Present concern of microbial infections biology essay

Science, Biology

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Contents

extraction techniques 314. 2. 1 The Student’s T test 334. 3 Viable counts from ‘ in use’ T "" towels344. 4 Partial identification of microorganisms38384. 4. 1 Partial identification of Bacterial species384. 4. 2 Partial identification of Yeasts and fungi 414. 5 Persistence of bacteria after laundering414. 6 Minimum inhibitory concentration (MIC)424. 6. 1 Mann Whitney U test 434. 7 Relevance of findings 444. 8 Future work 454. 9 Limitations of the study 464. 10 Impact of research on the public474. 11 Conclusion 47

List of figures

List of Tables

Abstract

1. Introduction

1. 1 Microorganisms and their ability to cause infectious disease

The term ‘ Microorganisms’ refers to a collection of small organisms, visible only with the aid of microscopy. Antonie van Leeuwenhoek was one of the first to observe these in the 1600s using his own microscopic design (Porter, 1976). The ability of such a microorganism to invade a host and multiply is known as infection. Early work conducted by microbiologists including Pasteur and Koch facilitated research on the microbial causation of infectious disease. Pasteur controverted the theory of ‘ spontaneous generation’ by identifying that the process of putrefaction required living organisms and thus provided a basis for the ‘ germ theory’. Koch’s formulation of the postulates and identification of the microbial causes of diseases, including

anthrax and T. B, proved this ‘ germ theory’ accurate (Baron et al., 2006). Following this development in microbiology, substantial improvements in medicine have occurred. The development of antibiotic therapy to combat infectious disease is one of the most beneficial. Alexandra Fleming’s penicillin discovery has found a cure for many bacterial infections and has led to the development of other antibacterial agents (Sternbach et al., 1992). The immunisation programme has allowed eradication of certain diseases by preventing their development and spread, for example the last case of smallpox was recorded in 1980 (Strassburg, 1982).

1. 2 Present concern of microbial infections

Although this progression in medicine has improved public health vastly over the last two centuries, areas of concern remain. Opportunistic infections are currently a major cause of mortality, particularly in the immunocompromised. Therefore emphasis on hygiene to control both the development and the spread of such diseases is necessary. There is also growing concern over the ability of some microorganisms to resist the inhibitory action of antibiotic treatment (Tenover et al., 1996). This resistance has led to the development of infections which do not respond to treatment; individuals are infective for longer periods of time and so the spread of such infections is easier, particularly in those who are critically ill. There has also been an increase in mortality linked to this (Tenover, 2006). Antibiotic resistance was first noticed in hospitals where excessive drug therapy was utilised (Levy, 1998). Over the years overuse of broad spectrum antibiotics has caused rapid progression of this problem. Common

microorganisms showing resistance include Staphylococci, Enterococci, Klebsiella pneumoniae and Pseudomonas spp (Tenover, 2006). The introduction of new antibacterial drugs to help relieve resistance and treat such infections has fallen by 50% over the last 20 years (Spellberg et al., 2004). Another method which may help overcome the resistance issue is to emphasise on public health. Educating the public on areas where potentially pathogenic microorganisms may harbour and on their elimination is important in order to reduce the number of infections arising from the domestic environment. Controlling infectious disease as such may help reduce antibiotic prescribing and stop the production of resistant bacteria.

1. 3 Microbial infections arising from a domestic setting

Particular attention given to home hygiene in the 19th century led to a great decline in morbidity and mortality associated with infection. Adaptation of stringent food preparation methods showed the greatest drop in Salmonella related disease (Stanwell-Smith, 2003). However, at present there is still great concern in the high number of cases of other intestinal infectious disease (IID) and respiratory infections linked to the home environment (Bloomfield et al., 2012). Such infections are most likely to infect high risk groups including the immunocompromised; such as those on invasive therapy, and the immunodeficient; such as the elderly, neonates, pregnant women and recently discharged hospital patients. Research indicates that 1 in 6 UK residents are within these high risk categories (Stanwell-Smith, 2003).

1. 3. 1 Intestinal infectious disease (IID)

The World Health Organisation (WHO) currently estimates over a million cases of food borne illness in the UK annually. Although many cases may be asymptomatic and self-limiting, there are a high number which cause hospitalisation and in severe cases, mortality (FSA, 2011). Research based in England and Wales showed that after commercial catering services and residential settings, private homes were the third most likely areas for IID contraction (Hughes et al., 2007, Stanwell-Smith, 2003). A study conducted in Netherlands showed up to 80% of Salmonella and Campylobacter cases were contracted from private homes (Hilton et al., 2000). A number of other studies have also indicated that private homes are responsible for the greatest number of food borne illnesses; therefore emphasis has currently shifted away from catered services to private settings (Scott, 1996, Roberts, 1982). Stanwell-smith (2003) research found that although cases of IID have decreased significantly; Campylobacter and salmonella are still the most prominent causes of food borne disease within the domestic setting (Stanwell-Smith, 2003). This is shown in figure 1. 1 below. Figure 1. 1: The incidence of gastrointestinal infections in England and Wales (Stanwell-Smith, 2003). Although the majority of these cases are linked to direct contact with food products, there is substantial evidence that cross contamination within the kitchen can cause such bacteria to spread to surfaces, cloths and other cleaning utensils (Humphrey et al., 1994, Cogan et al., 1999, Bloomfield et al., 1997). Therefore not only is adequate food preparation important but hygiene within the domestic setting is also important. A study showed that 34% of hamburger related infections caused

by a strain of Escherichia coli could have been prevented by particular hand hygiene measures and by appropriate cleaning of work surfaces (Mead et al., 1997).

1. 3. 2 Respiratory infection

Respiratory disease is usually airborne and is most commonly spread via infected people within the home. However there is evidence that insufficient hand washing may lead to contamination of surfaces, door handles and cloths causing the virus to proliferate (Reed, 1975, Winther et al., 2007). Research shows that common viruses found within the home include Rhinovirus, Parainfluenza virus and Influenza virus (Kramer et al., 2006, Boone et al., 2005). Influenza virus is accountable for a large number of fatalities annually in high risk groups; therefore it is crucial that measures are taken to decrease its spread. A US study showed that 59% of surfaces including telephone receivers, refrigerator handles, and kitchen faucets were contaminated with Influenza virus (Boone et al., 2005). Furthermore complicated respiratory tract infections such as pneumonia, bronchitis and tuberculosis, may also spread via similar pathways. Particular concerns are for those with underlying respiratory conditions such as Chronic Obstructive Pulmonary Disease (COPD) or Cystic Fibrosis (CF). Denton et al conducted a study which showed that 42% of homes with CF children were contaminated with Stenotrophomonas maltophilia which may cause respiratory complications and mortality. Sites included dishcloths, sponges, kitchen surfaces and the washing machine (Denton et al., 1998).

1. 4 Common bacteria found in a domestic setting

Although most bacteria found within the home are non-pathogenic, research has shown that pathogenic bacteria also exist. The first comprehensive study looking at opportunistic microorganisms within the domestic setting was conducted by Finch et al. A sample of 21 homes was inspected for the presence of microorganisms at various sites within the home. A large number of Micrococcus and Bacillus spp were found and pathogenic bacteria including Staphylococcus spp, Pseudomonas spp, Escherichia coli, Campylobacter, Corynebacteria and Salmonella was also collected (Finch et al., 1978). This research has been supported by many other studies (Scott et al., 1982, Speirs et al., 1995, Josephson et al., 1997).

