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Individual Research Project Dissertation

A critically comparative investigation of the performance of disinfectants and detergents during surface cleaning for reduction in microbial burden under controlled laboratory conditions

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Abstract

Background: Hospital-Acquired Infections are a major challenge faced by the health industry and hospital cleanliness plays an important role for controlling the rates of infection. This study aimed to determine the effects of detergent-based compared with disinfectant-based cleaning on surfaces contaminated with bacteria that are common causes of hospital-acquired infection over a 24-hour period under controlled conditions in a laboratory.

Hypothesis: There is a significant difference between the use of detergent and the use of disinfectant during surface cleaning with regard to the removal of microbial burden.

Methods: Plastic surfaces were contaminated with Staphylococcus aureus (*S.aureus*) and Escherichi coli (*E.coli*) before cleaning with a detergent, a disinfectant and distilled water. The surfaces were sampled for bacterial colony count at 0, 1, 2, 4, 7 and 24 hours after cleaning. The colonies of *S.aureus* and *E.coli* were counted from each sample.

Results: There was a significant reduction in average colony counts of *S.aureus* after using both detergent and disinfectant compared to the control samples at each time point to a factor of 1900 after 24 hours. The average ACC for the samples cleaned with detergent and disinfectant were significantly different from each other at every time point when analysed using a one-way repeated measures ANOVA test for each condition. A regrowth of bacterial colonies was observed between hours 0 and 2 after cleaning with detergent, and between hours 0 and 7 after cleaning with distilled water, however no such regrowth was observed after cleaning with disinfectant until a gradual increase between 7 and 24 hours after cleaning which was observed across all conditions. The overall average ACC per plate after 24 hours when they were cleaned with detergent or disinfectant was 9.8 and 8.6 ACC respectively, compared to an average of 19,000 ACC per plate of the control samples. *E.coli* colonies were not able to be measured in this study.

Conclusion: Both detergent and disinfectant reduce bacterial colonies on plastic surfaces to a similar degree over a 24 hour period, however a statistical similarity using a one-way repeated measures ANOVA cannot be confirmed from the study due to its' limitations in statistical analysis. Due to this, a significant difference was found between samples cleaned with disinfectant and detergent at all time points. This study gives evidence that disinfectant may be more appropriate for hospital cleaning; in particular for outpatient scenarios due to its' short-term benefits in the few hours after cleaning where no regrowth of bacterial colonies were observed. Further research is required in this area to confirm the findings of this study.

Mitigation plan

Event/Issue	Potential/actual Impact on project	Action(s) taken to mitigate impact on project outcomes	Remaining impact
I was unable to get access to the microbiology laboratory after 11th March 2020 and so I was unable to complete the experiments.	A key aspect of the project was using results and data obtained from the laboratory experiments to discuss the findings. I was unable to produce results for *E.coli* bacteria in the first and only repetition and thus I have only data from *S.aureus* bacteria.	I used the limited data I collected and discussed the findings with reference to insufficient repetitions to draw reliable conclusions. I also made greater use of existing data from previous projects than was initially planned. I changed the scope of the project from focussing on primary data to using a combination of primary and secondary data.	The limited data I produced cannot be deemed reliable and thus some interpretation and conclusions may be incorrect due to a lack of experimental data.
Inability to access technical staff and module tutor for guidance related to the project.	I was unable to easily access the technicians that supported my project and my dissertation tutor to ask questions and discuss the findings of my project.	I used email and online video calling to communicate with the technicians and my dissertation tutor after the 11th March.	The difficulty of this compared with face to face contact impacted the project with delays of information and possible misunderstandings.
Inability to access study spaces for continuing with the report.	I was unable to access library facilities where I would usually complete written work. I do not have a suitable space for this at home and so this has impacted my ability to complete the project in the same time frame.	I moved to my family home where there is more available space to complete written work than my university accommodation.	Working at home rather than in a suitable study space has impacted the time frame in which I have been able to complete the work.

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List of Abbreviations

HAIs – hospital-acquired infections

S.Aureus - Staphylococcus aureus

MRSA - Methicillin-resistant Staphylococcus aureus

E.coli - Escherichi coli

C.difficile - Clostridium difficile

CPE - Carbapenemase-Producing Enterobacteriaceae

UTI – urinary-tract infection

ICU – intensive care unit

WHO – World Health Organisation

Cfu – Colony-forming unit

IPA - Isopropyl Alcohol

DBTH - Doncaster and Bassetlaw teaching Hospitals

UHDB - University Hospitals of Derby and Burton

ACC – Aerobic Colony Count

1. Introduction

Hospital-acquired infections (HAI's) are a major challenge faced by the health industry. They are defined as any infection which has been contracted in a health-care facility and presents 48 hours after admission or within 30 days of discharge (WHO, 2011). The definition, which initially focussed on infection transmission whilst in an acute-care hospital, has evolved to include a range of settings including family medicine clinics and ambulatory care (Haque M, 2018). The rise of antibiotic-resistant bacteria has allowed HAI to remain one of the biggest causes of death in most countries (Haque M, 2018). In the US in 2002, there were around 1.7 million instances of HAI resulting in 99,000 fatalities (Klevens, et al., 2007); making it the sixth leading cause of death in the US (Kung HC, 2008). According to the European Centre for Disease Prevention and Control, around 4,131,000 patients are affected by a HAI every year in Europe (WHO, 2011), and cross continent prevalence studies have found 7/100 patients in advanced countries and 10/100 patients in emerging countries acquire an infection whilst in hospital (Danasekaran R, 2014). The risk HAI's pose to public health is great and the control and minimisation of the routes of transmission is an important area of research and development in modern infection control.

The scope of HAI's ranges greatly however high frequency of infection is associated with the use of invasive devices such as in ventilator-associated pneumonia, surgical site infection, catheter-associated urinary tract infections and central line-associated bloodstream infection (WHO, 2011). These are transmitted through and catalysed by cross-contamination between patients and health workers, the degree of patient susceptibility, implants and prostheses (Messina AF, 2014). Medical innovation has allowed for more invasive treatment, which in turn provides more opportunity for infection as a possible route of transmission (Filetoth, 2003). The growing proportion of immune-compromised patients in hospitals has resulted in an increasing number of patients at a higher risk of acquiring a HAI (Filetoth, 2003); 500,000 critically ill patients acquire an infection whilst in hospital every year in intensive care units (ICU's) alone (Nuvials X, 2015). In ICU's, antimicrobial resistance rates are high due to antibiotic overuse, improper isolation practice and the frequent use of invasive devices (Mehrad, et al., 2015), putting these patients at particular risk when exposed to HAI's. Thus, reducing possible reservoirs for infection is increasing in importance to control infection transmission.

The increased risk of HAI has significant economic impact on the health industry. Studies have found that adult inpatients remained in hospital 2.5 times longer after developing a HAI, incurring greater costs by approximately three times more than patients who do not contract an infection (Plowman R, 2000). In Europe, HAI's cause 16 million surplus days of hospital care, amounting to a direct financial loss of €7 billion annually (WHO, 2011).

Despite the increasing risk and economic impact on the health industry, the complete elimination of HAI is beyond the scope of medicine (Filetoth, 2003). However, approximately one third of HAI's are thought to be preventable (Yokoe, et al., 2014). Research is required to assess infection control practices to inform the allocation of infection control resources. A likely cause of HAI is inadequate use of disinfection leading to patient to patient transmission (Ekizoglu, et al., 2003). The frequent disinfection of high-risk reservoirs such as hand touch sites including bedside tables and lockers are imperative to the prevention of infection transmission (Bhalla A, 2004). Research and development into reducing routes of transmission and possible reservoirs for HAI is imperative to reducing the overall impact of HAI on the health industry. In the UK, cleaning regimens differ between hospital trusts, but in general most specify the use of detergent once-daily to clean near-patient furniture and beds. However, cleaning routines have been found to be largely inconsistent due to wards often being

run at or over capacity (Bogusz, et al., 2013). Regular cleaning may also not provide sufficient protection against repopulation by air-or hand-borne organisms (Dancer, et al., 2009).

Research into the effects of disinfectants and detergents on the effective cleaning of hospital surfaces has been largely inconsistent and experimental data is hospital based. The aim of this report is to provide a laboratory investigation under controlled conditions into the effectiveness of disinfectants and detergents on hospital-prevalent pathogens in order to reduce potential reservoirs for their transmission.

2. Aim

To investigate and critically compare the effect of disinfectants and detergents against Staphylococcus aureus (*S.aureus*) and Escherichia coli (*E.coli*) recontamination in controlled laboratory conditions.

3. Objectives

- By investigating existing literature regarding HAI, *S.aureus*, *E.coli*, and studies carried out on detergent and disinfectant based cleaning within hospitals, critically analyse the findings and produce a literatiture review.

- Determine the most prevalent bacteria found in hospitals that are a major cause of HAI.

- Investigate the effectiveness of each cleaning method on the gram-negative and gram-positive bacteria by producing a statistical analysis on the laboratory results.

- Make note of any regrowth and decline at around eight hours after surface wiping.

- Explore how the laboratory statistical analysis might relate to studies completed in hospital environments by comparing the data obtained in the study with existing data from field experiments.

- Conclude whether the data obtained is transferrable to a hospital environment and can be used to help guide cleaning protocol within hospital institutions in order to minimise the risk of HAI transmission.

4. Literature review

4.1 Introduction and scope

Research into the appropriate cleaning methods and products used for hospital decontamination is continually developing and changing. Ongoing research into different bacteria and viruses that increase the risk of HAI as well as research into routes of transmission of these pathogens affect the integrity of current cleaning products and solutions.

This literature review will investigate current knowledge and experimental studies into HAI, the bacteria that are responsible for it and detergent and disinfectant use and effectiveness; especially in hospitals. The identified continuation of research into this area gives scope for experimental practice. The conclusions drawn from the experimental data can be compared to the current cleaning practices in UK hospitals.

The literature will be systematically reviewed and relevant studies and information that affected the project set-up and analysis are included in this report. Cited articles have been reviewed and all reports with experimental evidence that have been used within this project are included and referenced.

4.2 How do Hospital-Acquired Infections occur?

The prevalence of HAI's in Acute care hospitals is around 6.4% causing over 206,000 infections every year in England, with the top three infections in 2016/17 being Pneumonia at 25.7%, urinary-tract infections (UTI's) at 18.9% and surgical site infection at 18.3% (UHDB, 2020). The Health and Social Care Act 2012 gives a legal obligation for healthcare personnel to act on embedding a culture of prevention for HAI's (UHDB, 2020). Some infections such as MRSA require screening, however many other organisms pose a risk for HAI such as Clostridium difficile (*C.difficile*), Norovirus and influenza, and so minimising the reservoirs with which they provide the means for infection is necessary to reducing the rate for HAI's (UHDB, 2020).

A possible reason for the transmission of HAI's is inadequate hand hygiene when entering and leaving a patient zone. This could result in the cross contamination of pathogens between patients and potential infection (Russotto, et al., 2015). Inanimate surfaces can also cause cross-contamination between patients, including near-patient surfaces as well as equipment items such as stethoscopes (Russotto, et al., 2015). Gram-positive and gram-negative bacteria have been found to be able to survive for up to several months on dry inanimate surfaces and so their potential to cause infection between patients is high (Kramer, et al., 2006). However according to (Boyce, 2007), pathogens must demonstrate characteristics that allow it to survive for prolonged periods of time on environmental surfaces as well as the ability to colonize the hands of healthcare personnel (Saka, et al., 2017). Equipment items commonly used in ICU's have been found to show the same susceptibility profiles of those isolated from patients and thus the role of inanimate surfaces in the transmission of HAI's is important (Russotto, et al., 2015). According to (Dancer, 2008), factors that affect cross-contamination rates include the type of organisms, source and destination surfaces, humidity level and size of inoculum.

A study by (Saka, et al., 2017) found that factors that reduced the instance of HAI's included enhanced cleaning, the use of no-touch methods for disinfection, and the use of self-disinfecting surfaces. However, according to (Weber & Rutala, 2013), multiple studies have shown that less than 50% of surfaces in hospitals are cleaned using adequate chemical germicides as well as the inadequate cleaning of hospital equipment that is transported across the hospital frequently. It has been found that the education and measurement of effectiveness of room cleaning with immediate feedback to services personnel has been effective in improving cleaning standards and led to less instances of HAI (Weber & Rutala, 2013), and thus education and monitoring of cleaning standards is effective in minimising the effect of HAI's. In this project, the effectiveness of the cleaning methods and products used within healthcare environments will be investigated to optimise their ability to remove bacteria on potential reservoirs as a means of reducing HAI instances. If implemented correctly, as investigated by (Weber & Rutala, 2013), improved cleaning methods may be able to reduce HAI to a greater extent.

