CROSS CONTAMINATION BETWEEN THE CLINIC AND LABORATORY DURING DENTURE FABRICATION

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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, in partial fulfilment of the requirements for the degree of Master of Science in Dentistry.

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DECLARATION

I, Krystle L'Oréal Moodley, declare that this research report is my own work. It has been submitted for the degree of Master of Science in Dentistry in the Faculty of Health Sciences at the University of the Witwatersrand, Parktown, Johannesburg, South Africa. It has not been submitted before for any other degree or examination at this or any other University.

…….
This 9th day of July 2019

ABSTRACT

Purpose: Contamination during denture fabrication can occur at any point between the laboratory and clinic. Contaminated denture surfaces are reservoirs and vehicles for both opportunistic and pathogenic microorganisms between patients and dental personnel. This study assessed the level of cross contamination between the clinic and the laboratory, as well as the efficacy of a currently used disinfectant.

Method and materials: Samples of denture debris, impressions, trial bases, primary and master casts and articulators were collected with sterile cotton tipped swabs after completion of each procedure prior to disinfection (if carried out) and then after disinfection. At least 10 samples were collected for each stage of denture manufacture. Common aerobic bacteria were isolated on several agar media. A disc diffusion test was used to determine the antimicrobial activity of Germicide 3 and Chlorhexidine disinfectants. Microbiological results were descriptively analysed, using both quantitative and qualitative analyses. The presence and absence of contamination was determined by a Chi-squared test.

Results: Mixed flora were present in 87% of the samples from the clinic. Streptococci had the highest prevalence (71.5%), with a cell count of >100 cfu/ml (32.6%) and the least prevalent microorganism was Lactobacilli (11.7%). In the laboratory the most contaminated area was the pumice area and the least contaminated area was the grinding wheel. Disinfection in the clinic resulted in a reduction in numbers but not elimination of the microorganisms present. The mean zone of inhibition of Germicide was greater with *S. aureus* and *S. mutans*. The zone of inhibition was comparable for both disinfectants with *Candida*. However, the zone of inhibition with Lactobacilli was greater with the 0.2% Chlorhexidine gluconate than the Germicide.

Conclusions: Streptococci, Lactobacilli, *Candida,* aerobic gram-negative bacteria and *S. aureus* were present at every stage of denture fabrication, and were also present in every area of the laboratory. No disinfection occurred in the laboratory. The disinfectant used in the clinic reduced the level of microorganisms but did not eliminate them. There is a clear need for improved protocols in the clinics to prevent cross contamination, and a protocol must be established in the laboratory as a matter of urgency.

ACKNOWLEDGEMENTS

"Rejoice always, pray continually, give thanks in all circumstances; for this is God's will for you in Jesus Christ"- 1 Thessalonians 5:16-18 NLT

I would like to express my sincerest gratitude to:

"The delicate balance of mentoring someone is not creating them in your own image, but giving them the opportunity to create themselves." - Steven Spielberg

To my Supervisors, Professors Peter Owen and Mrudula Patel. Thank you for your gentle guidance, patience and willingness to share your years of wisdom and knowledge with me. I have grown so much observing you and have learnt not only lessons to be a better academic but many life lessons as well.

"My child, listen when your father corrects you. Don't neglect your mother's instruction. What you learn from them will crown you with grace and be a chain of honour around your neck" – Proverbs 1:8-9 NLT

To my family, you are my true north. Thank you for your unending love and unwavering support. Thank you for allowing me to follow my dreams, for praying for me and keeping me calm and grounded.

"The heartfelt counsel of a friend is as sweet as perfume and incense" Proverbs 27:9 NLT

To my work family, thank you for being a sounding board, for always being a phone call away and assisting in every way possible to make this research a reality. Thank you for being a voice of reason and a calm between the storms. Your selflessness has not gone unnoticed.

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CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

1.1. Introduction

Denture fabrication is a multi-step process involving both clinical and laboratory procedures. Cross contamination can occur at any stage of this process. Clinical contamination may include normal flora, pathogens and opportunistic microorganisms. These are transferred to the laboratory in cases where there is inadequate disinfection and may occur at any of the stages of denture fabrication prior to being transported to the laboratory. Transmission can occur by direct contact or aerosols created during clinical and laboratory procedures, and microorganisms can be inhaled or ingested from contaminated prostheses. Patients, clinicians and dental laboratory staff are at risk if there is cross contamination. Proper infection control strategies must therefore be implemented to prevent disease transmission by disrupting one or more links in the chain of possible infection (CDC, 2016).