1. 4. 1 Staphylococcus spp

The domestic environment shows contamination of Staphylococcus spp, particularly S. aureus and S. epidermis (Speirs et al., 1995, Scott et al., 1982, Finch et al., 1978). Staphylococcus aureus is often carried by individuals within the nasopharyngeal cavity as part of the normal microbial flora. However it has the ability of causing both toxin-mediated and non-toxin mediated damage in immune deficient individuals and can often lead to systemic infections including infective endocarditis. Food poisoning can also occur if the toxin is ingested. Other common infections caused by this gram positive bacterium include impetigo and conjunctivitis (Hugo et al., 1995). A study of 251 domestic homes conducted by Scott, Bloomfield and Barlow, extracted 60 samples from various areas within the homes; including the kitchen and the bathroom. It was found that although the majority of

contaminants were non-pathogenic, some potentially pathogenic species did exist. Staphylococcus aureus showed a percentage frequency of 31-35% (Scott et al., 1982). A similar study focused particularly on the kitchen region within 46 homes. Samples were taken from various sites, of which wet areas around the sink and cloths used for wiping surfaces and drying equipment showed the highest colonisation. Of these 46 homes all showed some Staphylococcus spp persistence (Speirs et al., 1995). Methicillin resistant Staphylococcus aureus (MRSA) is currently a major concern not only within hospital settings but also within the domestic environment. Recently discharged individuals may act as carriers of this organism and therefore it is important to emphasise to the public the importance of good hygiene measures (Bloomfield et al., 2012).

1. 4. 2 Pseudomonas spp

Pseudomonas aeruginosa is an opportunistic pathogen, only causing disease in those with compromised host defence systems including patients with cystic fibrosis, cancer or HIV diseases and those using intravenous drugs. It can lead to infections including malignant external otitis, endocarditis, pneumonia, septicaemia and meningitis (Bodey et al., 1983). Pseudomonad bacteria have also showed up in research relating to the domestic environment. Scott et al (1982) found numerous Pseudomonas spp in the kitchen and bathroom at varying sites. P. aeruginosa was amongst these and the study also showed the persistence of many other unidentifiable pseudomonas bacteria. Common sites within the kitchen for P. aeruginosa were the sink u-tube, the draining board and the dishcloth; however

frequency at each site was low (Scott et al., 1982). Speirs et al (1995) also found similar results. This study showed that Gram negative bacteria as such collated near the sink region and Gram positive bacteria such as S. aureus persisted on cloth materials. The most common pseudomonad found was P. aeruginosa at 15. 2%. (Speirs et al., 1995)

1. 4. 3 Listeria spp

Listeria is a pathogen which normally contaminates dairy products. Pregnant women are at particular risk as infection may lead to miscarriages, premature births or serious illness in new-borns (Hugo et al., 1995). A study conducted by Beumer et al (1996) found that listeria spp was present in 101 out of 213 homes. Listeria monocytogenes was present in 45 homes. The dishcloth showed high contamination, 40 out of 108 dishcloths sampled contained listeria spp of which 18 showed the presence of listeria monocytogenes (Beumer et al., 1996). Speirs et al also demonstrated its ability to grow at low temperature as they extracted L. monocytogenes from 2. 2% of fridge surfaces (Speirs et al., 1995).

1. 4. 4 Escherichia coli

E. coli is a type of enterobacteria which may persist in undercooked food and if ingested may cause enteritis in young children leading to fatal GI symptoms such as bloody diarrhoea and dehydration. It also has the ability to infect the urinary tract and bladder leading to infections such as pyelitis, pyelonephritis and cystitis (Hugo et al., 1995). Studies have shown its existence within the domestic environment, particularly due to cross contamination from raw meat (Speirs et al., 1995, Scott et al., 1982). An

experimental study where untrained participants were asked to prepare a meal showed up to 10% contamination of E. coli on surfaces after the meal had been prepared. This study demonstrated that improper hand cleanliness and undercooked meat were common sources (Kennedy et al., 2011). Scott et al (1982) found E. coli to persist in regions of the kitchen including the sink, the u-tube, the draining board and the dishcloth (Scott et al., 1982).

1. 4. 5 Klebsiella spp

Klebsiella bacteria often colonise the human gut, the pharynx and the skin. K. pneumonia is an opportunistic pathogen which may cause bronchopneumonia, if colonisation of the respiratory tract occurs. Several studies have shown that this bacterium persists in the domestic environment (Scott et al., 1982, Finch et al., 1978).

1. 4. 6 Salmonella and Campylobacter

Serious strains of Salmonella spp including Salmonella typhimurium may lead to the typhoid fever, whilst strains such as S. Typhimurium and S. Enteriditis have a link to bacterial food poisoning (Hugo et al., 1995). As identified earlier salmonella and campylobacter are the greatest causes of food borne illness (Stanwell-Smith, 2003). The most common origin of such bacteria is raw food however research shows that many other sites within the kitchen can become contaminated. Cross contamination of Salmonella Enteriditis PT4 around the kitchen on to utensils and surfaces via contaminated egg shells has been identified (Humphrey et al., 1994). Although the kitchen dishcloth and sponge have been identified as potential vehicles for such bacteria in the kitchen (Cogan et al., 2002); results within

real domestic settings suggests no contamination of these items with these bacteria (Hilton et al., 2000, Bloomfield et al., 1997, Speirs et al., 1995).

1. 5 Sources, dispersal and persistence of microbes in the domestic setting

Research has shown that although the majority of IID cases are food borne there is still a high number linked to cross contamination. Figure 1. 2 shows that food borne contraction has decreased whereas non-food borne has increased (Hughes et al., 2007). The mode of transmission for these is said to be person to person spread or from surfaces to person. Similarly respiratory infections such as the common cold are often said to be spread by aerosol transmission but now there is growing evidence that a significant proportion are via hands and surfaces. This suggests that improved standards of personal and domestic hygiene may prevent outbreaks of both IID and respiratory infections (Bloomfield et al., 2012). Figure 1. 2: A comparison of the outbreaks of food borne and non-foodborne cases of IID (Hughes et al., 2007). A number of studies show that water accumulating areas in the kitchen such as the sink area, drainage boards, u tubes, dish cloths, sponges and other cleaning items not only promote microbial growth but may also harbour opportunistic pathogens (Finch et al., 1978, Scott et al., 1982, Josephson et al., 1997, Speirs et al., 1995, Erdogrul et al., 2005). The period of which the bacteria reside on these surfaces depends on the bacteria’s properties. Research shows that some bacteria are able to survive on surfaces, cloths, sponges and utensils for a number of days (Erdogrul et al., 2005). In particular it was found that on dry inanimate surfaces Klebsiella would normally last around 90 minutes, Salmonella a day, Campylobacter up

to 6 days, E. coli from 1. 5 hours to 16 months, S. aureus 7 days to 7 months and P. aeruginosa could last from 6 hours to 16 months (Kramer et al., 2006).

