to Harries *et al.,* 2009, HIV and TB are overlapping epidemics that are creating an immense burden of disease in sub-Saharan Africa.

Pathogens are continually introduced into the home by people (who may have an infection or may be asymptomatic carriers of infectious organisms), contaminated food and domestic animals, and sometimes in water or via the air. Exposure of surfaces to pathogens may take place either by direct contact with contaminated objects or indirectly through airborne particles (Kusumaningrum *et al.*, 2002). According to Zucker *et al*. (2000), bio-aerosols consist of all airborne particles of biological origin, i.e., bacteria, fungi, fungal spores, viruses and pollen fragments, including various antigens. The transport and ultimate settling of bio-aerosol are

affected by their physical properties (size, density and shape of droplets) and environmental factors which include, air current magnitude, relative humidity and temperature (Stetzenbach *et al*., 2002).

Airborne bacteria and fungi can cause a variety of infectious disease and have allergic and toxic effects. In the context of healthcare settings; bio-aerosols can cause occupational hazards and nosocomial infections. Nosocomial infections transmitted by the airborne route, especially fungal infections such as aspergillosis, have been reported by Dykewicz (2001) to be the major source of morbidity and mortality in immuno-compromised patients. Bio-aerosol exposure assessment in a healthcare setting such as a hospice is therefore crucial in the effort to reduce the level of airborne contaminants

1.8 ANTIMICROBIAL RESISTANCE

For more than half a century, antibiotic drugs have made it possible to treat potentially life-threatening bacterial infections. They have turned bacterial infections into treatable conditions rather than the life-threatening compounds they once were. However, the increasing prevalence of antibiotic resistance

poses a serious threat to healthcare, and people living with HIV/AIDS are at particular risk (Manges, 2001). Furthermore, the emergence of multi-drug resistance bacteria has created a situation where there are few treatment options available for certain infections (WHO, 2005). According to Ghandhi *et al.,* 2006, poor management of TB has led to the emergence of multi-drug-resistant (MDR) TB and extensively drug-resistant (XDR) TB in patients co-infected with HIV, threatening to sabotage management strategies for both HIV and TB. One of the major disadvantages of antimicrobial use in animals is the potential development of antimicrobial-resistant zoonotic food-borne bacterial pathogens and the subsequent transmission thereof to humans as food contaminants. Lately, the effectiveness of many antibiotics is diminishing dramatically in the face of increasing resistance amongst various types of bacteria. Antibiotic resistance in food-borne pathogens has become a reality, although substantial qualitative and quantitative differences do exist (Teuber, 1999). Schlundt (2002) identified *Campylobacter, Salmonella, Yersinia,* pathogenic *E. coli and Listeria* as the major food-borne bacterial pathogens. Infection with any one of these bacterial strains, if resistant to antibiotics, will cause delays in the administration of appropriate therapy and may affect the outcome negatively (Molbak, 2005). Infections caused by resistant bacteria have been shown to be more frequently associated with increased morbidity and mortality than those caused by susceptible pathogens, which poses a serious public health concern (Helms *et al*., 2002; Travers and Barza, 2002; Varma *et al*., 2005. It is now collectively

acknowledged that the use of antimicrobials in both animals and humans can select for resistant bacterial populations. Thus, addressing the issue of antimicrobial resistance is one of the most urgent priorities in the field of infectious disease today.

1.9 RATIONALE

South Africa is the country that is shouldering the heaviest burden in terms of HIV/AIDS, and this is having an increasingly negative impact on the economy. The virus compromises the immune system, making the person more vulnerable to opportunistic infections. Infected persons are more susceptible to many types of infection, including those brought about by disease-causing bacteria and other pathogens that cause food-borne illness.

It has been estimated by public health and food safety experts that millions of episodes of illness annually can be traced to contaminated food and water. Food and water safety is especially important to persons infected with HIV. In one study of patients with AIDS (Antony *et al.,* 1998), two-thirds were suffering from diarrhoeal disease, and the following enteric pathogens were identified:

Salmonella, *Shigella, Listeria, Yersinia, Cryptosporidium, Entamoeba histolytica* and *Campylobacter* spp*.* These pathogens have also been recognized as aetiologic agents in food- and water-borne diseases. Salmonellosis is estimated to be nearly 20 times more common and five times more likely to be bacteraemic in AIDS patients than in patients without AIDS (Celum *et al.,* 1987). Campylobacteriosis can also cause bacteraemia (blood infection) and can be difficult to cure in AIDS patients (Barnard *et al*., 1989). In an epidemiological study of patients with HIV infection, a close association was found between consumption of raw or partially cooked fish and antimicrobial-resistant *Mycobacterium avium* complex (Horsburgh *et al*., 1994). Quantifying the cross-contamination risk associated with various steps in the food preparation process can provide a scientific basis for risk management efforts in both home and food-service kitchens (Chen *et al.,* 2001). Several studies have found that various bacteria, including *Escherichia coli, Staphylococcus aureus* and *Salmonella spp* can survive on hands, sponges/cloths, utensils and currency for hours or days after contamination (Scott and Bloomfield, 1990; Jiang and Doyle, 1999; Kusumaningrum *et al.,* 2002). The aim of the study was therefore to identify the presence of potential microbial hazards such as *Staphylococcus* spp*., Escherichia coli.* and *Bacillus spp*. in hospice kitchens. The specific objectives of this study were to identify possible microbial contamination of bio-aerosols (chapter 2) and microbial hazards in the food-processing sections of selected hospices in the Eastern Cape and Free State provinces (chapter 3). The

antibiotic resistance of the identified food-borne pathogens was also determined (chapter 4). In order to realise the aim of this study, the following aspects were considered: food contact surfaces, including the hands of persons preparing the food, and microbial surface load versus bio-aerosols.

1.10 REFERENCES

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CHAPTER 2

FOOD-BORNE MICRO-ORGANISMS ASSOCIATED WITH AEROSOLS IN THE FOOD PREPARATION AREA OF HIV/AIDS HOSPICES IN CENTRAL SOUTH AFRICA

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2.1 ABSTRACT

Bio-aerosols consist of all airborne particles of biological origin, i.e., bacteria, fungi, fungal spores, viruses and pollen, including various antigens. Components of the airborne material can have a negative effect on the health of exposed individuals, both outdoors and indoors (Jones and Harrison, 2004). They play a significant role in indoor air pollution as they can be pathogenic with resultant allergic reaction following inhalation. Exposure to these biological entities and microbial fragments may results in adverse health effects of which HIV- infected persons being at particular risk (Ross *et al*., 2000). Since the kitchen is the focal area of food preparation, it remains imperative that the bio-aerosol level is kept at the lowest level possible – especially in a hospice. Therefore, proper design and ventilation of the hospice kitchen is essential in the effort to control airborne contamination and also prevent airborne infections in this setting. Samples of breathable air were collected at ten registered hospices in two provinces in central South Africa, namely the Eastern Cape (1) and Free State (9). All microbial samples were collected 1.5 metres above the floor on 65 mm RODAC plates by means of impaction on soft agar. The (SAS) Super-90 surface air sampler (PBI International, Milan, Italy) was used for this purpose. The average airborne particulates were measured at 7.90 $mg.m^{-3}$ inside, compared to 8.0 $mg.m^{-3}$ outside.

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Keywords: Bio-aerosols; hospice; kitchen design; food safety.

2.2 INTRODUCTION

As in any domestic setting, the safety and quality of food served in a hospice depend on the kitchen design, storage conditions, and food preparation practices of the food handlers. Micro-organisms can become airborne when droplets are generated while speaking, coughing, sneezing or vomiting. Most residential and hospice kitchens do not normally use air-filtration systems. This means that the principal factors governing the levels of airborne particles indoor are: indoor sources, outdoor particle levels, the deposition rate of particles on indoor surfaces, and the air exchange rate (Nazaroff, 2004). Exposure of building occupants to certain micro-organisms, and elevated concentrations of environmental organisms, could result in allergenic reactions, irritant responses, toxicosis, respiratory illness and other ill effects.

This is especially important in a hospice environment that accommodates patients with compromised immune systems due to infection with the human immunodeficiency virus (HIV). Tuberculosis (TB) is an archetypal example of a

disease that is transmitted by airborne route. Primary pulmonary TB is caused by the inhalation of droplet nuclei carrying the causative agent, *Mycobacterium nuclei* (MTB). For hardy bacteria such as mycobacterium TB, only a single organism is needed to cause disease (Haas, 2006). TB acts synergistically with HIV and increases the risk of primary TB infection developing into the active disease by a hundred fold (Davies, 1999). The world-wide occurrence of TB is high, with approximately one third of the world's population thought to be infected with MTB (Miller, 1996). Globally, TB is estimated to cause the deaths of three million people annually, and this figure is predicted to rise to five million by the year 2050 (Davies, 1999). Nosocomial infections similar to TB are a very real problem in healthcare facilities, with approximately one in 10 patients acquiring an infection during a hospital stay (Schulgen, *et al.,* 2000).

Although most nosocomial infections are generally associated with person-to-person contact, evidence is mounting that they are mostly transmitted via aerosol route. Unlike formal healthcare facilities that generally boast air filtration systems, informal healthcare facilities like hospices have none. With patients suffering from a plethora of diseases associated with a compromised immune system, contaminated air only serves to aggravate the problem. Therefore, the aim of this study was to quantify the load of indicator/common microbes and associated environmental factors in the air outside/inside a typical hospice environment.

Subsequently, the relationship between environmental factors and microbial concentration was established and compared with normal breathable air in the same environment.

2.3 MATERIALS AND METHODS

2.3.1 Sample collection

Samples of breathable air were collected at ten registered hospices in two provinces in central South Africa, namely the Eastern Cape and Free State. The sampling campaigns were conducted during the southern-hemisphere winter (dry months, April-September). Samples were collected in duplicate at each hospice in the morning (before and after food preparation) and also at lunchtime (before and after food preparation), both inside and outside the hospice kitchen for both sessions.

2.3.2 Air sampling procedure

All microbial samples were collected 1.5 metres above the floor on 65 mm RODAC plates by means of impaction on soft agar. The (SAS) Super-90 surface

air sampler (PBI International, Milan, Italy) was used for this purpose. The air sampler was calibrated at an airflow rate of 0.03 $m^{3}{\cdot}min^{-1}$ and all detachable parts were sterilised with 70% ethanol before use and between sampling runs (Venter *et al.,* 2004). Plate count agar (PCA) was used for the isolation of total viable aerobic count (Merck, SA). Samples were then placed in a cooler box and immediately transported to the laboratory. Subsequent incubation of the plates was done at temperatures of between 25 and 37 ℃ fo r periods ranging from 24 to 72 hours. All colonies were enumerated using the positive hole correlation method and expressed as colony-forming units per cubic meter of air sampled. Subsequent replica plating was performed using a replica plating device and sterile velveteen cloth to quantify the following micro-organisms: *Staphylococcus aureus*, *Pseudomonas* spp*., Bacillus* spp., coliform and total coliform.