4.3 Which bacteria should be investigated?

It is important in this investigation to test the efficacy of detergent and disinfectant on bacteria that commonly cause HAIs. Some of the most common bacteria responsible for HAIs include *C.difficile, S.Aureus*, and Carbapenemase-Producing Enterobacteriaceae (CPE) such as *E.coli* (Becker, 2018).

C.difficile is an aerobic spore forming bacteria that can cause toxic megacolon and sometimes occurs after use of broad-spectrum antibiotics (Wiengand, et al., 2012). In most cases, the source of C.difficile is from the patient's own bowel flora, however cross infection can happen after ingesting the bacteria following environmental contamination (UHDB, 2020) via the faecal-oral route (Becker, 2018). Colonisation rates vary from 3% in healthy adults to 66% in neonates (UHDB, 2020). *C.difficile* was described by (Wilcox, et al., 1996) in 1996 as endemic in many hospitals; a bacterium that threatens to severely limit the use of many antibiotics. *C.difficile* has been found to be resistant to many routine cleaning products and so further investigation into the effectiveness of detergent and disinfectant on this bacterium is much needed (Becker, 2018).

S. aureus is another pathogen worth investigating. It is a gram-positive anaerobe that produces surface proteins that adhere to host tissue without detection (Kelly & Monson, 2012). It is a normal part of the skin flora, however in healthcare settings can cause septicaemia and pneumonia among other infections (Becker, 2018). Most-gram positive bacteria, including S.aureus can survive for months on dry surfaces (Kramer, et al., 2006). Improper hygiene protocol is one of the main causes of transmission in a hospital environment, and so effective cleaning regimes are imperative to minimise infections (Becker, 2018).

Furthermore, following widespread use of antibiotics such as penicillin and methicillin in the last 60 years, S.aureus mutated to form a new strain of bacteria known as Methicillin Resistant Staphylococcus Aureus (MRSA) (Kelly & Monson, 2012). This pathogen is far more aggressive than the original S.aureus and is difficult to treat due to its resistance to many antibiotics. The transmission of MRSA occurs through direct contact with infected individuals and their environment, and can remain asymptomatic for over a year (Kelly & Monson, 2012). MRSA infections usually infect broken skin, where the bacteria are carried, causing cellulitis. (OUH, 2020). However more serious infections can result in septicaemia and pneumonia (Becker, 2018). Techniques to minimise the transmission of MRSA in the hospital environment have been implemented through widespread screening procedures on all inpatients followed by the isolation of infected patients. A study in Northern Scotland showed a decrease in S. aureus bacteraemia by 41% following the implementation of this technique (Lawes, et al., 2012). The Health Protection Agency found around 1185 cases of inpatients in England with MRSA bacteraemia in 2011; a reduction by around 84% since 2002 (Kelly & Monson, 2012). Despite these efficacious policies, there are still 12,784 MRSA infections each year in the UK (Public Health England, 2018). Perhaps better cleaning methods is part of the answer to improved MRSA transmission control.

Gram-negative bacteria, responsible for around 30% of HAI's (Hidron, et al., 2008), are highly efficient at up-regulating genes that code for mechanisms of antibiotic drug resistance; and so infections caused by this are of great concern in a hospital environment under antibiotic selection pressure (Peleg & Hooper, 2010). The recent acceleration of antibiotic resistance among this group of pathogens has reached a "pandemic scale" (Mehrad, et al., 2015). The CPE family including E.coli and are the most prevalent group of aerobic gram-negative bacteria to cause HAI (Peleg & Hooper, 2010), and can multiply in the presence of bile salts, using lactose as an energy source (Mehrad, et al., 2015).

CPE are bacteria carried on the skin and in the gut of around 25% of healthy people, however for vulnerable patients, the bacteria can cause UTI's, pneumonia and septicaemia amongst other infections (OUH, 2020). CPE has been found to be resistant to many antibiotics and minimising the possible route of environmental transmission within hospitals is important; particularly in ICU's where there are vulnerable patients (OUH, 2020). Investigating the use of appropriate cleaning products could provide useful insights to help minimise the transmission of the bacteria within hospitals.

One CPE bacteria is *E.coli,* a constituent of the human gut; a typical stool contains up to one billion bacteria per cubic centimetre (Berg, 2004), however some strains can cause serious infection. *E.coli* is the leading cause of UTI's in hospitals, and can also cause gastroenteritis and pneumonia amongst other infections. The strain O157:H7 is commonly found as the source of infection and it can cause hemolytic-eremic syndrome that may result in kidney failure (Becker, 2018). The route of transmission for E.coli depends on the strain, however the faecal-oral route through environmental contamination and the consumption of contaminated water are common (Davis, 2019). The use of appropriate cleaning products is necessary in preventing the environmental transmission of *E.coli* in hospitals and so investigating this bacteria would be useful for the conclusions of this project.

Conclusions made by the World Health Organisation (WHO) after multiple studies within Europe stated that *E.coli* and *S.aureus* were the most frequent pathogens to cause HAI's (WHO, 2011). Ideally, all possible bacteria and viruses that are responsible for HAI's would be investigated to ascertain the most appropriate cleaning products for near-patient sites, however the scope of this project only allowed for two bacteria to be investigated. The selection of the two chosen bacteria, *E.coli* and *S.aureus* was therefore influenced by conclusions of the WHO study and the availability of bacteria samples at the University of Leeds microbiology laboratory. The bacteria are gram-negative and gram-positive respectively and so investigating the effect of the cleaning products on both show results across a range of bacterial structures.

4.4 Detergents and disinfectants in hospitals

The choice between using disinfectant or detergent to clean hospital surfaces could define the extent of infection transmission within hospitals. Disinfectants are defined as a chemical agent used on inanimate objects to destroy pathogenic microorganisms such as bacteria and fungi but may be less effective in destroying spores (CDC, 2016). Solutions of disinfectants vary, however common disinfectants include chlorine-based disinfectant, alcohol and Peracetic acid. Detergents are defined as a class of surfactants, a substance which reduces the surface tensions of a liquid in which it is dissolved, with cleansing properties in dilute solutions that dislodge foreign matter from surfaces and retain it in suspension (Encyclopædia Britannica, 2020). Most detergents are akylbenzene sulfonates and they are classified as either anionic, cationic or non-ionic depending on the electrical charge that they carry (Helmenstine, 2020).

Neutral electrolyzed water has been evaluated as a novel disinfectant in the case of cleaning near-patient surfaces in a study by (Stewart, et al., 2014), and was compared to the effect of cleaning with detergent. Neutral electrolyzed water is produced through passing an electric current through water and salt, and it is based on a stable form of hypochlorous acid (pH, 6–8) (Thorn, et al., 2012). Cleaning using electrolyzed water within the acute care ward was found to reduce levels of MRSA microbes on near-patient surfaces with conclusions drawn that once daily cleaning is sufficient to maintain these levels due to a recontamination time of around 24 hours (Stewart, et al., 2014). The overall effect of cleaning using the novel disinfectant after 24-48 hours gave similar results to the

effect of cleaning with detergent and thus detergent could be sufficient for routine ward cleaning. Limitations to this study may include a Hawthorne effect whereby staff respond to a measure of cleaning activity by improving performance; it was unknown how well study sites were cleaned before initiative (Dancer, 2011). Contributions from air, ambient temperature and humidity remain unknown and their effect on recontamination could not be controlled (Stewart, et al., 2014). High risk reservoirs were found to be located on overbed tables; increasing the required frequency of cleaning for such spaces (Stewart, et al., 2014). The overall effect of cleaning near-patient surfaces using electrolyzed water disinfectant were found to be similar to that of detergent based cleaning, and so detergent alone may be a sufficient practice for surface cleaning high-risk areas, however disinfectants are recommended in busy high-risk clinics with a high turnover of patients (Stewart, et al., 2014). Studies that contradict the conclusions made by (Stewart, et al., 2014) include (Attaway, et al., 2012), who found that pre-cleaning levels of bacteria were reached three hours after disinfection, and (Aldeyab, et al., 2009) who found that MRSA recontamination at hand touch sites occurred 1-7 hours after cleaning with disinfectants.

A study by (Bogusz, et al., 2013) evaluated the length of time hospital surfaces took to become re-contaminated after cleaning with detergent. The detergent used in the study, Tuffie Detergent wipes, were the same as used in this project for the detergent sample and so direct comparisons can be made. Detergents were observed to provide physical removal of microbes without compromising significant cost and toxicity, as is associated with disinfectants (Bogusz, et al., 2013). Detergent cleaning complied with a proposed standard of Aerobic Colony Count for 24 hours, leading to a conclusion of once daily cleaning with detergents being sufficient on acute wards (Bogusz, et al., 2013). As also drawn from the disinfectant study by (Stewart, et al., 2014), overbed tables were found to be high-risk for bacteria contamination and require a greater frequency of cleaning (Bogusz, et al., 2013). Limitations from this study include the amount of patient belongings and foodstuffs on the study sites complicating data collection and results (Bogusz, et al., 2013). Unexplained resurgences at approximately eight hours followed by a decline at twelve hours have been attributed to damage inflicted on microbes through physical impact from vigorous cleaning (Bogusz, et al., 2013).

The unexplained resurgences of bacterial colony count at eight hours followed by a decline at approximately twelve hours after cleaning with detergent was not only found in the (Bogusz, et al., 2013) study, but in the detergent results of the (Stewart, et al., 2014) study. As previously mentioned, the (Bogusz, et al., 2013) attributed such results to the physical impact of vigorous cleaning however no comments were made on the resurgence in the study by (Stewart, et al., 2014). A resurgence was not found after using disinfectant products; only with detergent. An investigation under controlled conditions of detergent on contaminated samples could help to give an accurate reason for the described resurgence of colony count.

A study by (Hambraeus & Malmborg, 1980) investigated the effect of cleaning hospital toilets with disinfectant compared with detergent after each patient and after each day. The lowest number of bacteria, 0.1–0.3 cfu/cm², was sampled when disinfection was performed after each patient. The contamination level after cleaning with a detergent was 4-6 times higher than after cleaning with the disinfectant (Hambraeus & Malmborg, 1980). A study by (Danforth, et al., 1987) investigated the contamination of acute care nursing units after cleaning with either detergent or disinfectant. No significant difference was found between the contamination levels of all ward floors combined after using disinfectant and detergent. However, due to the date of these studies, investigating this again to find a significant difference may prove useful due to changes in cleaning practices, bacteria types of concern and cleaning product improvement over the last 30 years.

Relevant studies into the effects of detergent and disinfectant on HAI are largely field based and so the results from each case tend to differ. By replicating the effects in a laboratory study, external factors such as cleaning method, what happens to the affected area between samples and non-sterile environments for sampling can be controlled where they can't in hospital-based experiments. Current NHS guidelines for cleaning routines within hospitals vary between Hospital Trusts, however studies that assess the effectiveness of cleaning products can be used to guide Trusts when cleaning routines are developed.

This project compares the results of cleaning bacteria with disinfectant and detergent. There is also results provided for distilled water as a cleaning agent to determine whether the abrasive action of wiping the samples has an effect as well as the chemicals that the wipes are coated with. A control sample will be used to calculate how much bacteria is removed using each cleaning product.

The disinfectant used in this study was Tuffie Disinfectant Wipes; alcohol-based wipes containing a 70% solution of Isopropyl Alcohol (IPA) (Vernacare, 2015). IPA is a commonly used disinfectant within pharmaceuticals and hospital environments, and in concentrations of 60% to 90% has been found to be antimicrobial against bacteria, viruses and fungi (PAC, 2018). Concentrations below 50% has shown significantly worse antimicrobial effects, and concentrations above 90% do not significantly improve (PAC, 2018). The solution in this project uses 70% IPA which allows complete penetration of the cell wall, consequently coagulating the proteins and thus the microorganism dies (PAC, 2018). The water content of a 70% IPA solution is beneficial as it allows for slow evaporation and thus an increased surface contact time to increase the effectiveness compared to a stronger solution (PAC, 2018). In a study by (Kamath, et al., 2017) that investigated the cleaning practices of stethoscopes; a potential source of HAI, stethoscopes were cleaning using 70% IPA. The bacterial and fungal count reduced by 98-100% after cleaning with common isolates of *E.coli* and *S.aureus* (Kamath, et al., 2017). 70% IPA solutions were also assessed in efficacy of disinfection in a study by (Singh, et al., 2002) which looked at the disinfection rates of the pagers of healthcare personnel after cleaning. It was found that the total colony count of *S.aureus* bacteria was reduced by 94% after cleaning with 70% IPA (Singh, et al., 2002). The findings of this project can be related back to these studies after using the same disinfectant on the same common isolates under more controlled conditions in a laboratory.