1. 6 Ability of bacteria to bind to cloth materials

The fibres and intricate weaving of cloth materials makes them a good vector for viable bacteria. This has been of major concern particularly in the hospital environment where procedures are put in place to minimise infection risk (Neely et al., 2000). A comparison study of paper and cloth towels found that cloth towels harboured a greater percentage of microorganisms (67. 2%) compared to paper towels (33. 3%). Bacillus spp was the greatest contaminant for both, Staphylococcus epidermis and Corynebacteria were most prevalent on cloth towels and Staphylococcus aureus occasionally contaminated cloth materials. This study emphasised that in terms of public health paper towels are much safer to use (Robinton et al., 1968). Another study compared the bacteria binding and persistence properties of sponge dishcloths compared to cloth dishcloths (Hilton et al., 2000). This study showed that although sponge dishcloths harboured a greater number of Pseudomonas, klebsiella and Staphylococcus species it did not spread as many bacteria. Whereas a cloth dishcloth harboured less bacteria but spread was much easier. This was explained by contrast of the microstructure of both cloth types. The cavernous structure of the sponge provides a more protective environment for the microorganisms; therefore they are not removed easily. A cloth has a larger surface area and a smoother surface therefore bacteria are removed much easier (Hilton et al.,

2000). Adding to this, a study looked at the binding of opportunistic pathogens Pseudomonas aeruginosa and Staphylococcus aureus and found that both bound efficiently to acrylic, polyester and wool fibres of which polyester was the most contaminated. S. aureus did not bind to nylon fabrics however Pseudomonas aeruginosa did. Cotton showed the least contamination of such microorganisms (Takashima et al., 2004).

1. 7 T-towels as a source and vector of microorganisms

T-towels/dish cloths play an important role in the dissemination of bacteria within the domestic kitchen (Scott et al., 1982, Finch et al., 1978, Josephson et al., 1997). T-towels are often used during dish cleansing procedures, cooking procedures and surface cleaning processes. Gastrointestinal infecting species such as E. coli, Salmonella and Campylobacter may reside on dishes or surfaces from raw meat or infected individuals. S. aureus and Pseudomonas may be transferred from hands to tea towels, which are then used in the dish drying process causing bacterial spread. Figure 1. 3 illustrates how cleaning cloths are involved in the transmission of infection within the home. The cycle shows that sources of pathogens including infected people or contaminated food may transfer microorganisms to cloths. As such cloths are used for various processes within the kitchen they may act as vehicles causing dissemination of bacteria. This may eventually lead to the bacteria infecting a non-infected individual. This cycle is continuous and so the risk of spread is high (Bloomfield et al., 2012). Studies of the bacterial contamination within the home show the highest number of total coliforms and faecal coliforms in the kitchen and the largest carrier to be the

kitchen sponge and the kitchen dishcloth (Scott et al., 1982, Speirs et al., 1995, Rusin et al., 1998). A study conducted by Scott and Bloomfield (1993) showed heavy contamination of dish cloths within hours of first use. The continued use of such cloths showed transfer of pathogens between surfaces and cloths; emphasising their ability to act as vectors (Scott et al., 1993). Figure 1. 3 The spread of infection within the domestic environment and the ability of cleaning cloths to act as vectors (Bloomfield et al., 2012)

1. 8 Effect of laundering on tea towels

After usage, T- towels are often disinfected via the laundry process before they are utilised again, however research shows that laundering may not remove all traces of microorganisms. Studies have shown that some microorganisms causing ringworm, salmonellosis, adenovirus or hepatitis A may persist on fabrics if inadequate laundering procedures are used. Furthermore damp laundry may act as a habitat for bacterial growth. (reference)Staphylococcus aureus has shown to be resistant to the laundry process; this is of particular concern in the hospital environment, where such microorganisms may cause infection in immune deficient individuals (Walter et al., 1975). Studies show that water temperature employed during laundering is an important factor in the reduction of bacterial species. A cycle at 60°c may reduce S. aureus bacteria to below detectable levels. Length of the washing cycle and the soil load do not show significant effects (Jaska et al., 1980). Gerba and Kennedy (2007) emphasised the significance of detergent containing sodium hypochlorite (bleach) and the passage of washed items through the drying procedure; a reduction of 99. 99% of

enteric viruses such as rotavirus, hepatitis A and adenovirus were seen (Gerba et al., 2007). Similar results were noticed in earlier studies (Rusin et al., 1998, Scott et al., 1984). Scott et al (1984) found that the use of detergent and hot water alone was ineffective in reducing the bacterial load, whereas the use of hypochlorite or phenolic disinfectants proved to decrease the bacterial load by 50% after 90 minutes. However bacteria reestablished after 3 hours (Scott et al., 1984).

1. 9 The use of disinfectants

Often disinfectants or other cleaning agents are used to clean surfaces or areas within the domestic home. However there is much research suggesting that not all pathogenic microorganisms are sensitive to this and so they may still persist after its use. Josephson et al (1997) conducted a 3 phase study; phase 1 was without the use of disinfectant, phase 2 was with casual use of the disinfectant and phase 3 was with targeted use of disinfectant. Casual use of the disinfectant did not see any significant reduction in the bacterial load however targeted use of the disinfectant saw a dramatic decrease (Josephson et al., 1997). The effect of antibacterial dishwashing liquid was investigated on E. coli, S. aureus, Salmonella enteriditis and Bacillus cereus. In the suspension test, at 0. 5% S. aureus and B. cereus were absent whereas Salmonella and E. coli survived. At 2-4% all organisms were below the detection limit. In used sponges the antibacterial wasn’t as effective (Kusumaningrum et al., 2002).

2. Aims and Objectives

2. 1 Aims

The literature review has helped identify that there is a need to emphasise to the public the importance of hygiene within the domestic setting, both to reduce opportunistic infections and decrease antibiotic resistance. Due to the increase in more individuals receiving care at home rather than hospitals; the risk of infection from domestic settings is greater. Therefore it is very important to identify organisms within such environments and understand how vehicles can be eliminated. Although there has been much research carried out around this area; the focus around T �" towels in natural settings is limited and the studies available are dated. The aim of this study is to look at the ability of T-towels to harbour bacteria. As identified T-Towels play a major role in the cycle of infection transmission. This research project aims to observe and identify microorganisms which contaminate a T-towel over a function of time. The effect of laundry and disinfectants will also be considered.

2. 2 Objectives

The objectives of this study are as follows; Isolate microorganisms from contaminated T-towels over a period of 5 daysDetermine cell countsIdentify these microorganismsDetermine whether laundering is an effective method of removalConsider the effects of disinfectants on bacteria

3. Materials and Methods

3. 1 Preparation of Growth media and chemicals

Dehydrated Nutrient, Sabouraud and MacConkey media, obtained from Oxoid microbiology products limited (UK) was used to prepare agar plates. All agars were prepared according to the labelled manufactures instructions. A quantity of dehydrated media (11. 2g Nutrient agar, 26g Sabouraud dextrose agar, 20. 8g MacConkey agar) was added to 400ml of distilled water in a 500ml Duran bottle. After thorough shaking, the Duran bottles were sterilised by autoclaving at 121°c for 15 minutes. Once autoclaved the molten agar was left to cool slightly and was then poured into labelled agar plates using an aseptic technique. The plates were left to set, before inverting and storing at room temperature. Nutrient Broth was prepared in a similar manner; 6. 5g of dehydrated nutrient broth was added to 500ml of distilled water in a 500ml Duran bottle (as per manufacturers' instructions). After thorough shaking and complete dissolution, 50ml quantities of the mixture were transferred to 250ml Erlenmeyer flasks. The flasks were covered with foil and autoclaved at 121°c for 15 minutes. After autoclaving the mixtures were left to cool and stored at room temperature. Sterile saline solution of 0. 85% was prepared by adding 8. 5g of dehydrated sodium chloride to 1 litre of distilled water in a 1 litre Duran bottle. The solution was mixed and then sterilised by autoclaving in the similar manner as described above. All other chemicals and equipment used in the procedures involved in this experiment were sterilised by autoclaving; ensuring the results received were reliable and not due to unrelated effects.