2.3.3 Facility design

Assessment of the kitchen design and setting was done by means of visual observations and note taking. Simultaneous observation of safe food handling and storage practices was also conducted. (See table 2.1)

2.3.4 Quantification of environmental factors

The extrinsic environmental factors capable of influencing the survival of micro-organisms, i.e. temperature (area heat stress monitor, Questemp, SA) and relative humidity (whirling psychrometer, Airflow Instrumentation, SA), were measured, as were the factors that could influence the distribution of the assessed microbiota (Chang, *et al.,* 2001), namely airflow (airflow anemometer – LCA 6000 VT, Airflow Instrumentation, SA) and airborne particle (dust) concentrations (hand-held aerosol monitor - 1005/1060, PPM Enterprises, Inc) (Venter, *et al*., 2004). Positive and negative controls were included and all analysis and assays were repeated at least in duplicate according to Venter*, et al.,* (2004).

2.4 RESULTS AND DISCUSSION

2.4.1 Facility design

The results obtained from the technical investigations are presented in Table 2.1. From this, it is apparent that most hospices with the exception of a few e.g. Ons

plek and Bethlehem, complied with the requirements of good preparation and handling practices of food.

Table 2.1: Kitchen facility design and food handling practices in hospices around Central South Africa.

Occurrence	%
Kitchen facility design	
Ceiling	60
Ventilation through natural moving air	60
Air bricks with filters	50
Food handling practices	
Cleanliness/neatness of the food handler	70
Wearing of suitable protective clothing	50
Availability of hand washing facilities	60
Storage space for hygienic storage of food	50
Availability of easy to clean refuse containers	50
Regular washing of hands before/after food preparation	60

Overall cleanliness of the kitchen	
Environment conducive for cooking/preparing food	50

*The percentages and right hand values represent the level of conformance to Regulation 918 amongst all the hospices sampled (n=10).

The lack of air filtration systems due to financial constraints, may have contributed to the presence of bio-aerosol indoors. The majority of the investigated kitchens were also not designed to provide required barriers against moisture, temperature, pests, dust and associated microbes. Therefore limited control over the quality and safety of the food stored under the noted conditions would be expected.

2.4.2 Influence of extrinsic factors

The extrinsic factors that influence the viability and distribution of micro-organisms that prevail in various hospice kitchens are listed in figure 2.1. From these results, it is evident that on average, the hospice kitchens provide an environment that would sustain microbial viability concomitant to proliferation, given that a suitable substrate and sufficient time is available. The persistence of micro-organisms, the presence and density of pathogens and the potential

spread of microbial contamination from contaminated food in the household Kitchen have been extensively studied and re-examined. These studies indicated that domestic kitchen sites have been found to be repeatedly contaminated with a variety of bacterial contaminants, including *Listeria monocytogens* (Beumer, *et al.,* 1996), *Escherichia coli* and *Enterobacter cloacae* (Speirs, *et al.,* 1995). It is well known that dampness and other excess moisture accumulation in buildings are closely connected to observation of mould, mildew, or other microbial growth. Microbial growth has also been associated with building characteristics. In residences measures of microbial contamination have been found to be positively correlated with indoor temperature and humidity, age and size of buildings, use of wood stoves and fireplaces and absence of mechanical ventilation (Dharmage, *et al*., 1999). From this study it became evident that the evaluated kitchens boasted levels of relative humidity of 60 ± inside as compared to the average of 40 ± outside.

The relative humidity (RH) in one of the hospices was 100% and the dominant bacterial specie was *Bacillus cereus*, therefore showing an increase associated with an increase in RH. The average temperatures were below 20 ℃ outside and 15 ℃ inside (Figure 2.2); hence the prevalence of *Pseudomonas aeruginosa* (Fig 2.1) due to their ability to survive at low temperatures (Forsythe, 2000). The average airflow for the assessed kitchen was 0.4 $m.s^{-1}$ outside compared to the

average of 0.2 $m.s^{-1}$ inside. It is also interesting to note that the low airflow inside correlated with a high concentration of airborne particulates inside which was 8.04 $mg.m^{-3}$.

2.4.3 Airborne indicator organism presence

The presence of undesirable bio-aerosols is often associated with sick building syndrome (SBS) and building-related illnesses. Sources include furnishing and building materials, fungal contamination within wall and gaps at structural joints (Jay, 2000). Inadequacies in the building design and improper ventilation may contribute to poor indoor air quality. For purposes of this study, micro-organisms indicative of poor food manufacturing practices are defined as indicator organisms. In the formal food industry, the presence of *Escherichia coli* is indicative of faecal contamination, while the presence of *Staphylococcus aureus* points to extensive human handling, and total viable aerobic organisms is a sign of poor process hygiene. The presence of these organisms in the breathable air of the various hospice kitchens could also be attributed to the aforementioned practices, as food processing is the core business of the kitchens in this setting.

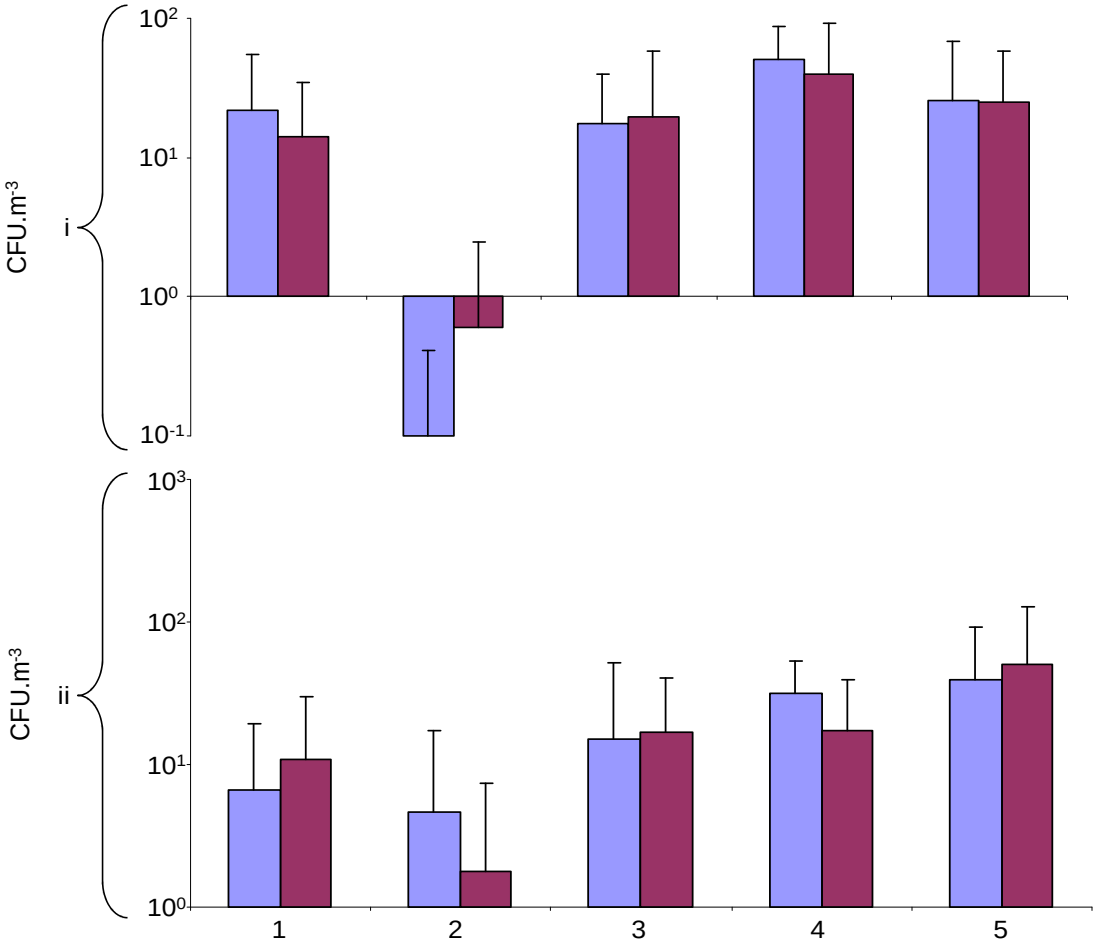

Figure 2.1: The average culturable airborne microorganisms (*Staphylococcus aureus* (1), *Pseudomonas* (2), Coliform (3), *Bacillus* (4) and Total Coliform (5)) isolated from breathable air inside and outside the hospices before (i) and after (ii) preparation of food

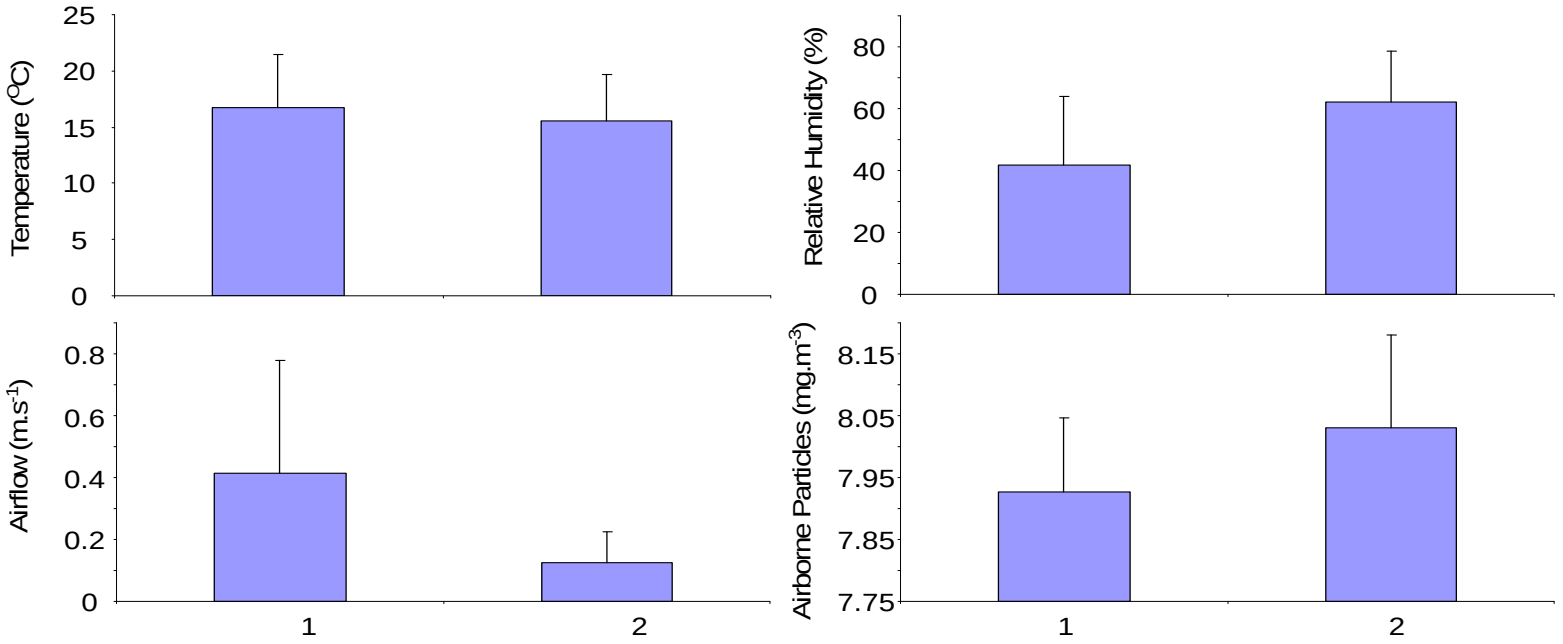

Figure 2.2: The Environmental factors quantified outside (1) and within (2) hospice kitchens.