1.2. Contamination of dentures

Dentures are reservoirs of both opportunistic and pathogenic microorganisms (Da Silva et al., 2008). According to Glass et al., (2010), wearing a denture for eight hours was enough to heavily contaminate the intaglio surface as microorganisms penetrated the porosities within the acrylic surface.

Contaminated dentures can therefore be a vehicle for cross contamination between patients and dental personnel (Assery et al., 1992; Pavarina et al., 2003). During denture delivery, recall visits and repairs, the denture is adjusted both at the chairside and in the laboratory. It

has been found that there are multiple routes of contamination in dental laboratories such as the felt discs, pumice and contaminated hands (Da Silva et al., 2008).

Pavarina et al., (2003) reported that both oral and non-oral pathogens associated with local and systemic disease have been cultured from both contaminated prostheses and dental laboratory pumice. It has also been found that microorganisms from impressions can be transferred to the dental cast and remain viable within the gypsum cast for up to a week (CDC, 2003). Glass et al., (2001) found a wide spectrum of microorganisms on the denture base and that a denture's appearance had no correlation with the amount of contamination present; no two dentures harboured the same quantities or types of microorganisms.

Debattista el al., (2010) reported on the bacterial cross contamination from the clinic to the dental laboratory, and assessed occlusion rims, trial bases, repairs, additions and new complete dentures. In addition the study assessed the pumice and hydroflask in the laboratory. Only aerobic bacteria were reported on and it was found there was a clear indication of cross contamination between the dental clinic and laboratory. It was recommended that all items be cleaned of blood, saliva and debris using 1% sodium hypochlorite solution before delivery to the laboratory and pumice slurry should be made using the same solution.

Candida is a commensal fungus associated with denture stomatitis – an inflammatory process involving the oral mucosa of the denture-bearing area. According to Rodriguez-Archilla et al., (1996) persistent or recurrent denture stomatitis is associated with increased systemic levels of Interleukin 2 which can be harmful. Glass et al., (2004, 2010) reported that *Candida* penetration into acrylic had been reported as early as the 1980s. Latib et al., (2018) found that

the depth of penetration of *C. albicans* increased as the length (days) of exposure increased and in addition these cells which had penetrated the denture surface remained viable and were not affected by disinfectants.

Lactobacilli are usually found in association with *Candida* hyphae and are often considered commensals. Lactobacilli have been implicated in dental caries, rheumatic vascular disease, septicaemia and infective endocarditis (Harty et al., 1994). Staphylococcus aureus, a Gram-positive coccus, has been found to be associated with angular cheilitis, parotitis and mucositis (Smith et al., 2003). It is also found to be the most prevalent microorganism associated with nosocomial pneumonia (Imsand et al., 2002). *S. aureus* is important because of its pathogenicity and its resistance to drying, heat and some groups of disinfectants (Da Silva., 2008). Pseudomonas aeruginosa, a Gram-negative bacillus and *S. pneumoniae* are associated with bronchopneumonia caused by aspiration from denture plaque (Li et al., 2000; Imsand et al., 2002). Bacillus cereus and Bacillus subtilis are Gram-positive sporulating bacteria that are resistant to some commonly used disinfectants (Da Silva et al., 2008). Streptococcus mutans has a role in the aetiology of carious lesions (Assery et al., 1992). All these microorganisms have been associated with dentures and denture stomatitis.

1.3. Infection control protocols

1.3.1 Centers for Disease Control 2003

The CDC (2003) has recommended that dental prostheses, impressions and other prosthodontic materials be thoroughly cleaned, disinfected with an “Environmental Protection Agency (EPA) registered hospital disinfectant with tuberculocidal claim” and subsequently rinsed properly. The most beneficial time to disinfect is shortly after removal from the patient’s mouth to ensure that drying of blood and other bioburden does not occur.

Personal protection equipment and clothing should be removed before leaving the work area. When there is no prior communication of disinfection stipulated, laboratory staff should perform cleaning and disinfecting before handling. Items which do not have contact with the patient but are prone to be contaminated e.g. articulators, should be cleaned and disinfected between patients. Pressure pots and water baths are also potential microorganism reservoirs and they should be disinfected and cleaned between patients.