3. 2 Bacterial Cultures and Maintenance

The bacteria investigated in this experiment were cultured on the media described in section 3. 1. Nutrient agar was used as a general media to provide a suitable environment for growth of most microorganisms. Sabouraud dextrose agar carries an acidic pH therefore provided an ideal environment for the isolation of dermatophytes, other fungi and yeast. MacConkey agar was an ideal medium for the isolation of coliforms and intestinal pathogens carried in water, dairy products and biological specimens. The sub cultured agar plates were maintained by storing at temperatures of 2 to 8°c.

3. 3 Cell counts and reproducibility testing

A strain of Escherichia coli was grown on nutrient agar at 28°c and then used to inoculate a flask of nutrient broth. This was placed in the rotary incubator overnight at 37°c. Serial dilutions were performed using the prepared culture, as shown in Table 3. 1.

Dilution

Culture

0. 85% Sterilised Saline

10-20. 1ml of neat culture9. 9ml10-40. 1ml of the 10-2 dilution9. 9ml10-51ml of the 10-4 dilution9ml10-61ml of the 10-5 dilution9ml10-71ml of the 10-6 dilution9mlTable 3. 1: Serial dilutions of prepared E. coli cultureAliquots (0. 1ml) of dilutions 10-5, 10-6 and 10-7 were plated out in duplicate onto nutrient agar. The complete procedure was repeated twice to ensure reproducibility. After overnight incubation at 28°c, cell counts were

performed; dilutions giving reliable counts (30-300 cells) were established and reproducibility was assessed by comparison of counts between repeats.

3. 4 Cell Retrieval in laboratory conditions

Prior to natural setting experimentation, two techniques were compared to find the most suitable method which would effectively extract microorganisms from the T- towel surface and give reproducible results. Before carrying out the double experiment, an E. coli contaminated T-towel was prepared. The T- towel was a basic all-purpose T-towel made from 100% cotton (All about home®- Prestige Imports UK ltd). Four 2cm2 squares were cut from the T-towel and sterilised by autoclaving at 121°c for 15 minutes. Sterilised tweezers were used to aseptically transfer the T-towel squares into sterile universals containing 0. 1ml E. coli inoculated nutrient broth (incubated overnight at 37°c) and 9. 9ml sterile saline. After 20 minutes the T- towel squares were removed from the solution and placed on individual uncovered petri dishes in the laminar air cabinet for 24 hours to dry completely. The dry T-towel squares were used in the following cell retrieval methods to extract the cells from the surface of the T-towel.

3. 4. 1 Griffin flask shaker method

Two sterile universals each containing one E. coli contaminated T-towel square, 5ml 0. 85% sterile saline and 15 sterile plastic beads were prepared. The universals were sealed tightly and attached to the arms of the Griffin flask shaker (See figure 3. 1 below). The shaker was set at the middle speed setting and universals were shook vigorously for 2 minutes. Duplicate samples of 0. 1ml (neat) were taken from the universals and plated onto

nutrient agar using disposable sterile spreaders. A 1/10 dilution (0. 1ml sample and 0. 9ml saline) and a 1/100 dilution (0. 1ml sample and 9. 9ml saline) were prepared. These were also plated onto nutrient agar using 0. 1ml from each of the diluted preparations. The universals were reattached and this process of sampling was repeated after a further 3 minutes (total of 5 minutes) of shaking and again after a further 5 minutes (total of 10 minutes) of shaking. All nutrient agar plates were incubated at 28°c for 24 hours, after which microbial counts, reproducibility and observations of any contamination were recorded. Figure 3. 1: Representation of a Griffin flask shaker (ref)

3. 4. 2 Tissue grinder method

Two sterilised 15ml Tissue Grinders were used in this method. One E. coli contaminated T- towel square was placed at the bottom of each tissue grinder along with 5ml of 0. 85% sterile saline. The glass rod was secured into the device and the T-towel was homogenised vigorously by hand for two minutes. This procedure was carried out on an isolated laboratory bench. Protective gloves and safety spectacles were used to protect the user. After 2 minutes 0. 1ml (neat) of the sample was extracted from the device and plated onto nutrient agar using a sterile disposable spreader. Dilutions of 1/10 and 1/100 were prepared and 0. 1ml of each dilution was plated onto an agar plate. The T-towel was homogenised further and the sampling procedure was repeated after a further 3 minutes (total of 5 minutes) and a further 5 minutes (total of 10 minutes). This procedure was repeated with the second tissue grinder device to test reproducibility. All nutrient agar

plates were incubated at 28°c for 24 hours, after which cell counts, reproducibility and observations of any contamination were recorded.

3. 5 Sample collection and viable counts ‘ In use’ tea towel

Once cell retrieval had been determined the practical could take place under natural domestic settings. An identical all-purpose T-towel made of 100% cotton was used in this method. Before exposure, the T-towel was sterilised by autoclaving at 121°c for 15 minutes. Once sterilised two 2cm2 squares were cut from the T-towel using sterilised scissors; scissors were immersed in 70% ethanol and then passed through a Bunsen burner flame. One sample was taken from the middle region whilst the other from the edge. These control samples ensured the T-towel was completely sterile and gave a day 0 measurement. The T-towel was introduced into the domestic kitchen on day 0 and householders were informed to use it as normal; they were unaware that the T-towel was involved in a microbiological experiment. On day 1 at approximately 2100 hours, two samples of 2cm2 squares were taken from the T-towel; one from the middle region whilst the other from the edge. Samples were collected whilst the T-towel remained in the domestic setting. Scissors were sterilised by dipping them in 70% ethanol. The 2cm2 squares were then maintained overnight in prepared sterile universals containing 5ml sterile saline stored at 2-8°c. The samples were then brought from the domestic kitchen into the laboratory. The Griffin Flask shaker method was utilised to extract the microbial cells. 15 sterile plastic beads were placed in each universal containing the ‘ in use’ T-towel samples. The universals were attached to the arms of the Griffin Flask shaker and the shaking procedure

began. Samples were taken after 5 minutes of shaking. Sterile pipettes were used to extract 3 x 0. 1ml (neat) samples from each universal and were then plated onto Nutrient, Sabouraud and MacConkey agar plates. 1/10 and 1/100 dilutions were prepared and again 0. 1ml solution was plated onto each type of agar. The plates were then left in the 28°c incubator overnight. These procedures were repeated for samples taken on day 2, day 3, day 4 and day 5. On day 6 the T-towel was laundered alone following the general household manner. Bold White Lily & Crystal Rain® 2 in 1 detergent and fabric softener, at a standard setting of 40°c was used when washing the T-towel. Once the wash cycle was complete the T-towel was left to dry naturally. Once dried samples were again taken using sterilised scissors from the middle and edge of the t-towel and placed in universals containing sterile saline. Neat and 1/10, 1/100 diluted samples were plated onto all three types of agar, and incubated in the same manner as described above. After incubation, microbial colonies were counted and each individual bacterial cell type was sub cultured onto appropriate agar plates. Light microscopy and some basic biochemical tests were used to partially identify the bacterial colonies.