In this scenario, the presence of *E. coli* could be due to faecal contaminants from the hands of food handlers, or from contaminated working surfaces and utensils. Therefore, it is of the utmost importance to observe proper personal hygiene, particularly with regard to hand-washing after visiting the toilet, in order to prevent contamination. However, three of the hospices that were investigated are without hand-washing facilities and are located in rural areas. As noted in figure 2.1 the average airborne particulates were 7.90 $mg.m^{-3}$ inside as compared to 8.0 $mg.m^{-3}$ outside. These results are comparable to those observed by Hargreaves *et al.,* 2003. The presence of *Bacillus spp* was also noted in the presence of these conspicuous airborne particulates which were dominant throughout the sampling period. This could be due to the fact that these are spore formers and bacteria in many cases are attached to larger airborne particles. According to Moir *et al.,* 2002 these spores can endure a wide range of extreme environmental stresses while retaining the capacity to return to vegetative growth almost immediately once the nutrient returns to the environment. It was therefore assumed that airflow in and outside the hospice kitchens were sufficient to carry dust particles as well as different bacterial population (Figure 2.1). In general, the average bacterial counts varied between 1×10^1 to 1×10^2 $cfu.m^{-3}$. Compared to the literature these counts were fairly low and apart from being possible allergens they are also associated with decreased lung function, increased respiratory symptoms such as cough, shortness of breath and asthma attacks (WHO, 2002). From the observed results it is apparent that the kitchens in question boast a resident bio-aerosol population that is not significantly influenced by the noted

environmental parameters. Though the source of the bio-aerosols assayed is not clear, *S. aureus* probably results from aerosols dispersed by the food handlers in the kitchens. It should also be noted that although microbial counts were low, the kitchens provided an environment conducive to microbial survival as aerosolised particles and subsequently as food contaminants. In general the kitchens had a lower temperature, increased RH, and higher airborne particle count.

2.5 Conclusion

Indoor air pollution is usually caused by the accumulation of contaminants from various indoor sources. The generation of pollutants within the indoor environment may come from primary sources such as fuel combustion for cooking, as well as emissions from fireplaces, stoves, cleaning products and chemicals stored in the home. It is therefore safe to assume that since all these factors were present at the study sites, they all contributed to the indoor air pollution discovered. Factors such as heating, ventilation, air-conditioning and household activities, e.g. cooking and cleaning, all play a crucial role in the wellbeing of a building's occupants. In a setting such as a hospice, where proper food storage and handling is not always possible due to lack of infrastructure, it is essential that special care is taken regarding the type of food stored and the packaging material used. Micro-organisms detected in the

indoor air of the hospice kitchens included in this study could be derived from the hospice occupants, but may also emanate from the outside environment. One such example would be *Bacillus cereus*, which is a common air- and dust-borne contaminant that readily multiplies in meat products. These organisms are able to withstand unfavourable conditions such as low temperature and heat due to their ability to form spores (Whyte *et al.,* 2001). A study by Nel *et al.,* 2003, reported rapidly increasing levels of *B. cereus* when a product was exposed to poor handling and processing procedures. A hospice, where patients are provided with accommodation, food and care, would be a typical example of this setting.

From the results it can be concluded that certain food preparation and storage facilities in the hospices studied are, according to the technical data, not suitable for this purpose. However, it appeared that some of the extrinsic factors influencing microbial viability were being governed. Specific attention should be given to the upgrading of the kitchen infrastructure – for example, it would be ideal to have separate rooms for the preparation of raw and cooked food, as well as a room used only for food storage purposes. The results presented in this chapter further identified the hospice occupants as possible sources of the organisms found in the hospice kitchens and surrounding environments, since the occupants were in some cases also responsible for preparing the food, while moving continuously between the kitchen, bathroom and bedroom. In terms of residency, it would thus be ideal to separate the patients from the food preparation facilities, including the kitchen.

Companies that regularly donate food to the hospices are further cautioned to avoid donating foodstuffs that are past their sell-by date, as the inability of the assessed kitchens to control humidity fluctuations only exacerbates the problem with regard to food safety and proliferation of microbial load. It was further noted that in South Africa, unlike in more humid countries, the facility design and geographical localisation have a limited effect on the resident bio-aerosol profiles. In order to address and verify the concerns raised in this chapter, it is recommended that the ability of micro-organisms to proliferate on the foods (high-risk) provided to the hospices should be assessed and the menus adjusted in accordance to season- associated changes in the extrinsic factors that would influence microbial viability and growth. It should further be noted that the occupants of the hospices concerned have very little knowledge of proper food storage and handling practices and are to a large extent not aware of the threats posed to them by the resident microbiota. This problem could be alleviated by providing the patients and food handlers with educational training in respect of proper food safety (storage and handling).

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CHAPTER 3

MICROBIAL CONTAMINATION ON HANDS AND SURFACES IN HOSPICE KITCHENS IN CENTRAL SOUTH AFRICA: A QUANTITATIVE EVALUATION

This chapter has been submitted for publication (partially / in full) to the Journal of food protection

3.1 ABSTRACT

Selected food-borne pathogens were isolated from various possible reservoirs in hospice kitchens in Central South Africa. Samples from foodhandler's hands and food preparation surfaces (cutting -boards, tables, sinks) were collected and the following microbiota were isolated: Total viable counts (TVC), Coliforms, *Escherichia coli*, *Staphylococcus aureus, Pseudomonas* and presumptive *Salmonella*. Respective mean TVC were in a range of 2 cfu.cm^{-2} to 5 cfu.cm^{-2} for both food contact surfaces and hands. Coliform counts, however, were present at a concentration of 3±1 cfu.cm^{-2} for surfaces and hands. These counts were lower than those recorded by Josephson (1997), although *E. coli* counts found on the cutting board (4 cfu.cm^{-2}) in this study were higher than those reported in a later study focusing on traditional home-based kitchen by Antonia *et al.,* 2007. This could have originated from either the bio-aerosols or from cross-contamination via the food handler's hands and the food. The findings of this study demonstrate that the extent of bacterial contamination is dependent on the hospice set-up with regard to kitchen infrastructure and the food handling practices of the occupants/food-handlers. It is also interesting to note that the microbial load and contamination level of hospice kitchen is different from that of traditional home-based kitchen. The national standard for the TVC on work surfaces in accordance with regulation 918 of 1999 is 100 cfu.cm^{-2} and all samples in the study conformed to this guideline.

Key words: kitchen, hygiene, food safety

3.2 INTRODUCTION

Illnesses resulting from the consumption of contaminated food remain a global public health problem. One of the major contributing factors in the spread of infectious disease is the kitchen. Within this setting, the primary sources by which pathogenic micro-organisms are continually and inevitably introduced into this setting are people, food, pets, water; insects and air (Beumer *et al.*, 1999).These are considered to be the primary sources of potential harmful micro-organisms in the home. Many foods brought into the kitchen are frequently contaminated with naturally occurring pathogenic micro-organisms. This is of great concern even in industrialized countries which have experienced their share of gastrointestinal infections, with up to 30% of the population suffering from food-borne diseases each year (WHO, 2007). Although many of the respiratory and gastrointestinal infections can be asymptomatic and self limiting, they still remain a major economic burden (Shuval, 2003). This is especially important since increasing numbers of people with reduced immunity to infection are now being cared for at home and also in the hospice environment. For this designated group, the consequences of food-borne infection may be hospitalization (with associated additional costs) or even death. Understanding the cross-contamination

route(s) of an infectious disease is critical to identify accessible targets for control strategies. For example, person-to-person transmission may be inhibited by proper hygiene, sanitary conditions and education. Good hygiene practices during food preparation is therefore essential in preventing cross-contamination of prepared foods from raw foods, and subsequent contamination of food by infected household members or domestic animals.

Early studies on bacterial contamination in the kitchen, which were conducted in the late 1960s, investigated the bacterial load on handtowels and the hygienic conditions of domestic dishcloths and tea towels (Speirs *et al.,* 1995). These studies indicated that domestic kitchen sites are repeatedly found to be contaminated with a variety of bacterial contaminants, including *Listeria monocytogenes* (Beumer *et al.,* 1996), *E. coli* and *Enterobacter cloacae* (Speirs *et al.,* 1995), *Salmonella* (Enriquez *et al.,* 1997), *Campylobacte*r (Josephson *et al*., 1997)) and *Staphylococcus aureus* (Hilton ,2000). Several kitchen sites, particularly wet areas including sponges / dish cloths (Beumer *et al.,* 1996), and sink drain areas (Salo *et al.,* 2000) appear to act as continuous reservoirs that harbours and encourages the growth of potential microorganisms (Table 3.1). Zhao *et al.,* 1998 also demonstrated that bacteria could readily be transferred to chopping-boards after cutting and handling contaminated chicken. It was found that large numbers of bacteria can survive on chopping-boards for at least four

Table 3.1: Microbial composition on various kitchen surfaces.

Surface	Bacteria	CFU	Reference
Dishcloths	*Listeria spp*	10^4	Beumer *et al.,* 1996
Sinks	*Listeria spp*	10^4	Beumer et al., 1996
Dishcloth	*Salmonella*	14%	Enriquez *et al.,* 1997
Kitchen sponge	*Pseudomonas*	36%	Enriquez *et al.,* 1997
Refrigerator compartment	*Listeria*	10^3	Hilton, 2000
Kitchen sponge	*S. aureus*	4×10^4	Hilton, 2000
Kitchen sponge	TVC	$20 - 6 \times 10^8$	Hilton, 2000

hours and can cross-contaminate fresh vegetables if the boards are not cleaned or disinfected. Likewise, pathogenic organisms can also be shed, or transferred by contact, into the environment by infected family members, or by carriers of pathogenic organisms who may show no symptoms of disease (asymptomatic). In South Africa, surveillance systems are still insufficient to accurately document the true level of infectious disease despite the emergence of infections with high mortality, such as human immunodeficiency virus (HIV). Food handlers play a major role in the prevention of food contamination during food production, distribution and handling. They are at risk of cross-contaminating raw and processed food stuffs and also inadequately cooking and storing foods (Humphrey *et al*., 2001; Cogan *et al*., 2002, Duff *et al*. 2003). Food- borne illness has been associated with

improper storage or inappropriate storage and/or reheating of food as well as cross-contamination (Redmond *et al.,* 2004).