NHS guidelines vary between trusts, however, since 2019 the current guidelines in the Doncaster and Bassetlaw trust specify that Perecide is to be used to clean bed frames, cradles, lockers and tables (DBTH, 2019). Perecide consists of tablets containing Tetraacetylethylenediamine (TAED) and sodium percarbonate which are stable chemicals that react in water to produce peracetic acid (Perecide, 2018). Peracetic acid acts as a disinfectant through oxidising the outer cell membrane and deactivating the microorganism (Lenntech, 2018). In the University Hospitals Derby and Burton trust, detergent and hot water or detergent wipes are recommended for most cleaning tasks including bed frames and lockers (UHDB, 2020). Actichlor plus tablets, a combination of detergent and disinfectant with chlorine, are used during outbreaks of viral gastroenteritis and cleaning of isolation rooms. Disinfectant wipes are used for convenience which are 70% alcohol as the wipes are used in this study (UHDB, 2020). However, it is specified in the trust's guidelines that such wipes are not suitable for *C.difficile* spores, where chlorine based disinfectants must be used. The results of this project could provide standardised recommendations for appropriate cleaning methods and products to ensure pathogenic decontamination in hospital wards.

4.5 Hypothesis

Due to the findings of this literature review, a hypothesis was proposed that there is a significant difference between the use of disinfectant and the use of detergent in surface cleaning with regard to the reduction of microbial burden; in particular *E.coli* and *S.aureus* bacteria.

5. Methodology

A description of the experimental methodology and preparation is outlined below. The laboratory study was completed in the University of Leeds microbiology laboratory in the Civil Engineering department.

5.1 Media and consumables prep per experiment

5.1.1 Media and solutions required:

- 1 x *S.aureus* culture
- 1 x *E.coli* culture
- 4 x 100ml Nutrient broth
- 50 x 9ml Ringers (1/4 strength) bottles
- 100 x 9ml Ringers + 0.5% Tween-20
- 140 x 10ml Ringers + 0.5% Tween-20
- 1 x 100ml Ringers + 0.5% Tween-20
- 4 x 250ml - Autoclaved distilled water
- Disinfectant for Spiral plater
- 4L (160 plates) of Mannitol salts agar plates
- 4L (160 plates) TBX agar

5.1.2 Equipment required:

- Inoculation loops (1 packet) – used to transfer the bacteria to the nutrient broth to inoculate
- 1 x 0.2ml box of autoclaved pipette tips
- Plate spreaders (1 packet) – used for spreading the solution into sample squares
- Timer
- Cleaning wipes;
    - Tuffie Disinfectant Wipes (alcohol-based wipes) - 70% solution of Isopropyl Alcohol BP (Vernacare, 2015)
    - Tuffie Detergent Wipes - Contains amongst other ingredients less than 5% cationic surfactants, amphoteric surfactants and EDTA (Vernacare, 2018). They contain a mixture of non-ionic constituents at neutral pH (Bogusz, et al., 2013).
    - Distilled water wipes
- 120 swabs – used for measuring remaining bacteria on samples at intervals after cleaning
- 6 x Plastic sheets with marked out squares
- Bunsen burner – used to create a sterile environment for serial dilutions and inoculation

5.1.3 Machinery required:

- Eppendorf® Centrifuge 5810 – used to separate the bacterial pellet from the solution
- Masterclave 09 – used to autoclave the Mannitol Salts agar and TBX before pouring them onto plates
- APS One – used to pour the plates of autoclaved Mannitol Salts agar and TBX

- Whitley Automated Spiral Plater (WASP) 2 – used to plate the bacterial dilution samples spirally
- ProtoCOL 3 plate counting machine – used to count the bacterial colonies on each plate after growth
- Stuart™ Orbital Incubator S150 – used to shake the bottles containing bacteria
- Refrigerator – used to inhibit growth of bacteria in nutrient broth
- Incubator – used to store the sample plates at 37°C for 24 hours before counting the colonies
- Cell culture hood – used to create a sterile environment for sampling
- LTE Scientific™ Touchclave-Lab K150 Autoclave – used to Autoclave equipment and solutions at 121°C

5.2 Preparation of equipment, reagents and consumables:

9ml Ringers solution stored at room temperature - one tablet of ringers was dissolved in 500ml of distilled water. Once fully dissolved, 50 volumes of 9ml were prepared of the solution into glass McCartney bottles and autoclaved at 121°C for 15 minutes.

Ringers + 0.5% Tween stored at room temperature - 5 tablets of ringers were dissolved in 2.5L of distilled water. Once fully dissolved, 12.5ml of Tween-20 was pipetted into the solution. The tween was allowed to dissolve into the solution 140 10ml bottles, 100 9ml bottles and one 100ml bottle were prepared of the solution and autoclaved at 121°C for 15 minutes.

Nutrient broth (Bottles and flasks) - 6.5g of nutrient broth was dissolved in 500ml of distilled water. 100ml of media was transferred into each flask and sealed with a foam bung and tin foil before autoclaving at 121°C for 15 minutes.

Autoclaved distilled water - the distilled water was autoclaved at 121°C for 15 minutes.

Disinfectant for spiral plater - one hypoclorite tablet was dissolved in 1L of distilled water and stored at room temperature.

Mannitol salts agar and TBX - the media was weighed out as specified on the packaging with distilled water and allowed to dissolve. The solution was autoclaved and poured out at 25ml per plate using the Masterclave 09 and APS One machines.

The distilled water cleaning wipes – the Tuffie alcohol wipes were soaked in distilled water and allowed to evaporate.

5.3 Methodology of experiment:

5.3.1 Day 1:

The cultures were inoculated. Using the inoculation loop in the presence of a blue flame to create a sterile environment, the nutrient broth was inoculated with *E.coli* and *S.aureus* in separate conical flasks. They were left in the bottle mixer for 24 hours.

5.3.2 Day 2:

Serial dilutions of the cultures were created. Using the prepared 9ml ringers, 1ml of the culture was pipetted into the bottle and shake once closed. The dilutions were repeated to 10^-6.

The cultures were plated out. Using the spiral plater, 2 plates each of the 10-4 and 10-6 dilutions of both cultures were plated out, and the pipette was disinfected between each plate. The plates were left to dry before turning upside-down and storing in the 37°C incubator for 24 hours.

5.3.3 Day 3:

The cultures were counted. The 10-4 and 10-6 plates of the cultures were counted using the plate counter. The cfu/ml was worked out for each culture.

A 15ml bacterial suspension was prepared. Using the counts obtained from the plates, a mixture was prepared consisting of a concentration of $1x10^9$ cfu/ml of the two bacteria when resuspended in 15ml of Ringers and Tween (0.5%).

The *S.aureus* and *E.coli* solution was centrifuged at 3000g for 10 minutes. In the prescence of a blue flame to create a sterile environment, the resulting liquid was discarded into a disinfectant tub. The bacteria were resuspended by flicking the pellet. 15ml of Ringers and Tween (0.5%) was added and mixed with the bacterial suspension.

The pipettes, inoculation loops and pipette tips were sprayed and wiped down with Trigene. The plastic surfaces were rinsed in autoclaved distilled water for 30 minutes. The sheets were sprayed with 70% methanol and left to dry in the hood for thirty minutes.

After disinfecting the plastic sheets and all other equipment in the hood, the bacterial suspension was pipetted onto the squares measured out on the plastic sheets. 1ml was pipetted onto each square. A new pipette tip was used for each square to ensure consistency.

Plate spreaders were used to spread the solution evenly across each square. A new plate spreader was used for each square and the spreading technique was uniform across all samples.

The plastic sheets were left to dry for 1 hour.

After 1 hour, the sample squares on all the plastic sheets were cleaned accordingly inside the hood. The control samples were not cleaned, the disinfecting samples were cleaned using the disinfectant wipes, the detergent samples were cleaned using the detergent wipes and the distilled water samples were cleaned using distilled water wipes. Each sample was cleaned uniformly to ensure consistency.

Each sample from the ‘Time 0’ sheet was immediately swabbed and the swab was then placed in the 10ml ringers and tween bottles. The bottles were then shaken for 30 minutes.

After 30 minutes the solutions were plated onto the Mannitol Salts agar and TBX using the spiral plater machine. The pipette was disinfected between each plate. After the plates had dried, the plates were stored upside-down in the incubator at 37°C for 24 hours.

The control samples were diluted by $\mathbf{10^{-3}}$ before plating out due to high concentrations anticipated of the control samples.

One hour after the ‘time 0’ swabs were taken, swabs were taken from the ‘1 hour’ sheet and the same process of shaking the samples and plating them out was repeated.

This was repeated for each time point; ‘2 hours’ ‘4 hours’ ‘7 hours’ and ‘24 hours’.

5.3.4 Day 4:

The ‘24 hour’ sheet was swabbed 24 hours after cleaning the squares. The same process was repeated for shaking the samples and plating them out and the plates were stored upside-down in the incubator at 37°C.

The plates that had been swabbed on day 3 were counted 24 hours after they were sampled using the plate counter.

5.3.5 Day 5:

The plates for time point ‘24 hour’ that had been swabbed on day 4 were counted 24 hours after they were sampled using the plate counter.

5.4 Data analysis

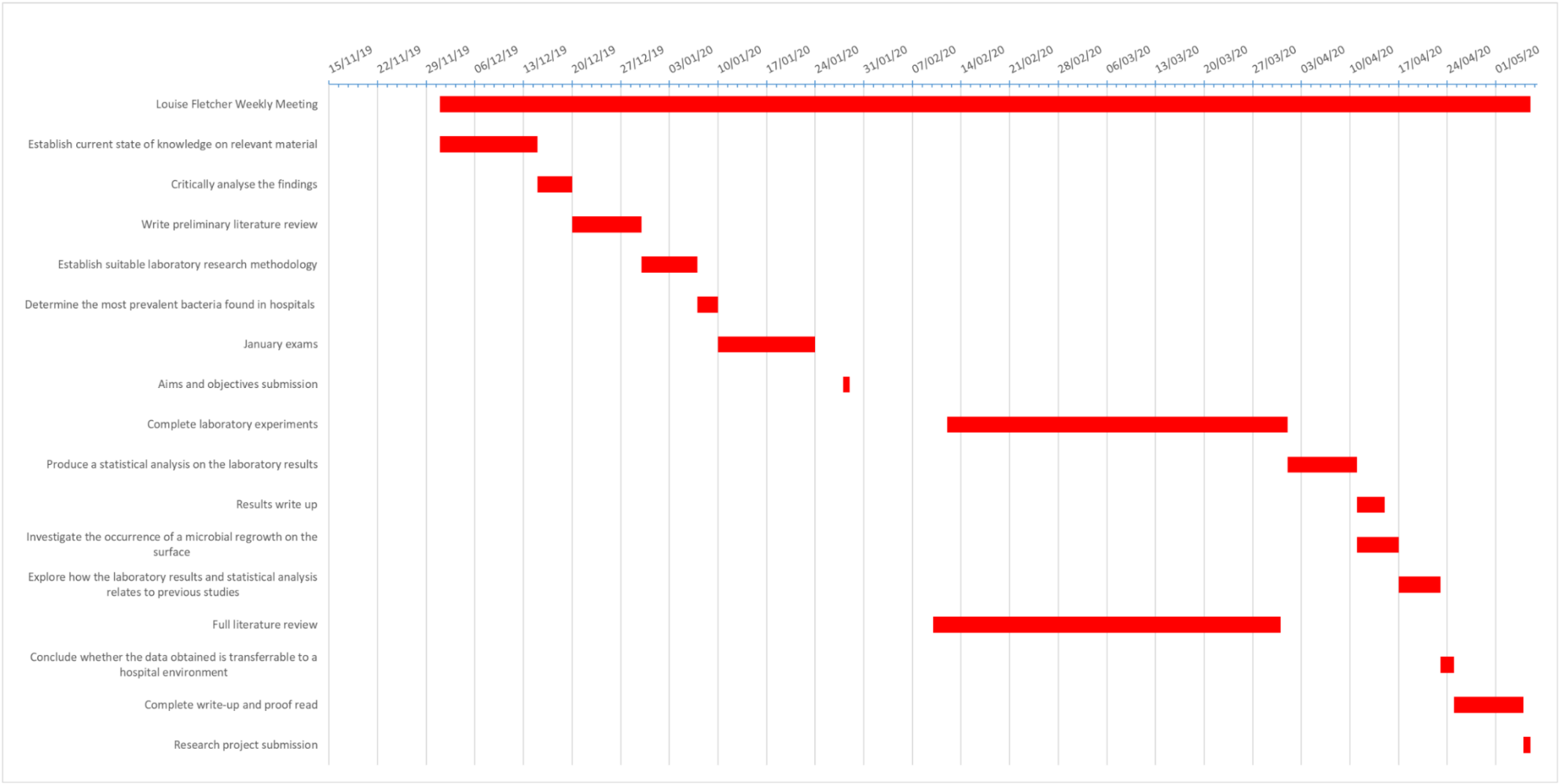
SPSS V24 computer software was used for all statistical tests.