3. 6 Gram staining procedure

To determine whether the unidentified bacteria species were Gram positive or Gram negative, the Gram staining procedure was carried out. A sterilised wire loop was used to transfer a drop of water to the glass slides; a minute sample of each of the unidentified bacteria was transferred and mixed into the drop of water placed on the slides, to produce a thin smear of bacterial

film. The slides were allowed to dry naturally for approximately 60 seconds after which they were fixed by passing over a Bunsen burner flame two or three times. Once cooled the slides were ready to undergo the staining procedure. The slides were placed on the staining rack and then flooded with crystal violet ensuring that the whole bacterial film was covered. This was left for 60 seconds after which the dye was poured off. The slides were then flooded with Lugols iodine and again left for 60 seconds before being poured off. Acetone was then added to the slides, but this time was rinsed off immediately with distilled water to avoid over decolourising. Finally the slides were completely flooded with Safranin and left to act for 60 seconds before being drained and washed with distilled water. The slides were blotted dry with absorbent paper avoiding the stained specimen. Once dry, the stained bacterial slides were ready to be examined (x100 oil immersion) under the light microscope.

3. 7 Oil Immersion light microscopy

To obtain high resolution microscopic images the oil immersion microscopy technique was used. One or two drops of immersion oil were placed on the stained specimen slides. The slides were then placed under a light microscope and the oil immersion (x100) objective lens was used to view the bacteria. Fine focusing was used to view images of distinguished cells. This procedure helped identify whether the cells were Gram positive or negative; Gram positive cells retained the purple colour from the crystal violet whilst Gram negative cells retained the pink colour from the Safranin. It also helped determine other cell characteristics, important for identification.

3. 8 Biochemical tests

To aid identification, chemical tests were carried out, exploring biological reactions the bacteria may undergo.

3. 8. 1 Oxidase testing

The oxidase test was used to determine whether the bacteria produced an oxidase enzyme, Cytochrome C, during the bacterial electron transport chain. A small sample of bacterial cells were placed onto the tip of the oxidase test strip containing the reagent tetra-methyl-p-phenylenediamine dihydrochloride, using a sterilised loop wire. A positive result due to reduction of the reagent was indicated by a colour change from pink to dark blue. A negative result showed no colour change.

3. 8. 2 Catalase testing

The catalase test was used to determine whether the bacteria produced a catalase enzyme which causes decomposition of hydrogen peroxide to oxygen and water. The test was performed by placing a small sample of bacterial isolate onto a glass slide placed in a petri dish. To this a drop of 3% hydrogen peroxide solution was added. A positive result indicating the breakdown of hydrogen peroxide was seen when bubbles of oxygen formed. A negative result showed no fizzing.

3. 9 Minimum inhibitory concentration (MIC)

MICs were used to determine the susceptibility of particular bacterial species to antimicrobial agents. The procedure helped determine the lowest concentration of antimicrobial required to inhibit bacterial growth after

overnight incubation (Andrews, 2001). Four types of bacteria extracted from the ‘ in use’ T- towel were investigated against two cleaning agents. The antimicrobial products used were Mr Muscle Kitchen lemon fresh antibacterial spray and an ASDA branded all-purpose antimicrobial spray. Broth cultures were made by inoculating four 250ml Erlenmeyer flasks each containing 50ml nutrient broth with single bacterial colonies. The flasks were then incubated at 37°c, 100rpm in the rotary incubator for 24 hours. The cultures were then diluted in sterile double strength nutrient broth to produce 1: 100 dilutions. A 96 well microtiter plate was used to perform each MIC; as shown in figure 3. 2. Multichannel pipettes were used to aseptically transfer 100µl of sterile distilled water into lanes A to F excluding column 12. Following this 100µl of 50% Mr Muscle antibacterial was placed in A1, B1 and C1; solutions were mixed by withdrawing and dispensing the solutions repeatedly. Then 100µl of solution from these wells was transferred to wells in lane 2 (A2, B2 and C2); the procedure was continued until column 12 was reached and the remaining 100µl was discarded. This was repeated for lanes D, E and F but in this case 100% Asda branded antimicrobial was used. This procedure produced doubling dilutions of the antimicrobials across the plate. Multichannel pipettes were then used to place 100µl of the bacteria/ broth mixtures into wells A1 to F1 following aseptic techniques. This was repeated for all columns up until column 12 and in each case rows G and H were not inoculated. Column 12 was used as the positive control. In this column, omitting rows G and H, 100µl of the diluted bacterial solution was added to which 100µl of sterile distilled water was mixed. This column had no antibacterial therefore allowed appearance comparison and minimised the

possibility of false negatives. Rows G and H were used as negative controls. Sterile nutrient broth was placed in these rows. These rows were not inoculated with the bacteria therefore any cloudiness would indicate unreliable results. This procedure was repeated for all four bacterial isolates, producing four microtiter plates each comparing the antimicrobial activities of the two cleaning agents. The plates were placed in the shaking incubator at 37c, 100rpm for 24 hours. Following this the plates were assessed for growth in the inoculated wells. The MIC was taken as the lowest concentration at which the wells had become cloudy.

A

B

C

D

E

F

G

H

A12 to F12: Positive controlMr MuscleAsda brandedRow G and H: Negative control1 2 3 4 5 6 7 8 9 10 11 12Figure 3. 2: Minimum inhibitory concentration, 96 well Microtiter plate.

4. Results and Discussion

The domestic kitchen is a major site for a number of different microorganisms, including potentially pathogenic bacteria. Currently the

increased concern in Intestinal infectious diseases and respiratory diseases in the domestic environment makes it a vital area of research (Bloomfield et al., 2012). Research into this area may help identify public health matters which can be portrayed to the general public, helping to eliminate the need for excessive antibiotic prescribing. This project explored the bacterial flora of a T. towel which has in previous studies shown to be a major vector for microorganisms within the domestic kitchen.

4. 1 Preliminary cell counts

Preliminary experiments were carried out to demonstrate the capability of utilising safe microbiological techniques to produce reliable and reproducible data. A laboratory strain of Escherichia coli was selected as the test microorganism due to research indicating its persistence within the domestic setting and its ability to cause intestinal infectious disease (Scott et al., 1982, Speirs et al., 1995). In this instance it was used to test reproducibility of cell count techniques. The ability to achieve reproducible cell counts was demonstrated by incubating the bacteria for 24 hours, performing serial dilutions, making growth media observations and selecting the most appropriate dilution to achieve an accurate count. In this case a 10-7 dilution was selected as on all other plates the colonies were too many to count (TMTC - counts of above 300 colony forming units (CFU)). Each dilution was plated out in duplicate and the procedure was repeated, producing four sets of results for each. Table 4. 1 shows the results achieved.