Several reports (Pinner *et al*., 1992) have documented a direct link between illness caused by *Listeria monocytogenes* in HIV-infected individuals and specific food vehicles. Kales and Holzman (1990) also discovered a higher prevalence of listeriosis amongst people infected with HIV than amongst the general population. The aim of this chapter is therefore to quantitatively evaluate the level of hygiene in the kitchens of selected hospices in central South Africa, specifically in terms of the food handlers' hands and the food preparation surfaces. The surface samples were obtained from the cutting boards, tables and sinks in each of the hospices.

3.3 MATERIALS AND METHODS

3.3.1 Sampling protocol

Bacterial samples from hands and food preparation surfaces were collected from ten registered hospices in South Africa – nine in the Free State Province and one in the Eastern Cape Province – over a period of six months during the southern-hemisphere winter (dry months, June-November). The hospices

chosen for purposes of this study were the Bethlehem, Ladybrand, Viljoenskroon, Smithfield, Ons Plek, Sunflower, Naledi, Tshepo, St Thomas and St Bernard hospices. Samples were collected from the tables, cutting-boards and sinks (appendix a) in the hospice kitchens, as well as the thumbs and three middle fingers of the food handlers. This was done daily between the hours of 08:00 and 14:00 – once before and once after the preparation of breakfast, and once before and once after the preparation of lunch – using 55 mm Rodac surface contact plates (Rodac Nunc, Denmark). The samples were kept on ice during transportation to the laboratory and analysed without delay (Bryan *et al.,* 1997).

3.3.2 Microbiological analysis

Total viable counts

It is generally accepted that TVC indicates the level of contamination of a particular foodstuff, as well as the presence of pathogens in a food-processing environment. For the enumeration of TVC, plate count agar (PCA) plates (Merck, SA) were incubated at 25 ℃ for 48 hours, and typical TVC were colourless colonies (Houghtby *et al.*, 1993; Voster *et al.,* 1994).

Escherichia coli and Coliforms

Escherichia coli are generally regarded as an indicator of pathogenic microbiota originating from faecal contamination and poor sanitation during food processing (Department of Health: South Africa, 2000). Violet-red-bile-MUG (VRBM) agar (Biolab, SA) was used for the quantification of *E. coli* and coliforms, and plates were incubated at 37 ℃ for 18-24 hours (Scharlau, 2000). Typical *E. coli* colonies were dark red with a diameter of two to five millimetres, while coliforms appeared as small pink colonies. With *E. coli* (ATCC 25922) being used as a positive control, a blank VRBM agar plate was used as a negative control, incubated at the appropriate temperatures.

Staphylococcus aureus

Baird-Parker Agar (Biolab, SA) with 50 ml egg-yolk tellurite emulsion (Merck, SA) was used and the plates were incubated at 35℃ for 48 hours (Fang, 2003). Typical *S. aureus* colonies were black with white margins surrounded by clear zones. For positive control, S. aureus (ATCC 25923) was used and a blank Baird-Parker Agar plate was used as a negative control and incubated at 35℃ for 48 hours.

Presumptive Salmonella

Brilliant green agar (BGA) (Scharlau, 2000) was used and the plates were incubated at 42℃ for 24-48 hours (Van Schothorst and Renaud, 1983). *Salmonella enteriditis* (ATCC 13076) was used as a positive control and a blank BGA plate was used as a negative control.

3.4 RESULTS AND DISCUSSION

3.4.1 Total viable counts

Total viable counts are used as an indicator of the general degree of contamination in a particular foodstuff (Aberle *et al*., 2001). Figure 3.1 represents the TVC on the various food preparation surfaces and the food handlers' hands, both before and after food preparation for the morning and afternoon meal sessions. Average TVC ranged from 2.5 cfu.cm^{-2} to 5 cfu.cm^{-2}. The results demonstrate a clear difference in the microbial contamination of the food preparation surfaces before and after food preparation. TVC observed in this present study were lower than the 10^3 cfu.cm^{-2} reported by Kusumaningrum (2003) for stainless steel surfaces. It is interesting to note that the average TVC (2.8 cfu.cm^{-2}) for the cutting-board increased after the

lunch preparation session. This could be due to the fact that any such food preparation utensil that is used regularly would become pitted and scratched over time, creating conditions conducive to the proliferation and retention of contaminants. The kitchen sink surfaces showed consistent contamination (average TVC of 3.4 $cfu.cm^{-2}$) at the time of the morning session, perhaps due to the constant presence of moisture. Noteworthy TVC measurements were taken from the hands of food handlers (75%) and from food contact surfaces (70%).

3.4.2 Coliforms

Coliform bacteria are organisms that are present in the environment and in the faeces of all warm-blooded animals and humans. They are members of *enterobacteriaceae*, which ferment lactose and produce gas, and are quantified as a measure of the adequacy of sanitation, as well as contamination during processing and/or post-processing recontamination due to cross-contamination by raw materials, dirty equipment or improper hygiene. Their presence is also a possible indication of faecal contamination (Buchanan, 2000).

The detection of coliforms is widely used as a means to measure the effectiveness of sanitation programmes, since their presence indicates substantial risk of the presence of food pathogens (Moore and Griffith, 2002).

Figure 3.2 represents the coliform counts measured on surfaces and hands, which ranged between 3 cfu.cm^{-2} and 4 cfu.cm^{-2}. Van Tonder and Lues (2007) reported similar results from a study conducted amongst food handlers in selected outlets of a retail group in the Western Cape Province of South Africa. According to Moore and Griffith (2002), surface specifications for coliforms after disinfection are not commonly available and general microbial target values of <2.5 cfu.cm^{-2} have been suggested.

3.4.3 Escherichia coli

Escherichia coli is a gram negative bacterium that is commonly found in the lower intestine of warm blooded organisms. Most *E. coli* strains are harmless, but some, such as serotype O157:H7 can cause serious harm to humans and are occasionally responsible for costly product recalls (Vogt and Dippold, 2005). The presence of *E. coli* is generally regarded as an indicator of pathogenic microbiota originating from faecal contamination and poor sanitation during food processing (Idowu and Roland, 2007).

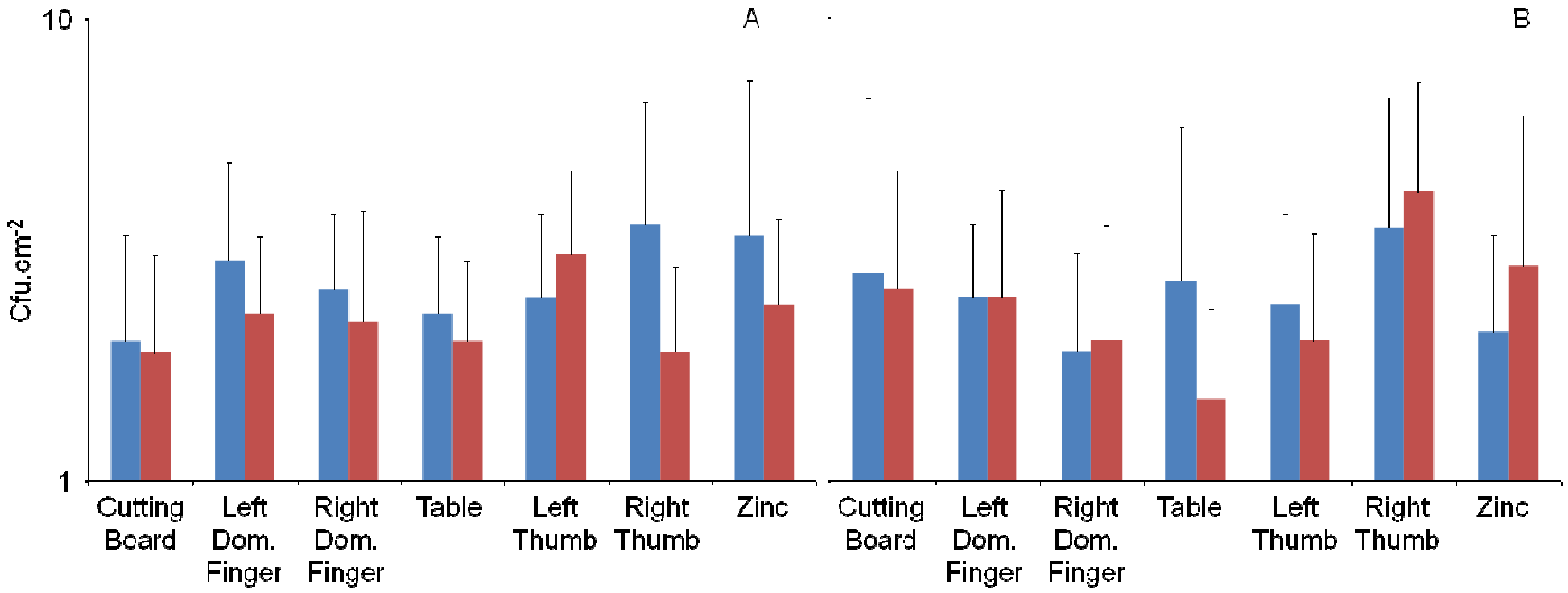

Figure 3.1: Total Viable Counts (TVC) isolated from food contact surfaces and hands. Before Preparation - ■ After Preparation - ■ . A – Breakfast Session – B – Lunch Session. According to regulations governing general hygiene requirements for food premises and transportation of food, Regulation 918 of 1999 no more than 100 viable micro-organisms per cm^2 on working food surface is allowed.

During this study, *E. coli* counts ranging between 2 cfu.cm^{-2} and 7 cfu.cm^{-2} were detected, as shown in Figure 3.2. The highest count was 7 cfu.cm^{-2}, which was isolated from the dominant left digit of the hand of a food handler. According to De Wit and Rombouts (1992), human hands are normally free from *E. coli*, and the presence thereof is thought to give a better indication of faecal contamination than the entire group of *enterobacteriaceae*.

3.4.4 Staphylococcus aureus

S. *aureus* is the predominant species involved in the outbreak of staphylococcal food poisoning due to cooked food products being handled by persons carrying enterotoxigenic staphylococci in their nostrils or on their skin (Angellilo *et al*., 2000). Fig 3.2(i) reflects *S. aureus* counts ranging between 2 cfu.cm^{-2} and 7 cfu.cm^{-2} . The highest count, i.e 7cfu.cm$^{-2,}$ was found on the hand of a food handler during the morning sampling session, prior to the preparation of food. It is interesting to note that this particular food handler also exhibited the highest *enterobacteriaceae* count (4 cfu.cm^{-2}). Aycicek *et al*. (2004) stated that due to the inherent character of this particular micro-organism, it is not possible to set an acceptable contamination level for *S. aureus* after proper hand-washing.