Repeated measures ANOVA tests and independent t-tests were used to obtain P values to test significance between the different conditions of the experiment. Independent t-tests may cause Type I errors whereby false-positives are overestimated, however the repeated measures ANOVA test is also used to minimise the possibility of Type I errors when drawing conclusions from the experiment.

When analysing the data, some anomalies were withdrawn from the dataset. The values of 0 colonies obtained from some of the control samples were removed from the results to avoid possible skewing. The presence of anomalies was attributed to the dilution process before plating the control samples out.

6. Gantt Chart

Please see the Gantt chart below for the timescales of the work.


15/11/19
22/11/19
29/11/19
06/12/19
13/12/19
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27/12/19
03/01/20
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17/01/20
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06/03/20
13/03/20
20/03/20
27/03/20
03/04/20
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17/04/20
24/04/20
01/05/20
Louise Fletcher Weekly Meeting
Establish current state of knowledge on relevant material
Critically analyse the findings
Write preliminary literature review
Establish suitable laboratory research methodology
Determine the most prevalent bacteria found in hospitals
January exams
Aims and objectives submission
Complete laboratory experiments
Produce a statistical analysis on the laboratory results
Results write up
Investigate the occurrence of a microbial regrowth on the surface
Explore how the laboratory results and statistical analysis relates to previous studies
Full literature review
Conclude whether the data obtained is transferrable to a hospital environment
Complete write-up and proof read
Research project submission

7. Results

Results of ACC (aerobic colony count) per plate were obtained for the *S.Aureus* samples only. No colonies of *E.coli* were observed across any of the samples in this experiment and this has been attributed to an error in preparation of the bacteria. The results were analysed in a one-way repeated measures ANOVA using SPSS software. A repeated measures ANOVA compares group means with different conditions in each group that are considered to be related, and is used when the data is normally distributed and there are equal variances between groups. Five conditions are met for a repeated measures ANOVA. For a repeated measures ANOVA, the independent variable must consist of 'related groups', the dependent variable must be continuous and normally distributed within the related groups, significant outliers should be discounted and sphericity must be able to be assumed (LAERD Statistics, 2019). For this reason, significant outliers of data were removed from the dataset, for example values of zero colonies on control samples. This error was attributed to the serial dilution necessary before plating out the control samples.

The null hypothesis for each one-way repeated measures ANOVA test was that there was no significant difference between the data points at each time point and each condition. The P values and values for the F-test obtained are summarised in the *Table X* below. The test uses a 95% confidence interval, where P values < 0.05 are considered significant.

Table 7.1.1 – P-values for the repeated measures ANOVA tests between the data points at each time point and for each condition

	P-value	**F**
All conditions	0.0	6.202
Disinfectant and Detergent and distilled water	0.0	4.702
Detergent and control	0.0	6.192
Detergent and distilled water	0.008	3.813
Disinfectant and detergent	0.004	4.155
Disinfectant and control	0.0	6.195
Disinfectant and distilled water	0.009	6.187
Distilled water and control	0.0	6.217

All conditions tested against each other showed a significant output with $P < 0.05$ in all cases, and thus the null hypothesis can be rejected. Each condition of cleaning product had a significantly different result when testing between the data points at each time point and in each condition. The F values, which are all greater than 1, further support a rejection of the null hypothesis as it gives evidence that the data from each condition test are not sampled from populations with the same mean. The plots for each ANOVA test are shown below through *Figures 7.1.1 – 7.1.8* and it can be seen at which time points the results may have differed significantly from each other to obtain the P values from *Table 7.1.1*.

A repeated measures ANOVA test was also programmed for a null hypothesis of no significant - difference between data points in each condition over all of the time points. The P values and values for the F-test obtained are summarised in the *Table 7.1.2* below.

Table 7.1.2 – P-values for the repeated measures ANOVA tests between the data points over all time points for each condition

	P-value	F
All conditions	0.0	74.665
Disinfectant and Detergent and distilled water	0.0	118.792
Detergent and control	0.0	74.8017
Detergent and distilled water	0.0	110.045
Disinfectant and detergent	0.023	7.840
Disinfectant and control	0.0	74.945
Disinfectant and distilled water	0.0	283.175
Distilled water and control	0.0	74.241

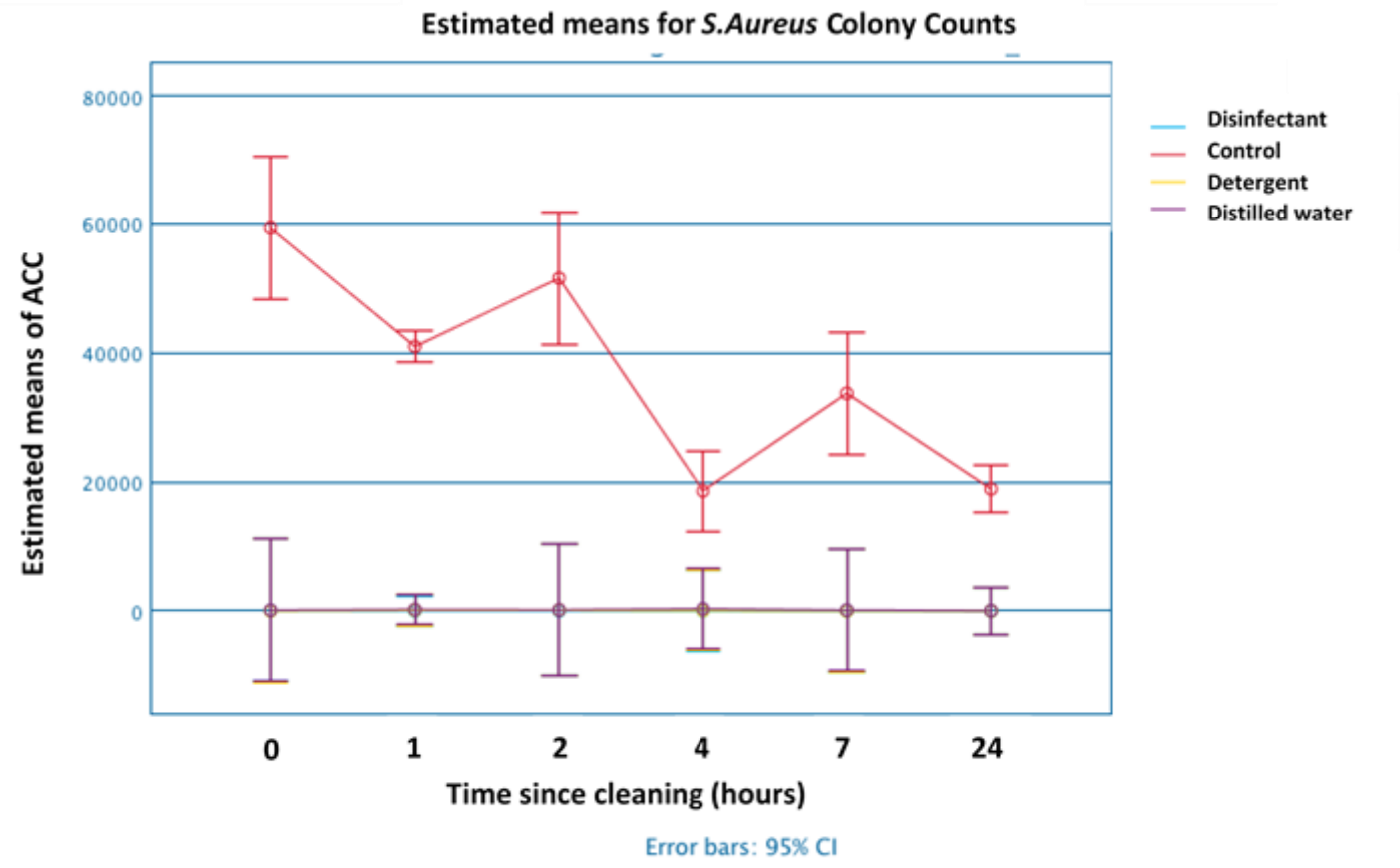

Figure 7.1.1 – Estimated means for S.aureus colony counts for control samples and samples cleaned with disinfectant, detergent and distilled water from repeated measures ANOVA test

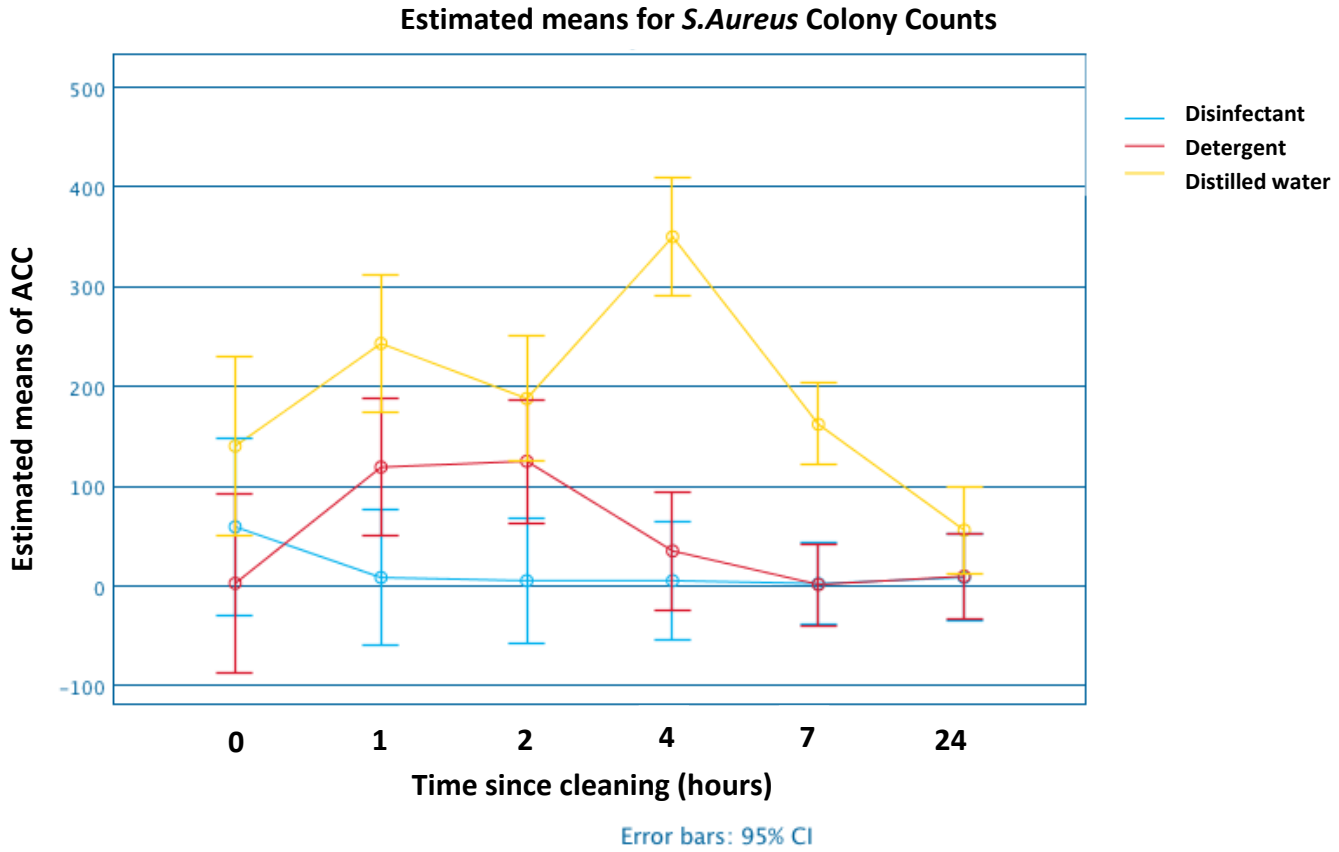

Figure 7.1.2 – Estimated means for S.aureus colony counts for samples cleaned with disinfectant, detergent and distilled water from repeated measures ANOVA test