Viable counts (log CFU/ml)

Attempt

Agar plate 1

Agar plate 2

Mean

Standard deviation

19. 699. 619. 650. 05729. 739. 719. 720. 014Table 4. 1: Viable counts for lab grown E. coli, to demonstrate the ability to achieve reproducible results. As the average viable count gave standard deviations lower than 0. 1, it can be concluded that the procedure was accurate and is an appropriate method to use in the experiment. All four counts from the 10-7 dilution were within a close range, emphasising that this method would give reproducible results. However after observation of other agar plates it was noticed that the 10-6 dilution plates showed signs of contamination. There were a couple of colonies which appeared darker in colour and did not resemble the E. coli type. Although the exact reason may not be determined, there are a variety of possible reasons such as; contamination of plates, pipettes or spreaders, inadequate sterility practices or contamination during the incubation period. To avoid this from occurring in the experiment, extra care must be taken to ensure everything used is sterile and is handled in the correct manner.

4. 2 Selection of microbial extraction techniques

An adequate method of extraction of microbial cells from the T- towels was necessary to ensure the highest number of cells was collected from the samples and the results received were accurate. Two methods were

compared for reliability; the griffin flask shaker method and the tissue grinder method (See tables 4. 2 and 4. 3).

Griffin flask shaker method

Time

(Minutes)

Dilution

CFU plate 1

CFU

Plate 2

Viable count (log CFU/cm2)

2Neat26212. 47

0. 101163. 03

0. 01133. 405Neat42432. 93

0. 1019143. 31

0. 011294. 1210Neat37412. 69

0. 1012163. 24

0. 01873. 98Table 4. 2: The viable count determined from the griffin flask shaker method over a period of 10 minutes.

Tissue grinder method

Time

(Minutes)

Dilution

CFU

CFU

Viable count (log CFU/cm2)

2Neat18242. 42

0. 10532. 70

0. 01103. 105Neat31282. 67

0. 10682. 94

0. 01243. 5710Neat31262. 55

0. 10582. 91

0. 01123. 27Table 4. 3: The viable count determined from the tissue grinder method over a period of 10 minutes. The results from each method varied slightly, on comparison of the results from the neat sample it can be seen that the griffin flask shaker method produced the highest count (See figure 4. 1). However the T test was used to statistically analyse the results and confirm this. Figure 4. 1: A comparison of the viable count achieved using the griffin flask shaker method and the tissue grinder method.

4. 2. 1 The Student's T test

This test can be used to determine whether the average viable counts recorded above are in fact significantly different between the two methods used. On rejection of the Null hypothesis that ' there is no significant difference between the number of microbial cells extracted using the griffin flask shaker method and the tissue grinder method'; the method giving the highest counts will be adapted. If the null hypothesis is accepted, the most practical and easiest method will be chosen to use in the study. Where degrees of freedom are equal to 2 and a probability of 0. 05 is selected the T values for the two tailed T test are as indicated in Table 4. 4 below.

Tcalculated

Ttable

8. 222. 92Table 4. 4: T value comparisonTable 4. 4 shows that the calculated T value was significantly higher than the predicted T value taken from ' The Standard Table of Significance' (Gravetter et al., 2009). The difference between the T values is +3. 92 therefore the T test rejects the null hypothesis and shows that there is a significant difference between the two approaches. By comparison of the figures achieved in the viable counts it can be concluded that the greatest extraction was with the Griffin flask shaker method. The results from this method were consistent when compared between each run. The highest counts were seen at 5 and 10 minutes. There was little increase in cell count after shaking for 5 minutes. In some cases there was a slight reduction in the viable count this may indicate that the further 5 minutes of shaking may have caused cell damage.

Therefore it was concluded that the experimental method of choice would be the Griffin flask shaker method and this would be run for 5 minutes on each occasion.

4. 3 Viable counts from ‘ in use’ T " towels

After an adequate technique of extraction had been found, the T- towel could be tested in domestic settings. A sterilised T-towel was used by a busy household of 8 individuals for 5 days; results were taken every 24 hours to determine a change in the bacterial flora of the domestic T- towel. After 5 days the T-towel was laundered. This simulated the normal routine within this particular household. The T" towel was used from Thursday to Monday, allowing incorporation of the weekend which for many families is a busier time of the week. The T- towel was placed in the domestic kitchen and the individuals within the household were asked to use the T " towel as they would normally. Individuals were unaware that the T- towel was part of a microbiology research study; this avoided the chance of any biased results. The T-towel was used in daily kitchen tasks such as dish/utensil drying, surface drying/wiping, during food preparation and covering prepared food. In particular this household used the T-towel primarily in the dish drying process. The T- towel was not removed from the kitchen when taking samples, as removal would not follow the normal household routine and risk of contamination from sources out of the domestic settings would be greater. Samples were taken using sterilised scissors and stored in sterile saline before taking to the laboratory the following day. This again helped resemble the natural pattern of use. Analysis of day 1 results showed that, colonisation

of the microbial species onto the T-towel was fast. All colonies including the yeasts and enteric bacterium had colonised the T-towel after 24 hours of exposure. This agrees with findings from previous research, that colonisation of cloth materials is rapid and related to the microstructure of the T-towel (Hilton et al., 2000). Figure 4. 2 shows the non-diluted nutrient agar plate taken from day 1. Microorganism CMicroorganism GMicroorganism BMicroorganism FFigure 4. 2: Day 1 colony growth on nutrient agarThe viable counts achieved are shown in the table 4. 5 below. Run 1 show the results for the samples taken from the central region of the T-towel whilst Run 2 shows those obtained from the samples taken from the edge of the T-towel. Growth media

Day of study of 'in use' T-towels

0123456Nutrient AgarRun 10. 004. 254. 445. 216. 08TMTC4. 21Run 20. 004. 194. 435. 026. 09TMTC4. 22Sabauraud AgarRun 10. 004. 014. 025. 066. 06TMTC3. 69Run 20. 003. 904. 035. 046. 03TMTC3. 75MacConkey AgarRun 10. 004. 204. 015. 255. 85TMTC3. 80Run 20. 004. 224. 154. 185. 77TMTC3. 81Table 4. 5: Viable counts in CFU/cm2 taken over a course of five days use of the T-towel and after laundering. The day 0 counts showed no contamination of the T towel prior to exposure to the natural environment, indicating that the sterilisation procedure of autoclaving at 121°c was of appropriate standard and effectively removed all microorganisms present there previously. The results clearly indicate a positive correlation between time and the viable count. In particular a rapid increase was seen at day 4. This may be linked to the growth patterns of the microorganisms but may

also relate to the increased usage of the T-towel over the weekend. However the results taken from all days showed no appearance of any new colony types over time. Although several dilutions were carried out, the day 5 plates were not sufficiently diluted; these plates were heavily colonised and distinguishing between single colonies was not possible. These plates were classed as TMTC (too many to count). In Figure 4. 3 below linear regression has been used to estimate the number of colonies which may have been present on day 5. The results from Run 1 on the nutrient agar have been used to demonstrate this relationship. The increase noticed in the results agrees with previous findings that cloth materials are able to give a protective environment for the microorganisms and therefore they are not easily removed. (Hilton et al., 2000)The small difference in cell counts between the two samples taken (middle and edge of T towel), maybe due to the lack of water, nutrients and heat it was exposed to. On comparison of all the results it can be seen that the samples taken from the edge of the T towel tend to have fewer colonies compared to those taken from the middle. This relates directly to the method of use when dish drying as the middle region tends to gain more contact with the dishes, the water and any residing food particles. Estimated viable count = 6. 900 CFU/cm2Figure 4. 3: Prediction of the counts on nutrient agar for day 5 use of the T towelDay 6 results are those found after laundering the T towel.