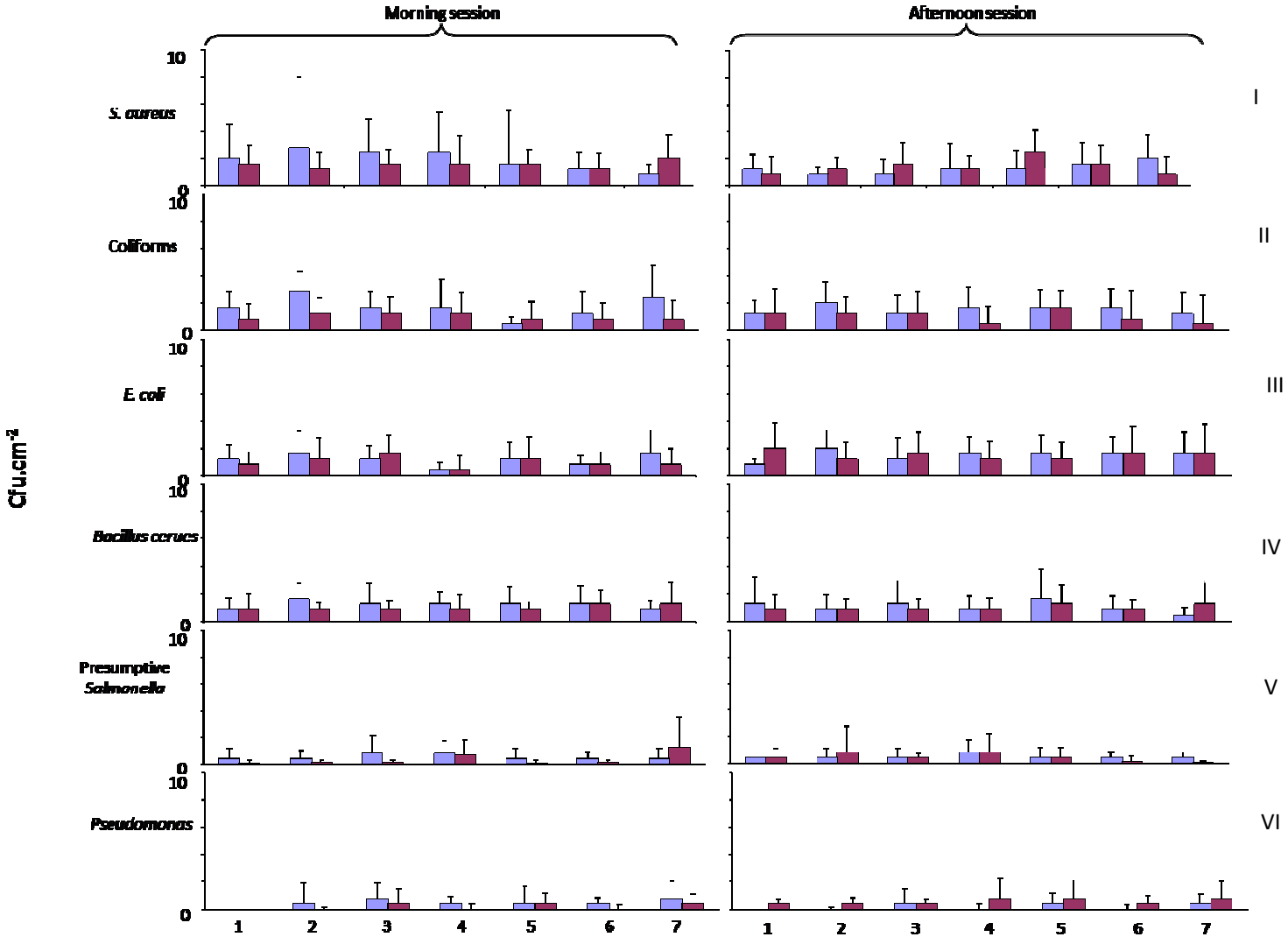

Figure: 3.2: Microbiological distribution of foodborne pathogens isolated from various food preparation surfaces/foodhandler's hands; Sampling points: 1= cutting board, 2= left dominant fingers, 3= right dominant fingers, 4= table, 5= left thumb, 6= right thumb and 7= zinc. Before Preparation - After Preparation -

Therefore, since some of the hospice kitchens involved in this study lack adequate design features, it is essential that safe procedures are implemented during the routine handling and preparation of food. At all of the hospices involved, it is common practice for food contact surfaces to be cleaned again in the afternoon before the kitchen closes for the night. The implication is that even though food particles are usually cleaned from kitchen surfaces, in accordance with good hygiene practices, the remaining bacteria attached to these surfaces are not visible to the naked eye and might therefore escape removal. According to Moore *et al.,* (2001) an inadequately cleaned surface can, if in contact with food, lead to cross-contamination and contribute to a product's microbial load, which might result in a shorter shelf-life.Therefore since some of the hospice kitchens were inadequately designed (See appendix A), safe procedures need to be implemented during routine handling and preparation of food. Common practice in all the hospices investigated is that cleaning of food contact surfaces is repeated in the afternoon just before retiring. The implication is that even though food particles are usually cleaned from surfaces when good hygienic practices are applied, bacteria attached to these surfaces are not visible to the naked eye and may therefore not be removed.

3.4.5 Pseudomonas spp.

Pseudomonas spp. are gram negative obligate aerobic rods and common food spoilage organism, especially found on the surface of ground meat. They are not associated with food- borne illness but are responsible for slime formation, off odour, off colour and greening in meat (Cramer, 2006). This microbe has a predilection for growth in moist environments, which is probably a reflection of its natural existence in soil and water. As shown in Figure 3.2(vi), *pseudomonas* counts in this study ranged from negligible to 2 cfu.cm^{-2} (measured on a kitchen sink). It is interesting to note that, true to its nature, the *pseudomonas* count was only prevalent on the sink surface in the morning prior to food preparation. The presence of *pseudomonas* is of great concern in a hospice environment, which caters for patients with impaired resistance to infection, and food contamination with bacterial pathogens can be lethal. Hospital studies show that *Pseudomonas aeruginosa* can form reservoirs of contamination in sink waste-pipes and can be a source of infection when splashes of contaminated water come into contact with human hands (Doring *et al.,* 1996).

3.4.6 Bacillus cereus

Bacillus cereus is a gram positive, aerobic, spore-forming bacillus commonly found in the soil, on vegetables and in many raw and processed foods. It is a common cause of food- borne illnesses in humans, and one of its most distinct features is the ability to produce heat-resistant spores. Furthermore, spores of *B cereus* have been found to have the ability to adhere to the surface of stainless steel which is a common component of processing equipment used in the food industry. The attached cells may subsequently form biofilms, and are more resistant to sanitizers (Frank and Koffi, 1990). Immuno-compromised individuals and those with underlying conditions such as cancer, rheumatoid arthritis are particularly susceptible to food-borne bacterial enteropathogens and nosocomial/opportunistic infections (Drobniewski, 1993). According to Mueller (1995), for these vulnerable groups, the number of enteropathogenic organisms required to cause illness may be significantly lower than for those with a healthy immune system. The *Bacillus* counts measured during this study ranged from 2 $cfu.cm^{-2}$ to 4 $cfu.cm.^{-2}$, as shown in Figure 3.2(iv). The highest count was found on the left dominant finger of a food handler after food preparation.

3.4.7 Presumptive Salmonella spp.

Salmonella infections belong to the most zoonotic diseases worldwide (Schlundt, 2002). With regard to food-borne salmonellosis, eggs and their products thereof are still the main entryway for *Salmonella* to enter the food chain. Gast and Holt (2000) reported that eggs contaminated with *Salmonella enteriditis* show only a relatively low number of *Salmonella* cells in their egg content, mostly fewer than 10 bacteria per egg. Many foods, particularly those of animal origin, have been identified as possible vehicles for transmission of these pathogens to human beings, who then spread them to food-processing and kitchen environments (Steinhart *et al.,* 1996). Figure 3.2(v) represents the average presumptive *Salmonella* spp., detected at levels ranging from undetectable to 3 cfu.cm^{-2} on kitchen surfaces and from undetectable to 2 cfu.cm^{-2} on human hands. Only one hospice had a presumptive *Salmonella* count in the order of 5 cfu.cm^{-2}, as detected on the cutting-board and table respectively. It is also interesting to note that this was the only hospice where pets (dogs) were allowed to roam freely in the kitchen. Moreno *et al.* (2006) established that cats and dogs can act as reservoirs of *Salmonella*, *Campylobacter* and other enteric pathogens. It can therefore be assumed that there is a link between high *Salmonella* counts and pets being kept at hospices.

3.5 COMPARISON OF HOME-BASED KITCHEN AND HOSPICE KITCHEN

Figure 3.3 illustrates the difference in the microbial proliferation of *E. coli* and *S. aureus* on the hands of food-handlers and cutting-boards in a hospice kitchen and traditional home-based kitchen. It is apparent from the results that the hospice kitchen displayed the high counts of *S. aureus* on the hands and cutting-board, as well as high counts of *E. coli* for the cutting board. *S. aureus* is normally found in the nose and throat of healthy individual. The high counts found on the hands of the hospice food handlers involved in this study can be attributed to the food-handling practices of the individuals concerned, as well as the environment and the occupants (patients). Johnson *et al*., 2005 proved that there is a link between poor hygiene and spread of *S. aureus*, including methicillin-resistant *Staphylococcus aureus* (MRSA). The data show that the organism is shed by infected persons, as well as by asymptomatic carriers, into the home environment, mainly from the skin surface (usually on skin scales) and the nose. It is also interesting to observe the high counts of *E. coli* and *S. aureus* on the cutting-board, which could have originated from the bio-aerosol or through cross-contamination via the food handler. In comparison with the cited literature (Antonia *et al.,* 2007), the results in this study show that *E. coli* maintained a high level of prevalence on the cutting board with every food preparation sessions. This could be ascribed to the type of material used, with most cutting-boards being made of wood and a few being made of

plastic. Ak *et al.,* 1994, demonstrated the relative ease with which a new chopping-board (whether wooden or plastic) can be decontaminated after being inoculated with micro-organisms and treated with chicken fat. However, a comparison between wooden and plastic chopping-boards by Welker *et al.,* (1997) showed that none of the plastic boards studied harboured any viable *E. coli*, but that six out of 39 wooden boards – especially those that were hand-washed – harboured low numbers of bacteria. It is also evident from this study (Figure 3.2) that the level of *S. aureus* present on the hands decreased as the day progressed, and therefore it can be speculated that this micro-organism was being passed to the food and the cutting-board.