In addition it has been suggested by the CDC (2003) that effective communication between the lab and dental personnel is necessary to enable proper disinfection procedures and ensure materials are not damaged or distorted by possible disinfectant over-exposure and to prevent duplication of disinfection procedures. They further suggested that written instructions should be provided with the following information: the methods of disinfection, type of disinfectant used and exposure time especially for communication with external laboratories. All final prostheses and appliances delivered to patients should be void of contamination, thus it is key that the lab and dental personnel communicate via writing as to who is responsible for the final disinfection process.

1.3.2 CDC 2016

In 2016 the Centres for Disease Control in the USA published summary guidelines based on the 2003 guidelines and emphasised that a new denture should be completely disinfected or sterilised before being delivered to the patient. This includes before handling and prior to returning it after adjustment procedures.

The CDC recommended proper administrative control of the infection control procedures such as implementation of policies and procedures for containing, transporting and handling contaminated instruments as well as continuous training for all dental health care personnel.

1.3.3 Australian Dental Association 2015

The current recommendations by the Australian Dental Association (ADA 2015) for impressions, involve thorough rinsing and immersion or spraying with a diluted detergent; however, neither dilution concentration nor detergents are specified. The same procedure is recommended for prostheses or any stage that has come into contact with the mouth. The guidelines also recommend standard precautions be applied for all stages in both the clinic and the laboratory, and in particular they specify that "when polishing appliances which have been worn in the mouth, repaired appliances or relined appliances, polishing pumice should be dispensed for individual use and the pumice tray cleaned after each use".

1.4. Disinfectants

Harrison et al., (2001) found that rinsing a denture with tap water was not sufficient to remove microorganisms, especially *Candida.* The study found that immersion type cleaners removed organic debris effectively and had minimal abrasiveness. Therefore disinfection is necessary. One of the ideal properties of a denture disinfectant is that it should not alter the prosthesis. Harrison et al., (2001) found that deterioration of the denture surfaces increased susceptibility to staining, plaque accumulation, and loss of surface detail, and made it harder to clean.

Numerous disinfectants have been investigated through the years for prosthodontic use. A variety of disinfectants suitable for prosthetic components and materials such as impression materials and denture components have been investigated as reported below.

Germicide 3 (Germiphene, Canada) is a surface disinfectant; its active ingredients are alkyl dimethyl ethylbenzyl ammonium chloride, benzalkonium chloride and isopropyl alcohol. It claims to be bactericidal, tuberculocidal, fungicidal and viricidal. Lambert and Kolstad (1986) tested the effect of Germicide when the active ingredient at the time was benzoic acid, on *Candida* and found that when combined with a detergent it was more effective in safely decontaminating dentures. However, to achieve decontamination it was reported that it needed to be soaked for six hours. Ultra-sonic enhancement decreased the decontamination time to ten minutes. Assery et al., (1992) found that 10 minutes was sufficient to thoroughly disinfect a denture, although disinfection is dependent on the material's ability to infiltrate denture porosities and penetrate the plaque biofilm.

Owen and Goolam (1993), reviewed disinfection of impression materials to prevent viral cross contamination. Based on the evidence at the time, the authors established protocols for the disinfection of different impression materials using materials that had been shown not to adversely affect the dimensions of the material or the surface quality of the cast.

Pavarina et al., (2003) tested the "effectiveness of an infection control protocol for cleansing and disinfecting removable dental prostheses". The study tested immersion solutions of the following: 4% chlorhexidine gluconate, 1% sodium hypochlorite, 0.48% biocide which is an iodophor and 3,78% solution of Amosan which is an alkaline peroxide. They used dentures which had been worn by the patients for at least 6 months; were scrubbed for 1 minute with

chlorhexidine, rinsed with sterile water; soaked in the disinfectant for ten minutes; and then rinsed again with 200mL sterile water for 3 minutes. They found that all the solutions significantly reduced the growth of microorganisms except Biocide which was not as effective in inactivating microorganisms. Biocide is an idophor solution and it was also found that iodophor solutions can discolour surfaces. Pavarina et al., (2003) reported that chlorohexidine was active against a broad spectrum of Gram-positive and negative bacteria, yeast, fungi, facultative anaerobes and aerobes and found that 4% chlorhexidine solutions were effective in the disinfection of dentures. The CDC (2003) guidelines described disinfection as a less lethal process of microbial inactivation that eliminates all recognised pathogenic microorganisms but not necessarily all microbial forms. The guidelines suggested that a disinfectant should be selected based on personal judgment and guided by label claims and government regulations. It recommended that the following factors be considered when choosing a disinfectant: "the degree of microbial killing required, the nature and composition of the surface, the item or device to be treated, cost, safety, and ease of use". The CDC requires all chemicals used in hospitals to be registered by the EPA. These chemicals must be both tuberculocidal and viricidal and must have a minimum proven efficacy against lipophilic and hydrophilic viruses.