4. 4 Partial identification of microorganisms

4. 4. 1 Partial identification of Bacterial species

Growth on various agar plates determined that the T towel had collected a large number of bacterial species including coli forms and intestinal pathogens. After the number of CFUs had been counted the plates were observed to examine the various types of colonies present. The colonies were classified according to morphological differences. The various types of microorganisms were then sub cultured and procedures including Gram staining, oxidase testing and catalase testing were carried out to partially identify these microorganisms. Table 4. 6 below categorises the different colonies from A to G and lists the results seen from various tests and observations. Also see figure 4. 2 above where some of the colonies of the microorganisms below have been identified on the agar nutrient agar plate from the day 1 sample.

Microorganism

Colony Appearance

Microscopic Observations

Enzyme tests

Colour

Size

Remarks

Gram stain

Shape

Growth

Oxidase

Catalase

A

PinkLargeIrregularMucoid-veRodSingle

-

+

B

YellowMediumRoundMucoid+veCocciClusters / Single

+

+

C

CreamSmall roundNon Mucoid+veCocciClusters / Single

-

+

D

PinkSmallroundNon Mucoid-veRodClusters / Single

+

+

E

CreamMediumroundNon Mucoid-veRodSingle

+

+

F

OrangeMedium roundNon Mucoid+veCocciClusters / Single

+

+

G

WhiteMedium roundNon Mucoid+veCocciSingle

-

+

Table 4. 6: Microorganism categorisation based on colony appearance, microscopic observation and chemical reactionsDue to the restrictions of this project, further analysis to help identify each of these microorganisms was not possible. However attempts have been made to predict the classification of some of the microorganisms.

4. 4. 1. 1 Microorganism A

After sub culturing this microorganism onto MacConkey agar and allowing growth at 37°c for 24 hours, the colony appeared pink in colour with a mucoid texture (See figure 4. 4). By comparison of these characteristics to those found in the classification chart from Oxoid limited, there is high resemblance to Enterobacter aerogenes (Oxoid, 2007). The results found in table 4. 6 above also agree with this species. The presence of this type of bacteria agrees with previous studies where sufficient numbers of Enterobacteria species were also found (Scott et al., 1982, Finch et al., 1978).

Pink/orange mucoid colonies

MacConkey agar

Figure 4. 4: Growth of Microorganism A on MacConkey agarEnterobacter species are part of the Klebsiellae tribe and normally colonise the gut as part of the human microflora. They are opportunistic pathogens responsible for a variety of nosocomial infections such as lower respiratory tract infections,

soft tissue infections, intra-abdominal infections and many more (Parija, 2009a).

4. 4. 1. 2 Microorganism B and C

This microorganism grew small pale pink non mucoid colonies on MacConkey agar. To confirm that the species was of the Staphylococcus genus it was sub cultured onto Mannitol growth media; which is NaCl rich allowing growth of this particular species. Figure 4. 5 below shows formation of yellow colonies on the pink media. As the media has not been acidified, the species can be classified as Staphylococcus Epidermis a non-mannitol fermenter (Oxoid, 2007). Microorganism C also resembles the Staphylococcus type however due to time restrictions in this project; complete identification was not possible (See figure 4. 6). Earlier studies have also indicated the presence of the Staphylococcus species within the kitchen. (Finch et al., 1978, Speirs et al., 1995, Scott et al., 1982)

Yellow non-mucoid single colonies

Mannitol agar

Figure 4. 5: Growth of Microorganism B on Mannitol agar

Cream non mucoid single colonies Staph epidermis

Nutrient agar

Figure 4. 6: Growth of Microorganism C on Nutrient agar. Staphylococcus Epidermis colonises the skin naturally and offers protection against many pathogenic species. However immunocompromised individuals are often at risk of developing infections such as Endocarditis in those with prosthetic

heart valves, intravenous catheter infections, wound infections and many more (Parija, 2009b).

4. 4. 2 Partial identification of Yeasts and fungi

The T towel had also collected a variety of yeast and fungi shown by the growth of cells on the Sabauraud agar plate in figure 4. 7. This study does not aim to identify or carry out any tests on the yeast or fungi found. Figure 4. 7 shows day 1 colonisation on the Sabauraud growth media. FungusYeast

Sabauraud agar

Figure 4. 7: Sabauraud growth media colonisation on day 1 of the ‘ in use’ study. Yeasts and moulds can be found in the domestic kitchen occasionally and are often associated with food spoilage in low pH products such as fruit. Some yeasts or fungi are opportunistic pathogens (Lund et al., 2000).

4. 5 Persistence of bacteria after laundering

To investigate how effective laundering is in reducing the T towel microflora, a wash cycle at 40°c was used. Although a significant reduction in the microflora was noticed, a high viable count still remained. The viable load on the nutrient agar decreased from 1207814 CFU/cm2 to 16106 CFU/cm2, showing a reduction of 1191708 colonies. From observation of the MacConkey agar plates it was seen that the small pink non mucoid colonies identified as Staphylococcus persisted after the cycle. Previous research conducted by Walter (1975) emphasises the ability of such microorganisms to persist after laundering and cause infection in individuals especially those within the hospital (Walter et al., 1975). The findings from this study that a

40°c cycle is not sufficient to remove all traces of Staphylococcus species is in agreement to those from the study conducted by Jaska (1980), in which it was found that total removal of Staphylococcus species requires a cycle at 60°c (Jaska et al., 1980). Other unidentified bacterial species also remained, including microorganism F of which there was very little reduction. The Sabauraud agar plates showed a high reduction in colony numbers however again some yeasts and fungi persisted, showing some resistance to the procedure. The biological washing powder used consisted of mainly anionic surfactants including Sodium Dodecylbenzenesulfonate and oxygen based bleaching agents including sodium carbonate peroxide. The presence of such ingredients explains the decrease in the bacterial load. There was no indication of any disinfectant present and therefore the results support previous research suggesting that phenolic disinfectants or sodium hypochlorite are necessary to sufficiently reduce the bacterial load (Gerba et al., 2007, Scott et al., 1984).

4. 6 Minimum inhibitory concentration (MIC)

Mr Muscle and Asda branded anti-bacterial agents were tested for their ability to inhibit bacterial growth. The MIC is the lowest concentration of antibacterial required to inhibit growth. Four types of bacteria mentioned in section 4 were tested against these products. Figure 4. 8 illustrates the MICs obtained for each of the four bacteria tested. The MIC results show that overall the Mr muscle branded antibacterial agent was more efficient in inhibiting bacterial growth compared to the Asda branded agent. To inhibit all tested bacterial growth with Mr Muscle a concentration above 0. 195%

would be appropriate, whereas a concentration above 1. 25% of the Asda branded would be required to effectively remove all of the tested bacteria. The results indicate the most resistant bacterial species is microorganism F as it requires the highest concentration of the antimicrobial agent and the least resistant is microorganism D. Microorganism B and C show similar MIC values; both of which were identified as Staphylococcus spp. Figure 4. 8: MIC of four of the microorganisms found within the domestic kitchen against two antibacterial agents.