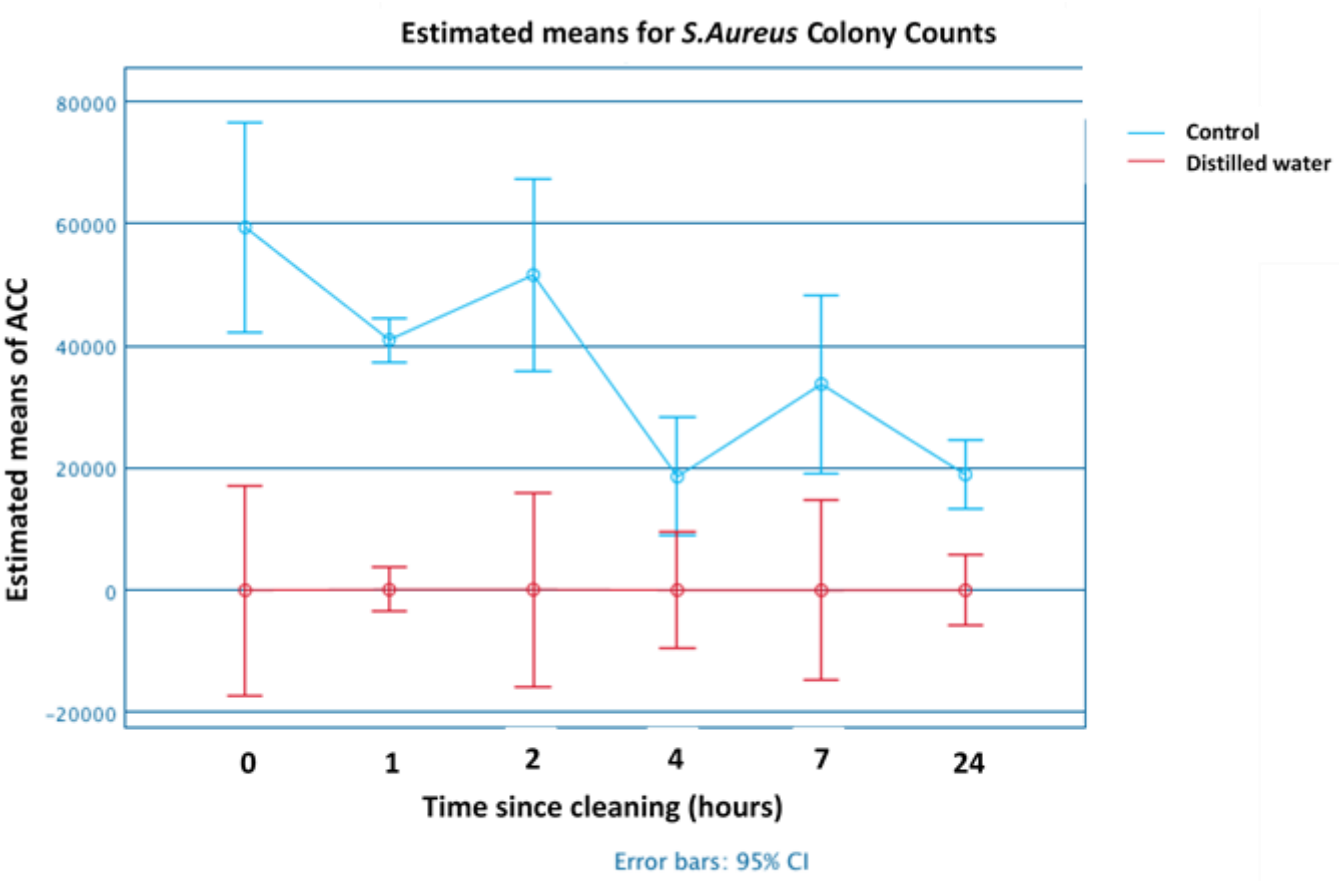

Figure 7.1.3 – Estimated means for S.aureus colony counts for control samples and samples cleaned with distilled water from repeated measures ANOVA test

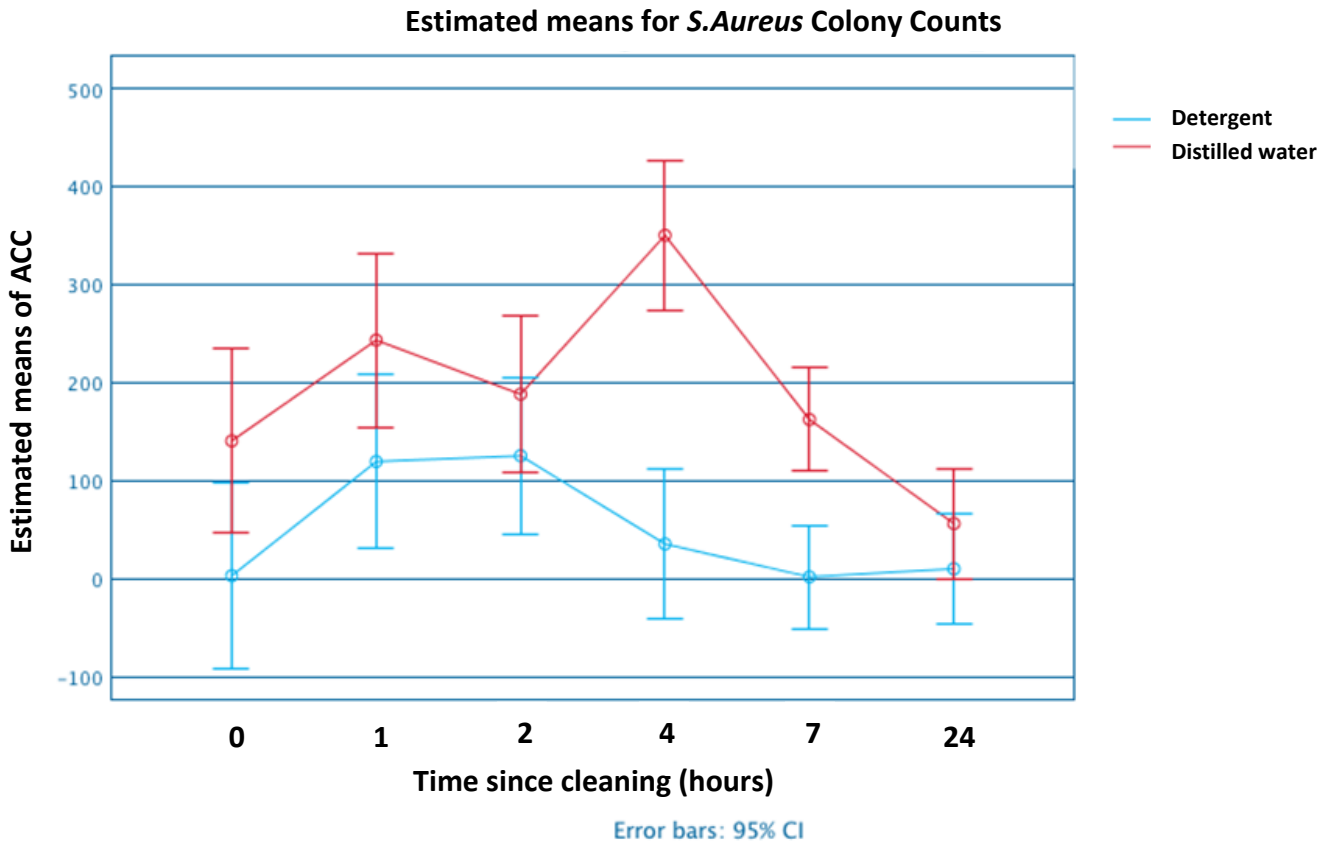

Figure 7.1.4 – Estimated means for S.aureus colony counts for samples cleaned with distilled water and detergent from repeated measures ANOVA test

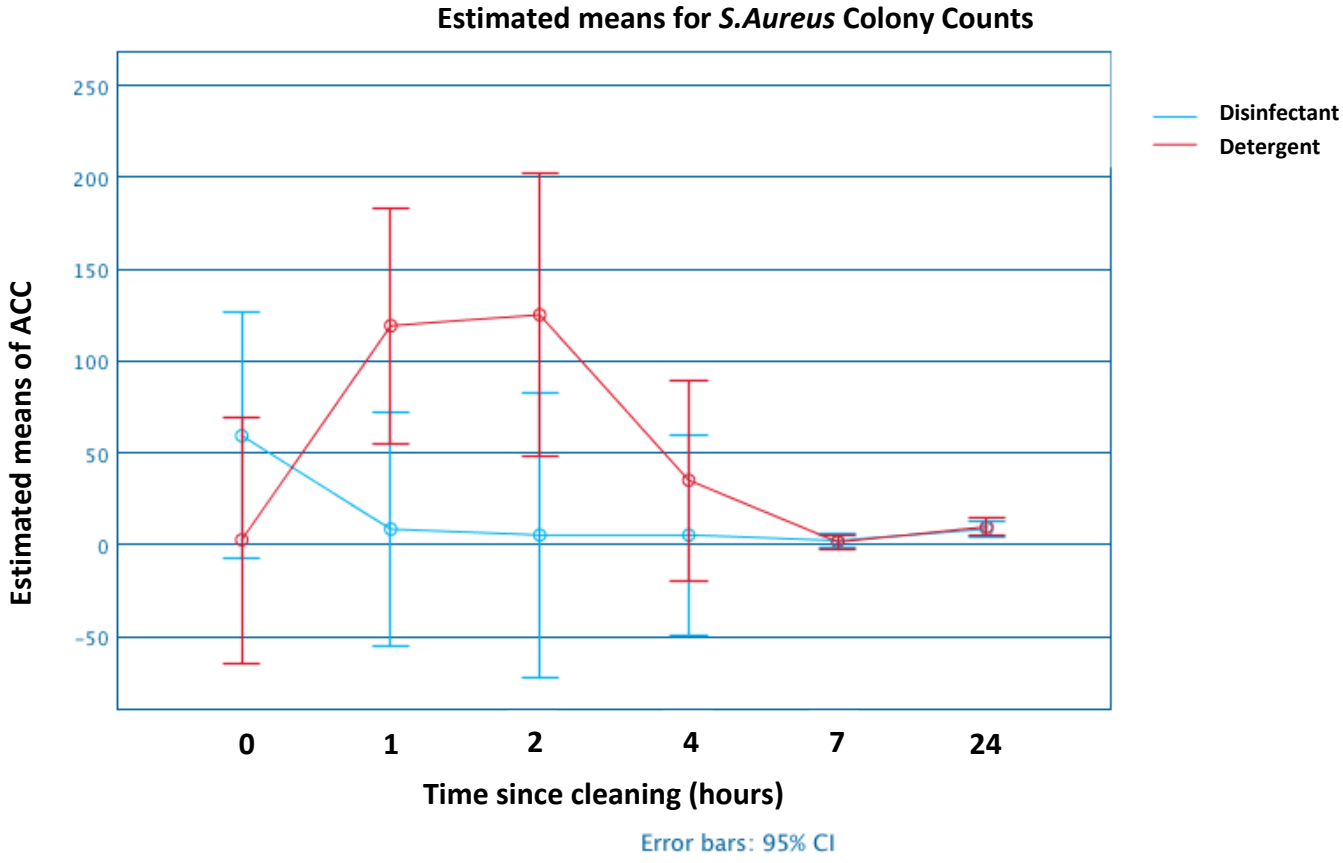

Figure 7.1.5 – Estimated means for S.aureus colony counts for samples cleaned with disinfectant and detergent from repeated measures ANOVA test