The disinfection of irreversible hydrocolloid impressions was tested by Rweyendela et al., (2009), where it was found that a chlorine dioxide solution was sporicidal after 1.5 minutes immersion. This is ideal for disinfection of materials like alginate because of its minimum contact time and excellent efficacy. Due to its sporicidal activity it is categorised as a high level disinfectant. Watamoto et al., (2013) stated that chlorine dioxide does not form toxic chemical derivatives and is thus safer for the human body than glutaraldehyde and sodium hypochlorite. The study also tested the efficacy of 0.02% chlorine dioxide, 2%

glutaraldehyde and 1% sodium hypochlorite and found that chlorine dioxide in conjunction with ultra-sonic cleaning achieved “complete removal of oral bacteria and fungi”. The disadvantage of this study is that only dental instruments were tested and not prosthetic items such as impressions and acrylic resin bases.

Egusa et al., (2008) also tested the efficacy of a variety of disinfectants on dental impressions. The action of the disinfectant toward Streptococci, Staphylococci, *Candida*, methicillin resistant *S. aureus* and *P. aeruginosa* was measured to determine efficacy. The study found that after disinfection microorganisms were still found on the impression and only the Hygojet system and benzalkonium chloride was effective in reducing colony growth. Both Lambert and Kolstad (1986) and Egusa et al., (2008) found that for Germicide to be effective it needed to be used in combination with another disinfectant/ detergent. Da Silva et al., (2008) tested the effectiveness of immersing acrylic resin blocks for 10 minutes in six different disinfectants on removing *Candida, S. mutans, S. aureus. E. coli* and *B. subtilis*, as well as their effects on acrylic resin. They concluded that 1% sodium hypochlorite, 2% glutaraldehyde, and 2% chlorhexidine digluconate were the most effective; however, it required ten cycles of 10 minute immersion.

Glass et al., (2011) found that disinfection methods such as denture soaking, sonication and immersion while using a vacuum, significantly reduced the numbers of microorganisms on the denture base. However, regular soaking of the denture was concluded as being most effective.

Valentini-Mioso et al., (2018) conducted a crossover randomised clinical trial to assess the effectiveness of various chemical hygiene protocols for the reducing the biofilm on complete

dentures. The study tested protocols which included water, 0.5% sodium hypochlorite, 0.2% chlorhexidine gluconate and 5% sodium bicarbonate solutions and found “that sodium hypochlorite and chlorhexidine can both effectively decrease microbial viability on denture surfaces and could be used together with mechanical tooth brushing”. However, the study tested chemical hygiene protocols where the dentures were taken home and disinfected over a period of weeks, not disinfection in a clinical situation during denture construction.

Thus it can be seen that protocols change depending on the disinfectant used and the level of decontamination one would like to achieve.

1.5. Denture fabrication steps

The steps involved in denture fabrication and possible routes of contamination between the dental clinic and the dental laboratory are shown in Fig. 1.1