4. 6. 1 Mann Whitney U test

The Mann Whitney U test was used to compare the effectiveness of the Asda branded antibacterial to the Mr Muscle antibacterial. Results are shown in table 4. 7.

Microorganism

Asda rank (R1)

Mr Muscle rank (R2)

B6. 52. 5C6. 54D2. 51F85T (Sum of ranks)23. 512. 5N (No of Samples)44U value)2. 5Table 4. 7: Results of the Mann Whitney U test for the MICs of Asda branded antibacterial and Mr Muscle branded antibacterial. The Critical value obtained from statistical tables is 0 at a probability of 0. 05. As the results show a U value above this, the null hypothesis suggesting there is no significant difference between the two types of antibacterial agents is accepted (Gravetter et al., 2009). So although figure 4. 8 shows that a higher concentration of the Asda branded antibacterial is required to inhibit bacterial growth compared to Mr Muscle, this test suggests that there is no

significant difference between the two types. This may be because the sample of bacteria it was tested against was very low. A greater number of MICs should be carried out to establish which antibacterial is more effective. However due to the time restrictions of this project the MICs were not repeated and the MIC for microorganism F showed a couple of cloudy wells in the negative control region. This indicates that these results are not very reliable, repeats should be carried out and the average MIC should be calculated to gain accurate data.

4. 7 Relevance of findings

In the past many studies have researched the microflora of T towels and expressed how they may be used as vectors causing dissemination of bacteria within the kitchen (Scott et al., 1982, Finch et al., 1978, Josephson et al., 1997). However much of this research is now dated and with the increased care of immunocompromised individuals at home there is an immediate need to identify the potential risk of infection from bacteria present within the home. This study has identified that public health is at risk from potentially pathogenic microorganisms present within the kitchen and has highlighted the need for further research to help prevent opportunistic infections arising from the home. The research conducted fulfilled the aim of this experiment to isolate and partially identify microorganisms found on a T towel used within a domestic kitchen. The five day study showed that microorganisms can contaminate a T towel within a number of hours and that colony numbers continue to rise with time. On identification of some of the bacteria it was found that opportunistic pathogens such as Enterobacter

Aerogenes and Staphylococci spp colonise the T towel within a short period of time. Such species have previously shown to exist within the domestic environment and may have a link to respiratory and intestinal infections in immunocompromised individuals (Scott et al., 1982, Finch et al., 1978, Speirs et al., 1995). Although laundering reduced the viable count considerably from 1207814 CFU/cm2 to 16106 CFU/cm2, many microorganisms still persisted including some pathogenic species such as Staphylococcus. The counts after laundering were similar to those found on day one of the study. This is an important factor to emphasise to the public as most individuals rely on laundering at environmentally friendly low temperatures to sterilise T towels. Guidance should be issued to ensure T towels and other cleaning cloths are effectively sterilised. Previous research has indicated that a 60°c cycle with a detergent containing a phenolic disinfectant or sodium hypochlorite may be effective (Gerba et al., 2007, Jaska et al., 1980, Scott et al., 1984). Further research into the antibacterial properties of regularly used cleaning agents within the kitchen suggested that not all cleaners are effective in inhibition of bacteria compared to others. Due to the limitations of this study only two cleansing agents were compared for efficacy and the Mann Whitney U test did not show a significant difference between the two types. The MICs obtained from this area also showed that some bacteria are more resistant compared to others. These findings are important as effective cleaning of surfaces within the kitchen may help eliminate bacteria responsible for opportunistic infections.

4. 8 Future work

Although all the objectives of this study were met time restrictions did not allow for complete analysis of the microflora. Therefore further research into this area is required to gain a complete understanding. Other techniques of analysis such as Serological testing, phage typing and polymerase chain reaction (PCR) or growth of the bacteria onto various agar plates may help gain more information and confirm the identity of microorganisms. If a complete analysis of the kitchen microflora and the items used within this can be achieved, it may provide many answers for causes of home acquired infections. This may ultimately help reduce the numbers of such infections by issuing appropriate home hygiene guidance.

4. 9 Limitations of the study

This study has helped highlight some very important areas in public health and the majority of results obtained coincided with results from previous studies suggesting some reliability; however the structure of this study may suggest otherwise. The findings from this study cannot be generalised to the larger population as such a small sample of only one household was used. Growth of microorganisms on T towels may vary in different homes depending on how often and in which ways they are used. Therefore in order to get a more representative sample, a larger number of homes should be studied. Not all techniques used in this study are completely free from contamination. During MIC determination and when carrying out the preliminary counts, the results showed signs of contamination suggesting the procedures used or the environments in which the investigations were

carried out were not adequately sterile. To overcome this approximately three times replicates of each sample should be analysed and an aseptic environment may be more ideal to gain reliable results. The simple extraction techniques such as grinding and mechanical shaking may not extract all types and numbers of cells. Also not all microorganisms grow on agar plates therefore the counts achieved may not be true to reality. Microorganisms grow at different temperatures and pH, these were not kept constant when performing the extraction steps or whilst plating out the preparation. Sterility was ensured by using Bunsen burner within close range, this may have increased the temperature within this area causing the bacteria to grow. To gain more reliable counts these factors should be considered and maintained if possible. The laundering procedure did not consider the effects a heavier load may have on removal of colonies. Therefore it was not true to reality as families are likely to wash the T towels with other items not just alone. More research into this area is required to understand how effective laundering is.

4. 10 Impact of research on the public

At present there is little information provided to individuals on the importance of public health. Studies such as these can help enlighten the public to the potential risks present within their home. This will in turn help reduce the number of cases of infection arising from the domestic environment. Not only will this help improve public health but will also impact on the current concern in antibiotic prescribing. Figure 4. 9 illustrates this effect. 1. Public education: Educate the public on possible areas of

microorganism contamination within the home. Highlight how the use of T towels provides a habitat for bacteria and how bacteria may potentially spread to other surfaces leading to contamination of food. 2. Improved hygienic measures within the home: issue guidance on appropriate use of cleaning materials within the home; kitchen sponges, dish cloths etc

3. Reduction in the presence of bacteria within the home and the number of infections arising from this setting.

4. Reduction in the prescribing of antibiotics and therefore a decrease in antibiotic resistance.

Figure 4. 9: Demonstration of the importance of the findings from this study and similar research.

4. 11 Conclusion

Overall previous research and this study have indicated that pathogenic bacteria persist within the domestic environment. Further analysis is required to gain an understanding of the types and numbers of microorganisms present. In the past there has been much emphasis on the importance of good quality water and personal sanitation although these areas remain as vital factors there is now evidence that public health may improve if good hygiene practices are utilised within the home. Appropriate guidance and education can improve public health and help resolve issues including escalating treatment costs and antibiotic resistance.