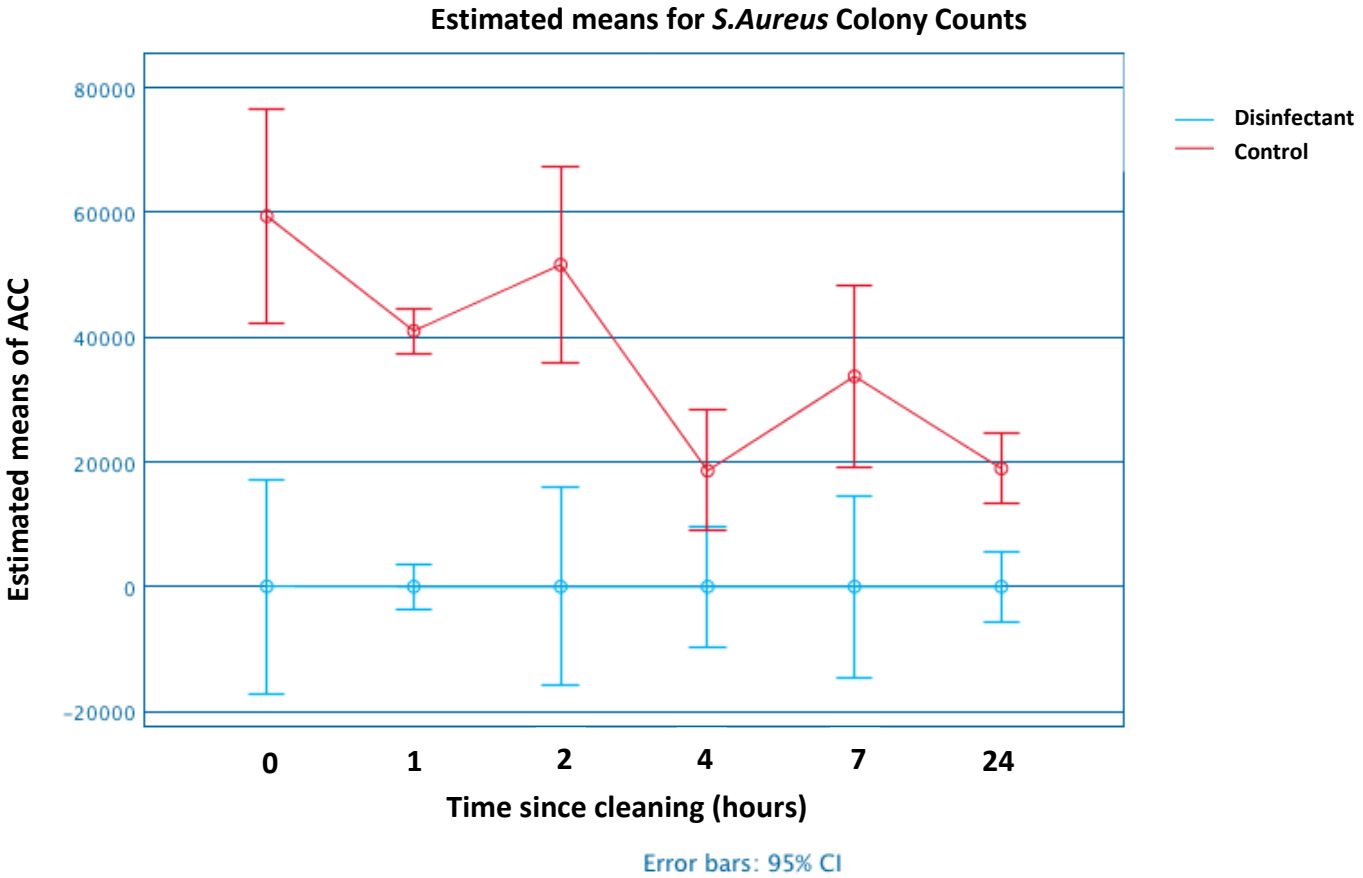

Figure 7.1.6 – Estimated means for S.aureus colony counts for control samples and samples cleaned with disinfectant from repeated measures ANOVA test

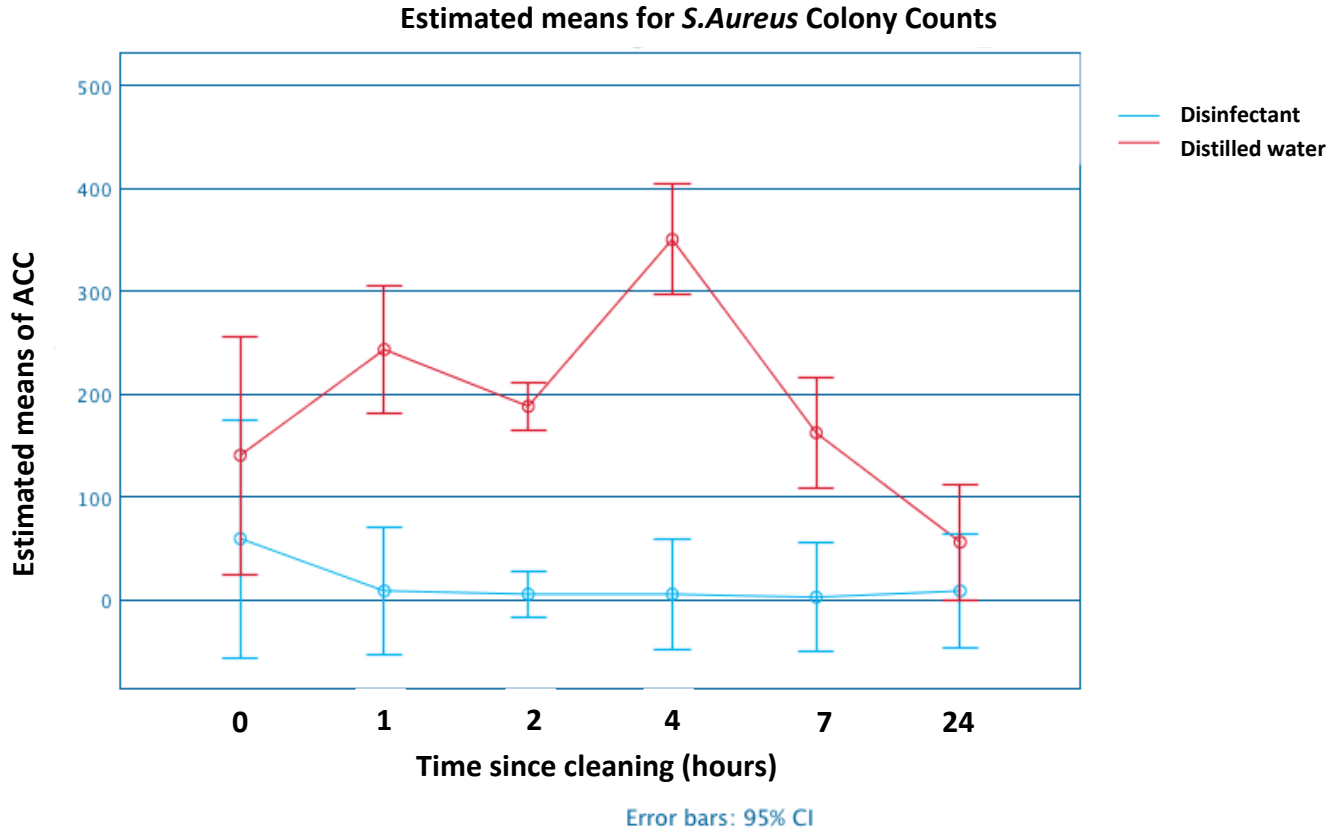

Figure 7.1.7 – Estimated means for S.aureus colony counts for samples cleaned with disinfectant and distilled water from repeated measures ANOVA test

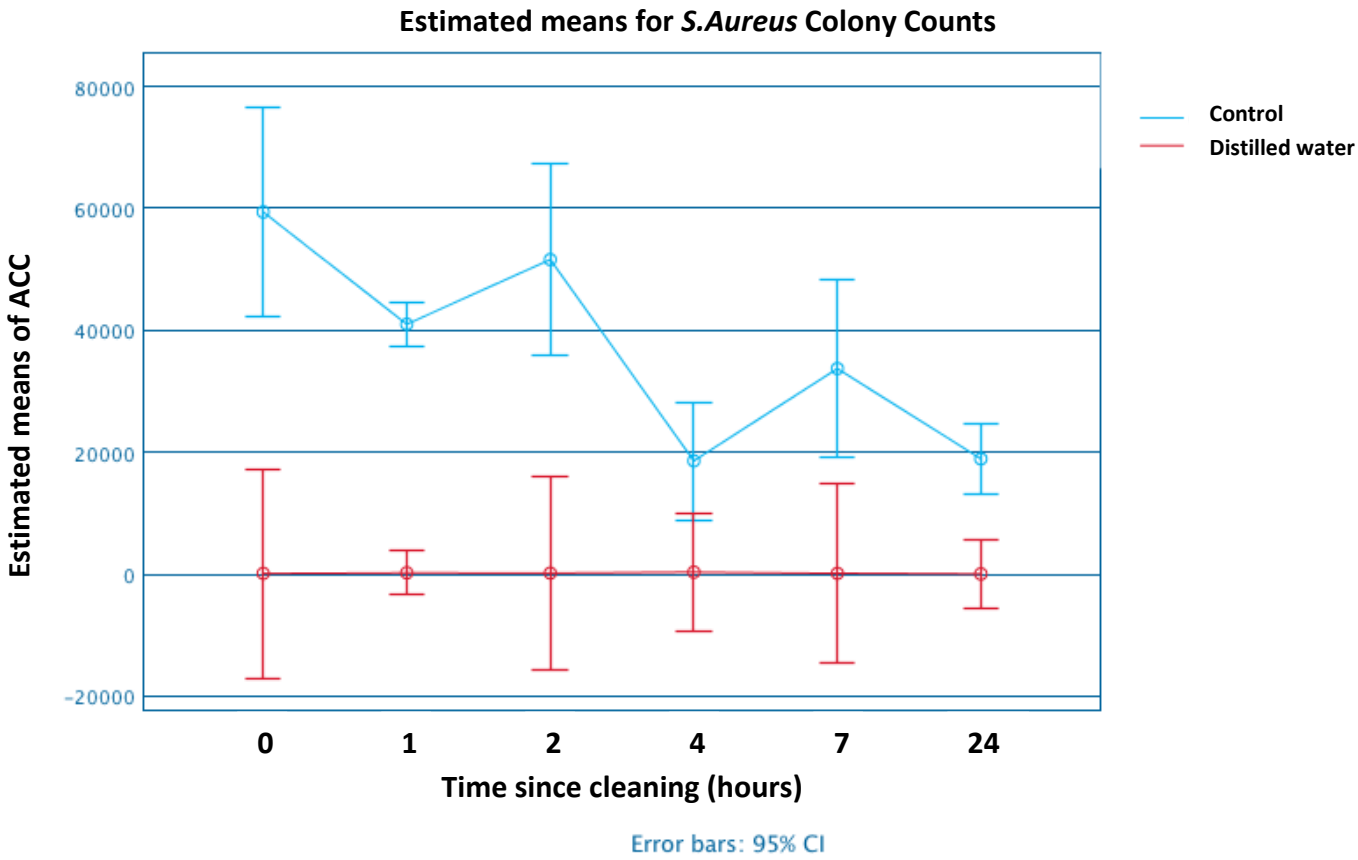

Figure 7.1.8 – Estimated means for S.aureus colony counts for control samples and samples cleaned with distilled water from repeated measures ANOVA test

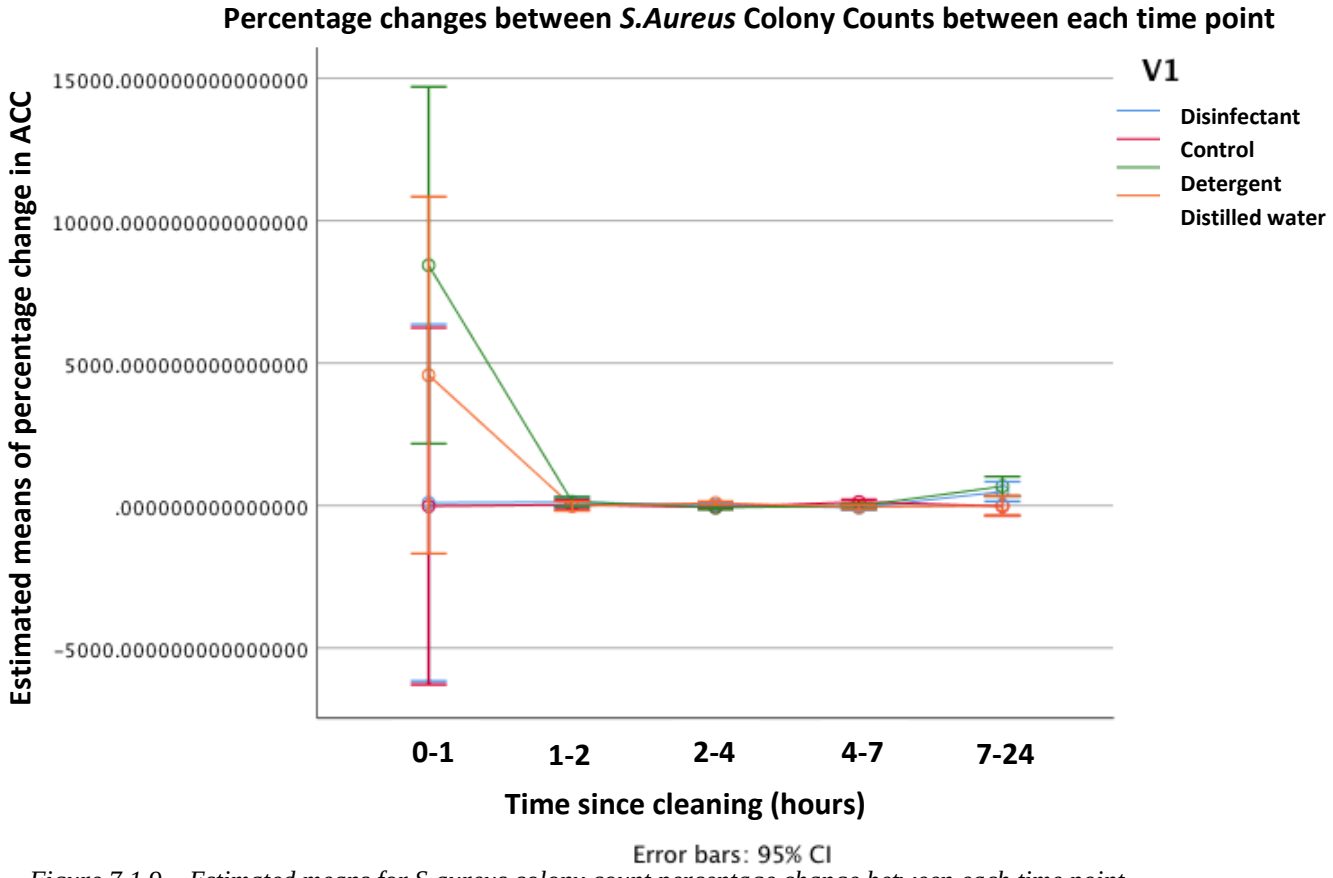

Figure 7.1.9 – Estimated means for S.aureus colony count percentage change between each time point

Figure 7.1.1 shows the profile plot of ACC per plate of each cleaning product condition and the control samples. The error bars represent a 95% confidence interval between repetitions of each condition. There was an overall reduction in ACC after use of the three methods of cleaning products; detergent, disinfectant and distilled water when compared with the control samples. All P-values for groups compared with the control samples were 0.0 and thus significantly different. This gives evidence that using either cleaning products significantly reduces the bacterial colony count than if there was no cleaning product used over the 24-hour period. This is shown in the significant difference of colony count for both cleaning products when compared to the control sample at each time point.