3.6 Conclusion

By law, hospital kitchens are required to be properly configured and managed in order to control microbial counts. They are generally well structured with a professional, functional layout. The infrastructure is cleanable and durable, and there is adequate signage to constantly remind staff to follow the proper procedures. The biosecurity system in a hospital kitchen is also well designed and properly managed – for example, the detergents and chemicals used are sourced from established suppliers and are designed specifically for use in a hospital kitchen.

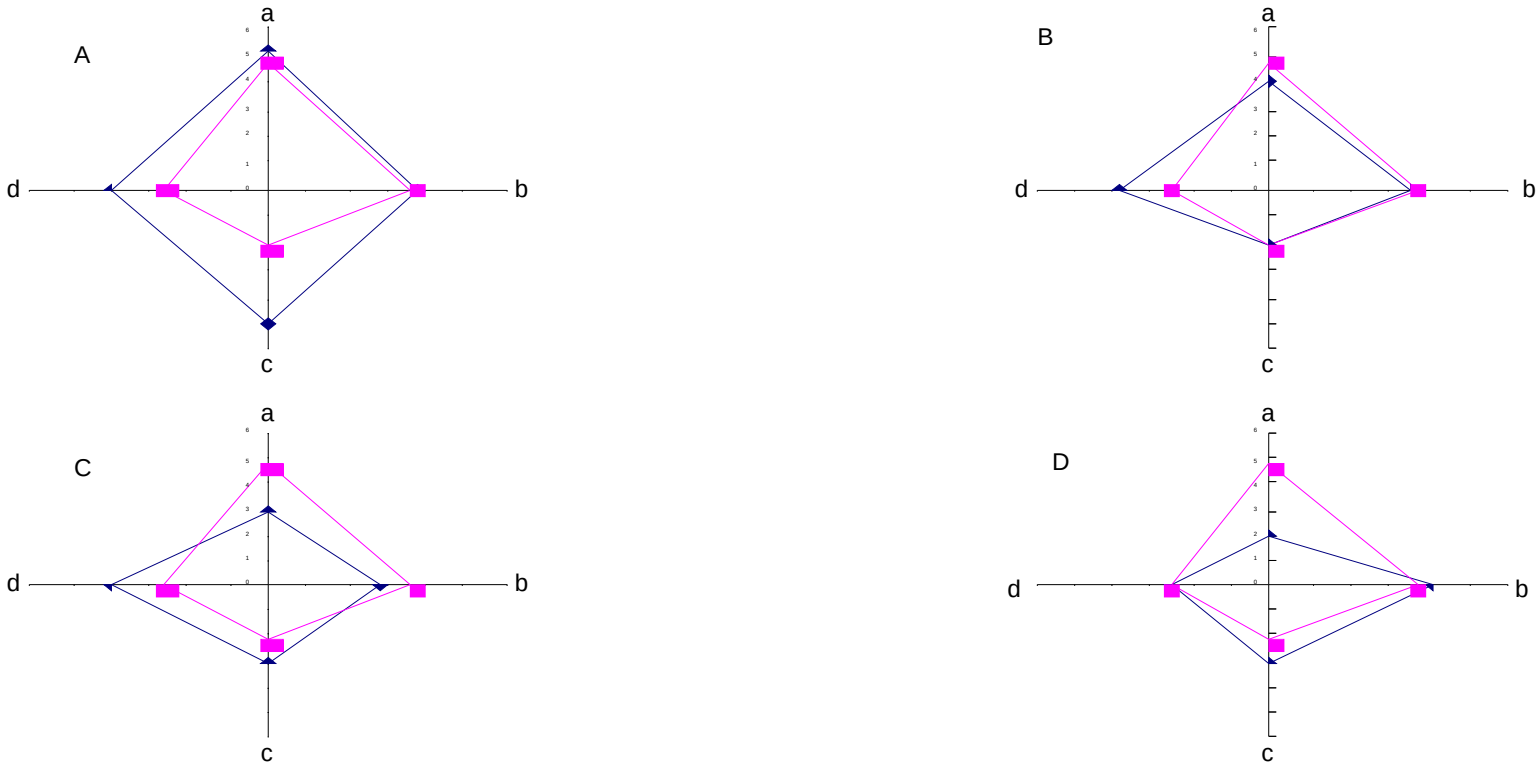

Figure 3.3: Comparison of selected microbial load (*S. aureus* hands – a; *E. coli* hands – b; *S. aureus* cutting board – c and *E. coli* cutting board – d) of average hospital kitchen and traditional home based kitchen as per information gathered from the literature (Taulo *et al*. 2007). (A – Morning before preparation; B – Morning after preparation; C – Lunch before preparation and D - Lunch after preparation)

The hospital is usually in a position to select suppliers, not only according to price but also in terms of quality, which is generally not the case for a hospice, which mostly lacks adequate financial and commercial resources.

Food handlers working in hospital kitchens are required to wear protective clothing and use specific equipment. Compared to the traditional kitchen, the location of the hospital kitchen and its proximity to the outside environment is better structured, and the washing and toilet facilities are of better quality. The dietary components of the food prepared in the hospital kitchen differ from those of the food prepared in a traditional kitchen, e.g. low-sodium foods for hypertensive patients and highly nutritious dishes. The cooking utensils also differ from those used in a typical kitchen. There are more rigorous and elaborate machines to wash the utensils, e.g. double sinks for washing and rinsing. Hospital staff members are properly trained in sanitation and hygiene practices, and the chefs are usually qualified. In addition, staff members are allowed to take sick leave and receive regular medical checkups. They also have reasonable working hours and can therefore take time off to relax and maintain their health.

Therefore, in order to manage and control the organisms identified during this study:

Staff members should start wearing aprons and caps to cover the head (see appendix a). It would be ideal to put health hazards signage in all the rooms including the kitchen so as to enhance communication. Meals are usually consumed in the kitchen; a situation that is not ideal keeping in mind that there are patients who often vomit involuntarily. Therefore, it is recommended that there should be separate dining area and that sanitation of surfaces should commence immediately after eating. Washing of utensils should not be concomitantly in washing basin with the same water that has cooled down. It would be ideal to rather fill up the sink with warm water, immediately induce the utensils to be washed and sanitize after washing. In a hospice everybody assists with almost all the task and it is thus recommended that with regards to food preparation they should be educated about food preparation with a single point of responsibility. Standard operating procedures will also be applicable e.g. to avoid cross-contamination at all costs (see appendix a). Finally, standard prerequisites that apply to any kitchen will also be relevant in this regard e.g. pest control, infrastructure including the wash basins.

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CHAPTER 4

ANTIBIOTIC SUSCEPTIBILITY OF BACTERIAL PATHOGENS ISOLATED FROM FOOD PREPARATION AREAS IN THE HOSPICE KITCHENS

This chapter has been submitted for publication (partially / in full) to the Journal of Antimicrobial Chemotherapy

4.1 ABSTRACT

Emergence of bacterial antimicrobial resistance in both medical and agricultural fields has become a serious problem worldwide. The implication is that greater emphasis must now be placed on preventative strategies such as hygiene, rather than relying solely on antibiotic therapy, and that these strategies need to be developed in healthcare settings such as the hospices and also in the community. The maintenance of good hygiene practices in hospices will result in fewer patients with infections requiring treatment with antibiotics. For purposes of this study, samples taken from food handlers' hands, bio-aerosols and food contact surfaces in hospice kitchens were screened for antimicrobial resistance. The minimum inhibitory concentration (MIC) of the following antimicrobial agents was determined: chloramphenicol, gentamycin, oxacillin, cefoxitin, tetracycline and nalidixic acid. In this study 100 food-borne pathogens identified to specie level were included. Susceptibility of *S. aureus* to gentamycin was evident (97%), followed by chloramphenicol (85%) and oxacillin (64%). *B.cereus and Pseudomonas* responded well to gentamycin and percentage susceptibility for both pathogens to this particular antimicrobial agent was 100%.However *S. aureus and Staphylococcus spp.* demonstrated resistance to nalidixic acid as only 5% responded to treatment for both pathogens.

Keywords: food-borne pathogens, minimum inhibitory concentration, antimicrobial agent

4.2 INTRODUCTION

The development of microbial resistance to antibiotics and the threat represented by this scenario in clinical practice has become a real concern. The emergence of antibiotic-resistant bacteria has been attributed to factors such as the misuse of antibiotics, increased attendance by children of day-care centres, use of antibiotics in the food industry, and an increasingly immuno-compromised population, use of antibiotics in the food industry, and an increasingly immuno-compromised population (Barza *et al.,* 2002). Microbial resistance to antibiotics in the clinic emerged soon after the first use of these agents in the treatment of infectious diseases, and it continues to pose a significant challenge for the health sector. According to Charlebois *et al.,* 2002, resistance to antibiotics is no longer confined to the organisms found in hospital settings and is increasingly identified in outpatient populations, the urban poor, daycare centres, and in people with no known risk factors for acquiring resistant bacteria.

According to Levy (1997), fifty years of increasing antibiotic use have created a situation leading to ecological imbalance and the enrichment of pathogenic bacteria resistant to multiple antibiotics. The development of antimicrobial-resistant food-borne bacterial pathogens can potentially compromise human drug treatments. Examples of antibiotic-resistant bacteria that have been associated with morbidity and mortality in the community setting include *Salmonella, Shigella*, community-acquired methicillin-resistant *Staphylococcus aureus* (caMRSA), and pneumococci (WHO, 1999). This is especially relevant for the very young, the elderly, and the immuno-compromised members of society. Patients with human immunodeficiency virus (HIV) or acquired immunodeficiency syndrome (AIDS) have a severely compromised immune system coupled with the concurrent emergence of opportunistic infections. Therefore, the development of resistant strains in this setting presents a major drawback with regard to treatment of this global epidemic.

Strains of food-borne bacterial pathogens that are resistant to a variety of antibiotics have become a major health concern (Kiessling *et al.,* 2002). The aim of this chapter is therefore to screen for resistant food-borne pathogens in the kitchens of selected hospices in central South Africa. Table 4.1 gives a brief summary of the pathogens and the antimicrobial classes to which they are resistant. The antimicrobial classes are considered by the World Health

Organization (WHO, 2007) to be of high importance and most relevant to public health.

Table 4.1: Foodborne pathogens and antimicrobial classes to which they are mostly resistant.