The control samples, initially at an average ACC of 59400 per plate, reduced in the first hour by an average of 18400 ACC per plate and peaked again in the second hour, rising by an average of 10600 ACC per plate. The ACC then reduced gradually to an average of 18900 ACC until 24 hours after the experiment started.

The alcohol disinfectant samples, with an average count at hour 0 of 60 ACC per plate appear to show a higher initial count for the colonies than the detergent samples which show an average count at hour 0 of 3.8 ACC per plate. The alcohol and disinfectant samples are compared in *Figure 7.1.5*, and have a P-value of 0.023 between each other as seen in *Table 7.1.1*, and so are significantly different from each other when considering the data point for each time point and each condition. After the first time point of 0 hours, the disinfectant samples then reduced by an average of 51 ACC per plate by the second time point of 1 hour and remained steady and low for the duration of the 24 hours with a final average count of 8.6 ACC per plate. The detergent samples increased in average ACC after hour 1 from 2.8 ACC per plate to 119 ACC per plate, then decreased until hour 7 to 1.6 ACC per plate. The average ACC per plate for the detergent samples then steadily increased to 9.8 colonies 24 hours after cleaning. The large difference in average ACC per plate between 1 and 7 hours after cleaning between the samples cleaned with detergent and disinfectant is evident in *Figure 7.1.5*. The samples cleaned with detergent experienced a peak in ACC per plate between these time points that the samples cleaned with disinfectant did not experience. The samples cleaned with alcohol can be seen to have a much steadier change in ACC per plate, with the majority of reduction in ACC occurring at hour 0 when compared with the control sample. The disinfectant seems to maintain its ability to inhibit the regrowth of bacteria over the 24-hour period much better than the detergent and distilled water.

The samples cleaned with distilled water, which are compared to the samples cleaned with disinfectant and detergent in *Figure 7.1.2*, also experienced the previously described regrowth that the samples cleaned with detergent experienced. However, the greater peak occurred 3 hours after the peak of the samples cleaned with detergent, with an average count of 350.2 ACC per plate compared to the peak of an average of 243.2 ACC per plate at 1 hour after cleaning with distilled water. The distilled water samples are a useful comparison to the cleaning products; the same abrasive action of cleaning was used without the chemicals used for cleaning. This can evaluate how much of the bacterial colony removal can be attributed to the abrasive action of cleaning rather than the cleaning chemical itself. A significant difference was found between the samples cleaned with distilled water and all other conditions, as seen in *Table 7.1.1*. This gives evidence of the abrasive action reducing the colony count without the aid of cleaning chemicals. There are two peaks of ACC at 1 hour after cleaning with distilled water with an average ACC per plate of 243 and at 4 hours after cleaning with distilled water with an average ACC per plate of 350, with a decrease in count after each peak. Overall, the distilled water had the highest bacteria count as a cleaning product at every time point of the experiment, seen in *Figure 7.1.2*. The ACC per plate after cleaning with distilled water more closely follows the ACC per plate of the samples cleaned with detergent than the samples cleaned

with disinfectant as seen in *Figure 7.1.2*. The ACC on the samples cleaned with distilled water appears to regrow and reduce at similar time points when compared to the samples cleaned with detergent, whereas the samples cleaned with disinfectant only start to regrow again 7 hours after cleaning. This may suggest that a proportion of the samples cleaned with detergents' reduction in ACC can be attributed to the abrasive action of cleaning rather than the chemicals used, and this action is responsible for a larger proportion of the effectiveness of the detergent samples than that of the disinfectant samples. This suggests that the action of the chemicals is more effective in reducing ACC in the disinfectant solution than the detergent solution.

The regrowth of *S.Aureus* colonies followed by a decrease in colony count despite no extra cleaning until 7 hours after cleaning with detergent has been observed in other studies. A study by (Bogusz, et al., 2013) investigated detergent-based cleaning at near-patient sites and found the same pattern of regrowth followed by a decrease of colony count which peaked at around 10 hours, as discussed in section 4.4 of this report. The colony count gradually started to increase again at around 14 hours after cleaning with detergent; after the aforementioned decrease following the peak of regrowth. The resurgence was described as a "microbiological phenomenon", that could have been caused either by the physical impact of vigorous cleaning where damaged organisms regain viability at around 8 hours, or a temporary inhibitory effect by a component in the detergent wipes (Bogusz, et al., 2013). This phenomenon was also observed in a study by (Stewart, et al., 2014), which observed a peak of regrowth at around 8 hours in colonies after cleaning with detergent, compared to no such peak observed after cleaning with disinfectant. No comments were made in the study on the resurgence of the colony count. In this investigation, the resurgence behaviour was observed not only after cleaning with detergent but with distilled water too, thus providing evidence that this peak can't be attributed to an inhibitory component of the detergent wipes suggested by (Bogusz, et al., 2013). The distilled water does not have the same chemical components as the detergent and so the regrowth experienced under both conditions was not a result of an inhibitory effect by a component in detergent. The resurgence was not observed with samples that were cleaned with disinfectant despite the same 'vigorous' physical cleaning method used across all three cleaning products, and so this provides argument against the suggestion by (Bogusz, et al., 2013) that the observed resurgence could be attributed to that. However, it could be argued that the disinfectant inhibits any effect that vigorous cleaning may have on the bacteria, and the disinfectant has an overriding effect on the colony count over the time period whereby the peak is observed.

The percentage change between each time point of the estimated means of ACC per plate of each condition are shown in *Figure 7.1.9*. It can be seen that the majority of percentage change of ACC per plate occurs between the first two time points, and some significant change is seen between 7 hours and 24 hours; in particular the samples cleaned with detergent and disinfectant. *Figure 7.1.9* suggests that the most significant change in ACC occurs in the first hour after cleaning, and thus the initial change in ACC from hour 0 is a defining factor of the appropriate cleaning method to choose.

8. Discussion

8.1 Key findings

The aim of this study was to compare the effect of disinfectants and detergents against *S.aureus* and *E.coli* recontamination in controlled laboratory conditions. The aim was achieved in the case of *S.aureus* bacteria only, and some interesting obsevations were discovered. Our results suggest that cleaning using a disinfectant reduces the bacteria colony count more than the detergent does in the first two hours after cleaning. 24 hours after cleaning, both the detergent and disinfectant samples had similar colony counts with an average of 9.8 and 8.6 ACC per plate respectively, however using disinfectant appeared to inhibit the effect of regrowth observed in the first 4 hours after cleaning with both detergent and distilled water. When statistically analysed with a one-way repeated measures ANOVA, significant differences were found between each condition at each time point, suggesting that disinfectant is the most effective cleaning product due to its greater overall reduction in ACC per plate at every time point with the exception of hour 0 after cleaning. The percentage change of colony counts between the samples cleaned with detergent and the samples cleaned with disinfectant were similar 1 hour after cleaning and onwards, suggesting the rate of change of colony count does not significantly differ after the first hour after cleaning with a change of cleaning product.

8.2 Strengths and limitations

This study has some strengths. Unlike the studies completed by (Bogusz, et al., 2013) and (Stewart, et al., 2014) previously discussed, this study has been completed under laboratory conditions and so external and control variables have been able to be controlled to a greater extent. In the study by (Bogusz, et al., 2013), delayed screening of cleaned areas due to patient belongings covering surfaces may have affected the reduction of counts at distinct time points. The study by (Stewart, et al., 2014) discussed some limitations including a possible Hawthorne effect of improved cleaning due to the presence of the study. This project, under laboratory conditions, eliminates the possibility of these limitations to the conclusions of the study. Controlled inoculation and swabbing were completed in the fume hood or in the presence of a blue flame to ensure sterile conditions and reliability of the data obtained.

This study has some limitations. The only bacteria with data available from this project is *S.Aureus*, and so the conclusions cannot be generalised for hospital-wide use; particularly in the case of gram-negative bacteria which has not been possible to provide results for in this study. Gram-negative bacteria could provide different results when cleaned with disinfectant and detergent to gram-positive bacteria and so the results cannot be generalised. Viruses often cause HAI; as seen in 2020 with the risk of COVID 19 spreading within hospitals. The conclusions provided from this study cannot be generalised to the effective cleaning of surfaces contaminated with viruses as this was not tested in this study. Further investigation into the effect of detergent and disinfectant on a range of bacteria and virus commonly associated with HAI is necessary before any conclusive evidence for improving the efficacy of hospital-wide cleaning is provided.

A limitation of this study is that the experiment was not completed due to COVID 19 disruptions in the lab. Five repetitions of the experiment were planned, however only one was completed. This resulted in a small sample size that might have affected the average means for ACC and variance results. Small sample sizes may skew statistical analyses due to the absence of a normally distributed dataset, allowing Type I errors where false positives occur. Due to laboratory closure experimental results for *E.coli* growth were not obtained. If these results for *E.coli* had been obtained there would be experimental data for both gram-positive and gram-negative bacteria and so more reliable conclusions could be drawn with regard to the cleaning products that should be used on hospital

surfaces. More repetitions of the experiment would have resulted in more reliable data and the possibility of anomaly elimination.

This project used plastic as the material for the surface of the samples. In hospitals, other surfaces may be potential reservoirs to bacteria that cause HAI including metal and wood. To obtain more valid results that can be directly related to improving the efficacy of hospital cleaning, further investigation into different surface materials for sampling is necessary.

A possible limitation of this study is using limited time points for sampling. It may be useful to observe any changes in colony counts between 7 and 24 hours after cleaning; which was not possible in this study. The regrowth rates of distilled water and detergent may have changed between these two time points. It may also have been useful to observe any significant changes in colony count up to 48 hours after cleaning to verify that daily cleaning of wards in hospitals is necessary; as was investigated in the study by (Stewart, et al., 2014).

8.3 Opportunities for further research

Further research could be conducted to identify the most appropriate composition of cleaning product for use in hospitals.

As well as completing the planned number of repetitions of the study, further possible research could include: investigating different solutions of disinfectant and detergent, testing the cleaning products' effect on a range of different bacteria and viruses, testing for a significant difference between different surface materials, and testing the swabbing efficiency of the experimental methods.

Further research could investigate the effect of disinfectant and detergent on other types of bacteria or virus. This report focuses only on the effect of disinfectant and detergent on *S.aureus* bacteria, when in reality there are many other bacteria and virus that can cause HAI's that may respond differently to disinfectants and detergents. This must be investigated before efficient and effective cleaning routines for hospitals are developed.

One such bacteria, which is a life-threatening cause of hospital-acquired gastroenteritis is *C.difficile*. A study by (Weber, 2010) looked at the relative resistance of *C.difficile* and norovirus to common disinfectants and alcohol-based antiseptics. *C.difficile* and norovirus survive for prolonged periods of time on inanimate surfaces and so environmental contamination plays a role in their nosocomial transmission. Infections have been associated with surface contamination in hospital rooms and health care workers hands (Weber, 2010). A study by (Wilcox, 2003) also looked at the effect of disinfectant on *C.difficile*, finding a significant decrease of *C.difficile* incidence from 8.9 to 5.3 cases per 100 admissions using hypochlorite for cleaning, providing evidence that using hypochlorite for environmental cleaning could be effective against *C.difficile* due to their sporicidal action. (Wilcox, 2003) also found that spore formation by *C.difficile* strains can increase due to exposure to detergents, and so cleaning using detergents where *C.difficile* contamination is likely is unfavourable.

For these reasons, further evidence for efficacious cleaning methods against *C.difficile* in laboratory conditions are required. A similar method to the study could be used.

Possible extension investigations could look into different types of surface materials. In this project, plastic was the material used as the surface to represent hospital bedside tables and lockers. Different surfaces found in a hospital environment include wood and metal. In a study by (Saka, et al., 2017), it was found that the pattern of contaminants according to the material make show a statistical

significance with P-value P=0.0044. It was found that *S.aureus* was the predominant pathogen found on aluminium surfaces, and so an investigation into the effect of the surface material on the regrowth rate found cleaning *S.aureus* samples with detergent and disinfectant in a lab environment could provide useful extensions to the conclusions made due to the findings of this report (Saka, et al., 2017).

Other extension work could look at different makes and compositions of disinfectant or detergent to investigate if there is a significant difference in the regrowth rate. The disinfectant used in this project was Tuffie disinfectant alcohol-based wipes which consisted of 70% solution of Isopropyl Alcohol BP (Vernacare, 2015), and the detergent used was Tuffie Detergent Wipes, containing amongst other ingredients less than 5% cationic surfactants, amphoteric surfactants and EDTA (Vernacare, 2018). As discussed in section 4.4 of this report, NHS guidelines vary between trusts, with the aforementioned Hospital Trusts using a combination of Peracetic acid (DBTH, 2019), chlorine-based disinfectant, 70% IPA wipes and detergent based cleaning products depending on the context of the clean (UHDB, 2020). A study by (Cadnum, et al., 2017) investigated a correlation between a change from a bleach disinfectant to a peracetic acid-based disinfectant and an increase in healthcare-associated *C.difficile* infection, however a defective product label that overstated concentrations of peracetic acid may be attributed to the increase in infection rates. A study by (Wheeldon, et al., 2008) found no significant difference between a chlorine-based disinfectant and peracetic acid on the sporicidal activity over a 60-minute period, however the peracetic acid was found to be favourable as an environmental disinfectant due to its' reduced occupational health hazards and odour-free nature compared to the chlorine-based alternative. An extension of this project could investigate the effect of peracetic acid and chlorine-based disinfectants currently used in NHS hospital trusts against the 70% solution of Isopropyl Alcohol disinfectant used in this investigation to determine if there is a significant difference in results.

An extension investigation into the swabbing efficiency of techniques and materials used in this project could provide the means for more valid results. The swabbing efficiency could be tested by changing the swab material, for example cotton, gauze or cellulose sponge, and swabbing on wet and dry surfaces. In a study by (Keeratipibul, et al., 2017), cellulose sponge swabs on a dry surface had higher efficiency of bacterial recovery than cotton swabs and polyurethane foam. Similarly, cellulose sponge swabs on a wet surface had a higher efficiency than gauze or cotton swabs. Swabbing on dry surfaces decreased the efficiency of all swab types to 30%. A study by (Hedin, et al., 2010) investigated the recovery rate of traditional cotton swabs compared to flocked nylon swabs and found a recovery enhanced up to three times using the flocked nylon compared to the cotton swab using *S.aureus* as a sample bacteria. The swabbing efficiency could have had an effect on the results produced in this project and thus a pilot investigation of swabbing efficiency could increase the reliability of the results produced.

9. Conclusion

In conclusion, this study provides evidence for the possible benefits of cleaning using disinfectant in hospitals as a means of minimising HAI. The results of this study can be seen to support the proposed hypothesis that there is a significant difference between the use of detergent and disinfectant during surface cleaning with regard to the removal of microbial burden. In this study, the disinfectant was observed to inhibit the regrowth effect found after using detergent and distilled water to clean; which was not only observed in this study but also in studies by (Bogusz, et al., 2013) and (Stewart, et al., 2014).

Inhibiting the regrowth of bacterial colonies after cleaning is important to minimise the risk of HAI and its' reservoirs, and thus the evidence provided in this study is in favour of disinfectant as a cleaning product as opposed to detergent. However, the importance of the first 2 hours after cleaning may depend on specific scenarios such as the moving of patients within a hospital compared with routine ward cleaning. The overall means of the bacterial colony counts of the repeated samples at 24 hours after cleaning did not differ by much between detergent and disinfectant cleaning, and thus detergent may be sufficient for routine ward cleaning scenarios. However, there was a significant difference found between samples cleaned with detergent and disinfectant at each time point using a one-way repeated measures ANOVA test, and thus further statistical analysis and repeated samples are required to investigate a statistically significant difference or similarity between the two cleaning products. Thus, conclusions cannot be drawn with regard to detergent cleaning after this investigation alone.

This study contradicts the claims made by (Stewart, et al., 2014) that the overall effect of detergent cleaning and disinfectant cleaning is not significantly different after 24 hours, and also the claims made my (Danforth, et al., 1987) that there is no significant difference between the microbial burden levels after cleaning with disinfectant compared to detergent. However, the suggestion by (Stewart, et al., 2014) that a disinfectant should be used to clean between patients in outpatient settings to alleviate contamination concerns may be seen to be supported by the evidence provided by this study due to the short-term regrowth rates of bacterial colonies after cleaning with detergent that are not observed after cleaning with disinfectant. This suggestion is further supported by the significant different found between detergent and disinfectant cleaning at all time points suggesting disinfectant reduces microbial burden to a greater extent.

The evidence provided in this study is not sufficient to advise the cleaning protocol of hospitals due to its' limitations in bacteria type, surface type and lack of repetitions. The findings of this study must be confirmed with further research into the effect of detergent and disinfectant on a range of bacteria and surfaces, with sufficient repetitions to ensure the reliability of results.

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11. Appendices

11.1 Risk assessments

Risk assessment The effect of cleaning on the survival rate of bacteria over time for two different types of material surfaces.	 UNIVERSITY OF LEEDS
Title: The effect of cleaning on the survival rate of bacteria over time for two different types of material surfaces.	Risk Assessment ID:
Risk Assessment Site: School of Civil Engineering	363765

Hazards and Risk Ratings

Hazard Type	How might the hazard cause harm	Who may be harmed	Control measures	Action by
High Temperatures	Users can sustain burns from: - carrying out the aseptic techniques using Bunsen flames. - hot molten agar.	Staff Students	- Training has been received on the proper way to pour plates and light the Bunsen burner. - Personal Protective Equipment. - The equipment will be used in accordance with the safety guidelines outlined in the user manuals. - For the Masterclave 09 (bioMérieux), the mixture will be cooled and maintained at 60°C, so it gets ready to be poured using APS One (bioMérieux). Once the cycle is completed, the plates will be allowed to cool and set before being relocated to a refrigerator.	Waseem Hiwar

COSHH substances

Substance	How might the hazard cause harm	Who may be harmed	Control measures	Action by
biological agent (Bacteria)	During the process of inoculation and swapping, any skin contact (hands) may result in human disease.	Staff	- Aseptic techniques and conditions will be maintained at all times when handling cultured plates.- Training has been received on safe handling of cultured plates.- Lab coat will be worn while carrying out microbial detection (inoculation, swabbing and spreading). Gloves will be added	Waseem Hiwar

			while conducting the quantification techniques.	

Disposal waste procedures
Please select the relevant waste stream category to be used for the disposal: Healthcare waste **Please give details of disposal method:** Procedure 1 - Disposal of healthcare waste – bags (Management of Healthcare Waste Streams in Health and Safety Services, University of Leeds)

Occupational Health
Do Occupational Health need to be notified and involved in this activity? No **Occupational health details:** **Date occupational health informed:** **Occupational Health person informed:** Not specified

Special arrangements
Do any people that are involved in this activity need additional arrangements? No **Please provide details:**

Exposure levels
Is exposure monitoring required for this activity? No **Give details of air monitoring required:**

Training
Is additional training required for this activity? No **Details of training required:**

Supervision
Is supervision required when carrying out this activity? Yes **Detail the level of supervision required:** In the first time of the experiment, technician required making sure everything in right way.

Communication of risk assessment findings to those involved			
METHOD	**YES**	**DATE**	**COMMENTS**
Local induction	No	Not specified	
Details or risk assessment discussed and agreed	No	Not specified	
Copy of risk assessment available	Yes	Not specified	Risk assessment for bioaerosol sampling and analysis (ID: 298003)
Controls covered by local protocols & procedures	Yes	Not specified	Working with substances hazardous to health. attached document as pdf. the approved list of biological agents. attached document as pdf Management of Healthcare Waste Streams. attached document as pdf
Safety Handbook location notified	No	Not specified	
Toolbox talk	No	Not specified	
Team meeting	No	Not specified	
Email circulation	No	Not specified	
Other	No	Not specified	

Review and sign off

Current status: Requires a review before: 14 May 2019

Date	User	Type
24 April 2018	Waseem Hiwar	Review
14 May 2018	Louise Fletcher	Sign off

[Next Risk Assessment review date]

Related documents

Filename	Date uploaded
Management of Healthcare Waste Streams .pdf	24 April 2018
the approved list of biological agents.pdf	24 April 2018
Working with substances hazardous to health.pdf	24 April 2018

Related actions

No related actions

Beth Macleod

11.2 Ethics Approval Form

This form should be completed by the student and passed to the supervisor prior to a review of the possible ethical implications of the proposed dissertation or project.

No primary data collection can be undertaken before the supervisor has approved the plan.

If, following review of this form, amendments to the proposals are agreed to be necessary, the student should provide the supervisor with an amended version for endorsement.

1. What are the objectives of the dissertation / research project?

   To investigate and critically compare the performance of disinfectant and detergents for reduction in microbial contamination during surface cleaning under controlled laboratory conditions

2. Does the research involve *NHS patients, resources or staff*? ~~YES~~ / NO (please circle).

   If YES, it is likely that full ethical review must be obtained from the NHS process before the research can start.

3. Do you intend to collect *primary data* from human subjects or data that are identifiable with individuals? (This includes, for example, questionnaires and interviews.) ~~YES~~ / NO (please circle)

   If you do not intend to collect such primary data then please go to question 14.

   If you do intend to collect such primary data then please respond to ALL the questions 4 through 13. If you feel a question does not apply then please respond with n/a (for not applicable).

4. What is the *purpose* of the primary data in the dissertation / research project?

5. What is/are the *survey population(s)*?

6. How big is the *sample* for each of the survey populations and how was this sample arrived at?

7. How will respondents be *selected and recruited*?

8. What steps are proposed to ensure that the requirements of *informed consent* will be met for those taking part in the research? If an Information Sheet for participants is to be used, please attach it to this form. If not, please explain how you will be able to demonstrate that informed consent has been gained from participants.

9. How will *data* be *collected* from each of the sample groups?

10. How will *data* be *stored* and what will happen to the data at the end of the research?

11. How will *confidentiality* be assured for respondents?

12. What steps are proposed to safeguard the *anonymity* of the respondents?

13. Are there any *risks* (physical or other, including reputational) *to respondents* that may result from taking part in this research? YES / NO (please circle).

    If YES, please specify and state what measures are proposed to deal with these risks.

14. Are there any *risks* (physical or other, including reputational) *to the researcher or to the University* that may result from conducting this research? ~~YES~~ / NO (please circle).

    If YES, please specify and state what measures are proposed to manage these risks.

15. Will any *data* be *obtained from a company or* other *organisation*. ~~YES~~ / NO (please circle) For example, information provided by an employer or its employees.

    If NO, then please go to question 18.

Beth Macleod

16. What steps are proposed to ensure that the requirements of *informed consent* will be met for that organisation? How will *confidentiality* be assured for the organisation?

17. Does the organisation have its own ethics procedure relating to the research you intend to carry out? YES / NO (please circle).

    If YES, the University will require written evidence from the organisation that they have approved the research.

18. Will the proposed research involve any of the following (please put a √ next to 'yes' or 'no'; consult your supervisor if you are unsure):

• Vulnerable groups (e.g. children) ?	YES		NO	X
• Particularly sensitive topics ?	YES		NO	X
• Access to respondents via 'gatekeepers' ?	YES		NO	X
• Use of deception ?	YES		NO	X
• Access to confidential personal data ?	YES		NO	X
• Psychological stress, anxiety etc ?	YES		NO	X
• Intrusive interventions ?	YES		NO	X

19. Are there any other ethical issues that may arise from the proposed research?

Please print the name of: I/We grant Ethical Approval

student Beth Macleod supervisor Dr Louise Fletcher

Signed:

(student)  (supervisor) MG hes

Date 28th January 2020 Date 27th January 2020