Pathogen	Origin	Symptoms	Drug resistance
Salmonella spp	Meat and products thereof and eggs	Acute gastroenteritis	Cephalosporin
Listeria spp	Ready-to eat foods (RTE)	Fever and diarrhea	Quinolone
Campylobacter spp	Chicken	Bloody diarrhea and abdominal pain	Quinolone
Escherichia coli 0157:H7	Fresh bovine, dairy products and raw cow milk	Mild to bloody diarrhea and urinary tract infection	Cephalosporin

Source: EFSA 2009

Staphylococcus aureus is a gram-positive bacterium that can colonize and infect humans. It can be harmlessly carried in the nostrils, throat and skin (particularly in areas such as the axilla and groin) (Kluytmans, 1997).*Staphylococcus aureus* continues to be a major cause of community acquired and health-care related infections around the world (Lowy, 1998). This is an organism that primarily infects people whose immunity to infection is compromised. The emergence of high levels of penicillin resistance followed by the development and spread of strains resistant to semisynthetic penicillins (methicillin, oxacillin) has made the therapy of staphylococcal disease a global challenge (Maranan *et al.,* 1997).

In 1997, the first clinical isolate of *S. aureus* with reduced susceptibility to vancomycin was reported from Japan (Hiramatsu *et al*., 1997). The vancomycin minimum inhibitory concentration (MIC) reported for this isolate was in the intermediate range ($8mg.ml^{-1}$) using interpretive criteria defined by the National Committee for Clinical Laboratory Standards guidelines (NCCLS, 2004).Health care associated strains of methicillin-resistant *S. aureus* (HCA-MRSA) principally affect frail and vulnerable individuals such as the elderly and immuno-compromised. This is of great concern in a hospice setting where most patients are in the final stages of disease and antibiotics have to be administered to fight off opportunistic infections. Of equal importance is the fact that most of these patients are now being cared for at home by a caregiver who may be a family

member. Reduced rates of infection and antibacterial resistance have been demonstrated where an approach to combining good hygiene and reducing the number of prescriptions has been set in place (Schmitz *et al*., 1998). The need for improved hygiene is thus emphasised in the effort to reduce the spread of antibiotic resistance.

Escherichia coli is a rod shaped Gram negative bacterium that is normally found in large numbers in the normal gut of all humans. There are many different strains of *E. coli* with different characteristics, all of which are constantly circulating in the community and evolving into new strains. *E. coli* is one of the most common bacteria causing gastrointestinal and urinary tract infections (UTIs) which can sometimes progress to cause more serious infections such as bacteraemia. ESBL (extended-spectrum β-lactamase) producing *E. coli* are highly resistant strains and one of the concerns is that these strains are spreading not only in hospitals, but also in the community. There is evidence to show that ESBL-producing strains are carried in faeces. Munday *et al.,* 2004, conducted a study to detect the presence of ESBL –producing *enterobacteriaceae* within the faecal flora of both community and hospital based patients in the city of York in the UK. For that study, 1 000 faecal samples from community- and hospital-based patients were submitted for screening for the diagnosis of diarrhoeal disease. Of these, 565 were from general practice

(community), 394 from hospital inpatients, 20 from hospital outpatients, and 21 from hospice patients.

4.3 MATERIALS AND METHODS

4.3.1 Sampling protocol

The study included 100 strains of various bacteria from the ten hospices in central South Africa. These bacteria were isolated from different food preparation sites (cutting-board, sinks, tables and hands). The 100 strains were identified by means of the API system (bioMerieux, France). In each case, MIC was determined using the agar dilution method of the Clinical and Laboratory Standards Institute (CLSI). Laboratory-standard powders obtained from Barker Medical were as follows: chloramphenicol, gentamicin, oxacillin, cefoxitin, nalidixic acid and tetracycline. The inoculum was prepared by direct suspension of colonies from overnight cultures (Mueller-Hinton agar), into 9ml saline solution to achieve a suspension equivalent to 0.5 McFarland standards. The plates containing doubling antibiotic concentrations (0.25-128 $mg.ml^{-1}$) were inoculated with 1×10^5 cfu/spot using a multipoint inoculator. MICs were read after 48h of

incubation at 35ºC. (MIC being defined as the lowest concentration of antibiotics to inhibit macroscopically visible colonies).

4.4 RESULTS AND DISCUSSION

The results of agar dilution susceptibility tests for the different microorganisms investigated are given in Table 4.2. Susceptibility of *S. aureus* to gentamycin was evident (97%), followed by chloramphenicol (85%) and oxacillin (64%). Tetracycline was, however, on the borderline of 52%.*Staphylococcus spp.* also displayed a similar pattern of susceptibility to gentamicin (90%), followed by chloramphenicol (85%) and lastly oxacillin (55%). *Bacillus cereus* was 100% susceptible to gentamicin, followed by chloramphenicol (85%), while oxacillin and tetracycline were both in the range of 62%. A similar pattern of susceptibility was also observed for the coliforms, namely gentamicin (80%), tetracycline (90%) and lastly chloramphenicol (60%). Of all the pathogens tested only *Pseudomonas* was susceptible to cefoxitin (78%), and it was also susceptible to gentamicin (100%), followed by tetracycline (85%) and chloramphenicol (71%).

In the case of *S. aureus*, a pattern of resistance was only observed for cefoxitin (20%) and nalidixic acid (5%). *Staphylococcus* spp. was found to be resistant to tetracycline (10%), as well as nalidixic acid (5%) and cefoxitin (5%). *B. cereus* also showed resistance to cefoxitin (7%) and nalidixic acid (30%). Coliforms displayed a similar pattern of resistance to cefoxitin (20%) and nalidixic acid (30%). *Pseudomonas* exhibited a resistant pattern of 43% to nalidixic acid. However, it is interesting to note the complete absence of resistance (0%) to oxacillin for both *Pseudomonas* and coliform.

Microbial resistance to antibiotics in health care settings emerged soon after the first use of these agents in the treatment of infectious diseases, and continues to pose a significant challenge for the health care sector. Resistance, which was once primarily associated with health care institutions, has firmly emerged as a problem in the wider community. This is evident in the spread of infection by methicillin-resistant *Staphylococcus aureus*, with associated deaths (CDC, 2000). Antimicrobial resistance of the investigated pathogens is of great concern, keeping in mind the compromised immune status of hospice patients. The primary cause of antibiotic resistance could be the overuse of antibiotics in both clinical and veterinary practice.

Table 4.2: Antimicrobial susceptibility of groups of food-borne pathogens isolated from the hospice kitchens.

Organism (n)	Antimicrobial Agent	% Susceptible	Susceptibility Breakpoint	MIC (mg.ml^{-1})		
				MIC_{50}	MIC_{90}	Range
***S. aureus* (34)**	Gentamicin	97	≤4	≤0.25	2	≤ 0.25-32
	Tetracycline	53	≤4	4	32	≤ 0.25-128
	Chloramphenicol	85	≤8	4	16	≤ 0.25-20
	Oxacillin	65	≤2	1.5	>128	≤ 0.25->128
	Cefoxitin	21	≤2	8	>128	≤ 0.25->128
	Nalidixic acid	6	≤4	64	>128	≤ 0.25->128
Coliforms (20)	Gentamicin	80	≤4	4	8	1-16
	Tetracycline	90	≤4	1	4	≤ 0.25-16
	Chloramphenicol	60	≤8	8	16	1-16
	Oxacillin	0	≤2	>128	>128	128->128

	Cefoxitin	20	≤2	8	64	0.5-64
	Nalidixic acid	30	≤4	8	16	3-34
***P. aeruginosa* (14)**	Gentamicin	100	≤4	4	4	2-4
	Tetracycline	86	≤4	2.5	64	0.5-64
	Chloramphenicol	71	≤8	8	16	1.5-20
	Oxacillin	0	≤2	>128	>128	>128
	Cefoxitin	79	≤2	2	16	1-64
	Nalidixic acid	43	≤4	6	32	1-32
***Staphylococcus* spp. (20)**	Gentamicin	90	≤4	2	4	0.25-10
	Tetracycline	10	≤4	8	64	4-128
	Chloramphenicol	85	≤8	8	16	4-16
	Oxacillin	55	≤2	2	8	1->128
	Cefoxitin	5	≤2	16	20	1.5->128

	Nalidixic acid	5	≤4	64	>128	4->128
***Bacillus cereus* (13)**	Gentamicin	100	≤4	≤0.25	2	≤0.25-3
	Tetracycline	62	≤4	1.5	8	≤0.25-32
	Chloramphenicol	85	≤8	4	20	≤0.25-22
	Oxacillin	62	≤2	1	>128	≤0.25->128
	Cefoxitin	7	≤2	6	>128	0.5->128
	Nalidixic acid	15	≤4	66	>128	2.5-> 128

Bacteria become resistant to antibiotics when they change or mutate, in ways that reduce or erase the antibiotic's effect on them (CDC, 2000). Furthermore, (Tenover, 2006) emphasized that resistance refers to the ability of bacteria to survive and even multiply despite the presence of an antibiotic at levels that could previously kill the bacteria or inhibit their growth. (Li and Nikado, 2009) outlines the following as the mechanisms through which bacteria acquires resistance to antibiotics: The bacteria's outer membranes may change in such a way that it no longer allows the antibiotic to enter the cell. The bacteria may develop biochemical pumps that remove the antibiotic from the bacteria before it can reach its target within the bacterial cell. Also, the bacteria's receptors may be altered so that the antibiotic can no longer engage them. Lastly, bacteria may create enzymes that deactivate the antibiotic.

This high rate of resistance increases the morbidity, mortality and costs associated with nosocomial infections (Varma *et al.,* 2005). Development of antimicrobial resistant food-borne bacterial pathogens can potentially compromise human drug treatments. Often, if a strain of bacteria develops resistance to one member of antimicrobial class, it develops at least partial resistance to some or all other members of that class as well. A study by (Brady *et al.,* 1993) revealed that exposure to one antibiotic, such as ampicillin, has also been found to increase the resistance of bacteria such as *S. aureus* to other

antibiotics- thus suggesting that antibiotic levels previously considered safe may actually be selecting for resistant populations of bacteria in our food supply. In areas of concentrated use such as healthcare facilities, this has led to longer hospital stays coupled with increased healthcare costs. The same considerations apply to hospices and family homes accommodating immune-compromised individuals for whom any infection can lead to life-threatening complications. In conclusion, the problem of antibiotic resistance amongst some food-borne pathogens serves to highlight the need for better infection prevention through good hygiene practices.

Resistance to cefoxitin was found in approximately 80% of all *S. aureus* isolates and coliforms, while 95% of *Staphylococcus spp* and 93% *B. cereus* isolates were resistant. Oxacillin resistance may be cause for concern, as none of the gram negative isolates were susceptible and only 50%-60% of the gram negative organisms was found to be susceptible.

Microbial resistance to antibiotics in health care centres emerged soon after the first use of these agents in the treatment of infectious diseases, and continues to pose a challenge for the health care sector. Resistance which was once primarily associated with health care institutions, has firmly emerged as a problem in the wider community. This is attested by the spread of infection by methicillin

resistant *S. aureus,* often resulting in death (CDC, 2000). Antimicrobial resistance of the investigated pathogens is a great concern keeping in mind the immune status of the patients at the hospices. The primary cause of antibiotic resistance could be the overuse of antibiotics both in clinical and veterinary practice.

Antibiotic resistance raises the morbidity, mortality and costs associated with nosocomial infections (Varma *et al.,* 2005). Contributing to the dilemma, the development of antimicrobial resistant food-borne bacterial pathogens can potentially compromise antibiotic treatments. A study by Brady *et al.* (1993) reported that exposure to one antibiotic, such as ampicillin, has also been found to increase the resistance of bacteria such as *S. aureus* to other antibiotics, suggesting that antibiotic levels previously considered to be safe may actually be selecting for resistant populations of bacteria in the food supply. In areas of concentrated use such as health care facilities this has led to increased hospital stays coupled with increased health care costs. The same considerations apply to hospices and homes in which there are immune-compromised family members for whom infections can lead to life threatening complications.

Emerging antibiotic resistance in community centers, such as hospices and care centers is increasingly encountered. Results from the study highlight the spread of resistance to these centers and also to environmental organisms and is a serious cause for concern as these community centers are not always equipped with the necessary infra-structure as well as skilled personnel to deal with the prevention of the spread of infection and more specifically the spread of resistant organism. The situation may justify serious attention from authorities in implementing procedures and relevant training of personnel in combating infection in these establishments.

4.5 Conclusion

Antibiotic resistance represents a major hurdle that severely undermines the ability of health care workers to control infectious diseases. The implication of this is that greater emphasis must now be placed on preventative strategies such as hygiene, rather than reliance on antibiotic therapy. The response to treatment of the investigated pathogens in this study was generally satisfactory with few exceptions as depicted in Table 4.1 and 4.2 respectively .In this study gram negative organisms (Coliform and *P.aureginosa*) are of great concern since they were both absolutely resistant to oxacillin. This is semi-synthetic penicillin

effective against penicillin-resistant infections, especially those of staphylococci (Klevens *et al.,* 2007). Pseudomonas resistance is of great concern in a hospice setting since; it is an opportunistic and primarily a nosocomial pathogen. Likewise, the resistance of coliform which in this study seems to have originated from hands and kitchen surfaces, poses a threat to the hospice occupants, keeping in mind their suppressed immune function. Therefore, good hygiene would be the recommended strategy to protect at-risk groups in this setting from infection, including infection with antibiotic-resistant strains of opportunistic pathogens.

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CHAPTER 5

GENERAL CONCLUSION

5.1 Introduction

Human immunodeficiency virus (HIV) and the resulting acquired immunodeficiency syndrome (AIDS) continues to be a major epidemic that is ravaging the South African population at large. The Joint United Nations Program on HIV/AIDS (UNAIDS) recently estimated that three million people are newly infected with the virus in South Africa alone. Hospices are non-governmental organisations that provide care to terminally ill patients, including those suffering from HIV/AIDS. Caregivers are encouraged to maintain high levels of hygiene and nutrition among the patients in order to strengthen their resistance in the continuous battle against opportunistic infections. Since HIV-infected patients are at increased risk of food poisoning and food-borne illness, and food safety with regard to quality is crucial, the aims of this study were as follows:

1) To quantitatively assess the level of hygiene of hands and surfaces in the kitchens of selected hospices in central South Africa;
2) To determine the microbial resistance of the identified pathogens.

In order to address these aims, the following aspects were considered: food contact surfaces – including the hands of food handlers – and microbial contamination of bioaerosols.

This study has been organised into four sections:

1) Chapter 1: Considering information gathered from the literature relating to hospice kitchens, including aspects such as the transmission of food-borne pathogens within this setting, bio-aerosols, and infrastructure.
2) Chapter 2: Establishing the contribution of moving air and the outside environment to the level of contamination in the kitchen.
3) Chapter 3: Quantifying the autochthonous micro-organisms in the hospice kitchen and on the hands of the food handlers/residents, including a comparison with a traditional home-based kitchen.
4) Chapter 4: Comparing the drug resistance of isolated micro-organisms with the findings presented in the literature.

5.2 Concluding remarks on the preceding chapters

From the results presented in Chapter 1, it can be concluded that the kitchen remains the focal point of food preparation. Hospices have been established to improve the quality of life for terminally ill patients, including those infected with HIV/AIDS. In an era of antiretroviral use as a means of reducing the viral load, food safety remains crucial in the fight against this virus, keeping in mind the compromised immune status of the patients. Micro-organisms are ubiquitous in nature, some responsible for food-borne illnesses others posing as opportunistic pathogens – hence the importance of hygiene interventions within the hospice setting. Hospice kitchens operate on a similar basis to traditional home-based kitchens with regard to infrastructure. Although the occupants of these settings exemplify hospital patients, this brings forth the question of whether a typical hospice kitchen will host different kinds of micro-organisms at different levels than home-based kitchens and whether the patients are contributing as a source. Another point to consider is the resistance level of the micro-organisms found in a hospice – i.e. are they perhaps more drug resistant than those present in a home-based kitchen?

Chapter 2 highlighted the fact that most of the hospices included in this study do not have proper food storage and preparation facilities due to limited

infrastructure. This also emanates from the fact that hospices are usually 'step-down facilities' donated by hospitals, or missionary houses donated by churches in some cases. Conditions in the rural hospices studied were found to be worse than those in the urban hospices, with the latter in many cases boasting advanced infrastructure.

The general lack of control over the environmental conditions within the kitchens and the buildings could lead to the presence of bio-aerosols and pathogenic micro-organisms (*Staphylococcus* spp. and *Escherichia coli*). It was also evident that some of the micro-organisms (*Bacillus* spp.) were being introduced into the kitchen by the occupants, amongst others, as counts were found to be higher

inside the kitchen than outside. In addition, environmental factors such as temperature, airflow, relative humidity and dust particles seem to play a role in the presence and distribution of micro-organisms.

In Chapter 3, the hygiene level of the hands of the food handlers and the preparation surfaces in the hospice kitchens was quantitatively evaluated and compared to the microbial load in a traditional home-based kitchen. Likewise, the microbial load on the hands of the hospice occupants and food handlers was

found to differ from that in a traditional kitchen. The make-up of this microbial load could pose a threat to the hospice occupants. *Pseudomonas aeruginosa*, for example, which is an opportunistic pathogen, will do more harm in a hospice than under other circumstances, since the patients have increased susceptibility to infection. High counts of *E*. *coli* and *S*. *aureus* were found on the cutting-boards used for the breakfast sessions in the hospices, and it is speculated that this could be due to cross-contamination via the food handlers' hands and the food served. It is evident from the results that hospices lack proper management systems regarding the prevalence of *E*. *coli*, as it was found on the cutting-boards with every food preparation session.

However, there was a twist of events in terms of the lunchtime sessions in the hospices. Whereas *E. coli* counts on the cutting-boards were found to be high prior to the preparation of food, a reverse pattern was observed after food preparation. This could be due to effective sanitation procedures being applied after each lunchtime session, thus reducing the microbial load attached to the cutting-boards.

The management system proposed for the hospice kitchens involved in this study differs from that of a traditional kitchen. Certain organisms are introduced into the kitchen not only by the occupants, but also through moving air. The

proposed management plan specifies that the movement of persons in and out of the kitchen should be restricted. However, it should be noted that hospice occupants, including workers, are more susceptible to upper respiratory tract infections and will thus contribute to airborne microbes, e.g. bacteria and viruses, through the act of coughing and sneezing. In this case, free-moving air is preferable to stagnant air.

As discussed in Chapter 4, some of the isolated pathogens obtained from the hospice kitchens were identified to species level and subjected to a battery of antibiotic resistance testing by employing the minimum inhibitory concentration (MIC) technique. Microbial resistance to antibiotics in healthcare settings emerged soon after the first use of these agents in the treatment of infectious diseases, and continues to pose a significant challenge to the healthcare sector. It would be expected that the organisms originating from the occupants in a hospice kitchen would be more drug resistant than those found in a traditional kitchen, because the hospice occupants would generally receive more medication than healthy individuals. Therefore, antibiotic resistance was assessed for some of the isolated micro-organisms. Response to antibiotic treatment was generally satisfactory, with a few exceptions. Gram-negative organisms (coliform and *P. aeruginosa*) were both particularly resistant to oxacillin, which pose an immense challenge in this particular setting. This can be addressed by emphasising hygiene as a core strategy for reducing antibiotic

resistance. Good hygiene and food-handling practices will most likely reduce the need to administer antibiotics to patients with infections, thereby diminishing the pressure that drives the ongoing emergence of resistant strains.

5.3 Conclusions

The objective of safe food handling and storage is to preserve the organoleptic property and integrity thereof while also protecting the food from pests and contaminating elements such as microbiota. In a setting like a hospice, where proper food storage and handling is not always possible due to lack of infrastructure and knowledge, it is essential that special care be taken regarding the type of food stored and the packaging material used.

Finally, it can be concluded that some of the food preparation and storage facilities within the hospices studied are, in terms of all technical data, not suitable for the purpose of food storage and handling. However, the hospices do seem to be governing some of the extrinsic factors, such as temperature and relative humidity, which influence microbial viability. Therefore, specific attention should be given to the upgrading of the kitchen infrastructure. For example, it would be ideal to have separate rooms for food preparation and dining.

The results presented in this study further identified the hospice occupants as possible sources of the organisms present in the kitchen and the surrounding environment, since in some cases, the patients also help to prepare the food and there is continuous movement by them between the kitchen, bathroom and bedroom. It would thus be ideal to separate the patients in terms of residency from the food preparation facilities, including the kitchen.

Companies that regularly donate food to the hospices are further cautioned to avoid donating foodstuffs that are past their sell by dates, as the inability to control humidity fluctuations in the hospice kitchens only exacerbates the problem with regard to food safety and proliferation of microbial load. It could also be speculated that in South Africa, unlike in more humid countries, the facility design and geographical localisation had a limited influence on the resident bio-aerosol profiles. In order to address and verify the concerns raised in this study, it is recommended that the ability of micro-organisms to proliferate on the foodstuffs (high-risk) provided to the hospices should be assessed and the menus adjusted in accordance with season-associated changes in the extrinsic factors that can influence microbial viability and growth. It should further be noted that the occupants of the hospices concerned have limited knowledge with regard to proper food storage and handling practices and are to a large extent not aware of the threats posed by the resident microbiota. This problem could be

alleviated by providing educational training to the patients and food handlers with regard to proper food safety.

5.4 Future research

The present study has revealed the following options for possible future research:

- The implementation of appropriate “educational programmes”, such as hygiene standards within hospices and antibiotic resistance.
- The need to control infection not just in healthcare settings or in association with food hygiene, but throughout the community. Infection control policies and guidelines must be based on the totality of evidence, including microbiological and other data. This is particularly important for hospice hygiene, since there is no intervention data available.
- The design of a biosecurity plan / model specific to hospices caring for HIV-positive patients.
- The implementation of the aforementioned model and the evaluation of its efficacy.

APPENDIX A