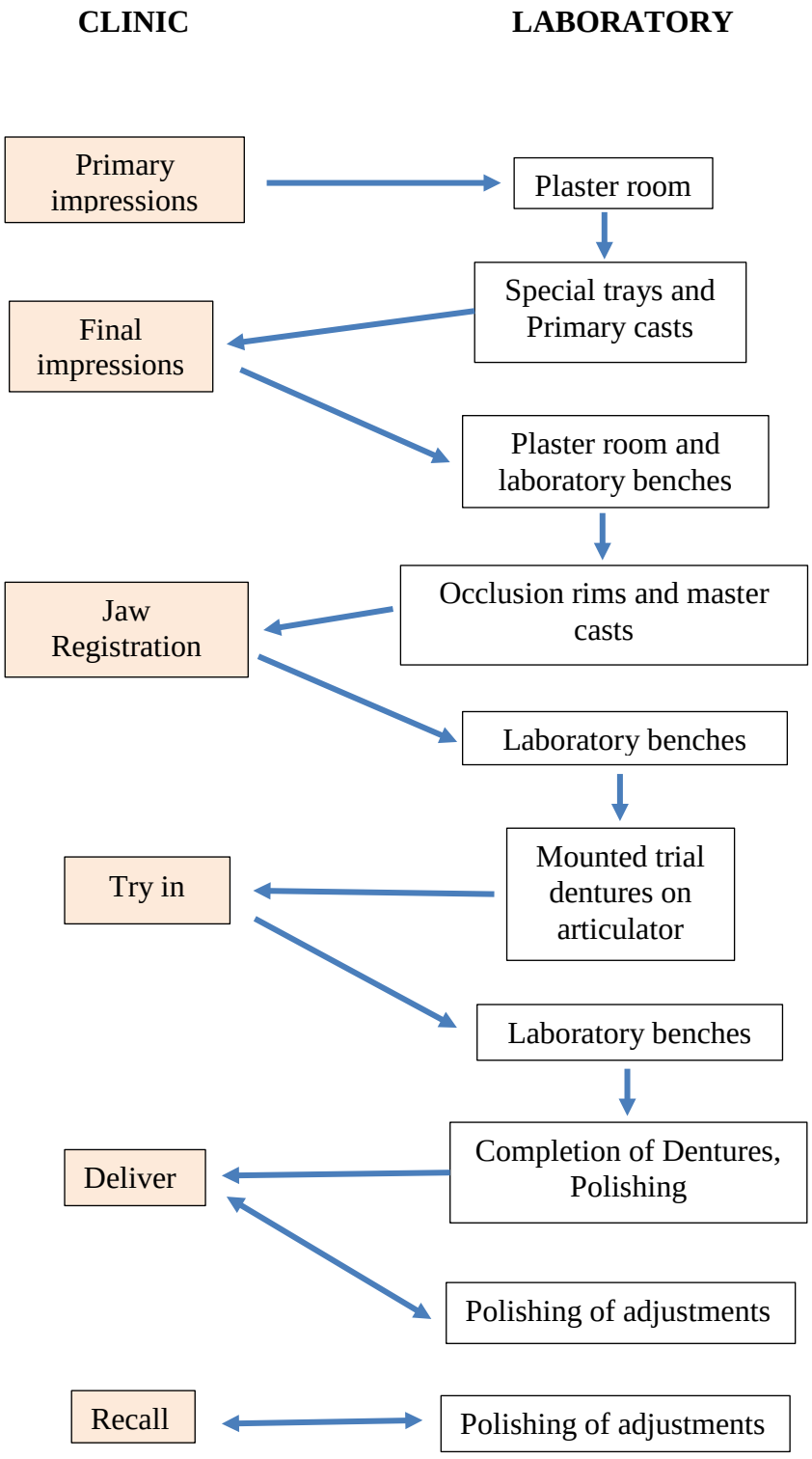

Figure 1.1 A flowchart representing the process of denture fabrication.

1.6. Current protocol

The current infection control protocol at the Wits Oral Health Centre recommended by the Department of Oral Rehabilitation is for impressions and prostheses to be carefully rinsed by placing under running tap water, and to be disinfected by immersing in a 48 ppm solution of chlorine dioxide for a maximum of 3 minutes. The use of chlorine dioxide was based on the studies by Rweyedela et al., (2009) and Patel et al., (2012). In addition the protocol recommends disinfection at each stage of fabrication and for the articulator to be wiped down with surface disinfectant before being transported to the laboratory. This protocol does not include disinfection of items from the laboratory or a laboratory protocol for disinfection. However, this protocol is currently not followed as the hospital is now using Germicide 3 (Germiphene, Canada). No studies have yet been carried out using Germicide.

The laboratory at the Wits Oral Health Centre has no specific or written infection control protocol as it is assumed that everything coming from the clinics is adequately disinfected. However, a protocol does exist that does not allow clinicians to wear gloves that were used in the clinics, no open shoes are to be worn, and a white coat must be worn at all times. When using cutting equipment, protective glasses are to be worn.

1.7. Purpose

No research has been done to assess the level of cross contamination between the clinic and the laboratory. This knowledge is important because it will guide the hospital and laboratory with regard to infection control education and reinforcement. In addition it will protect students, staff and patients from cross contamination.

CHAPTER 2: AIMS AND OBJECTIVES

2.1. Aim

1. To establish whether any cross-contamination between the dental clinic and dental laboratory during the procedures for denture fabrication occurs.
2. To determine if the disinfecting solution currently in use is effective.

2.2. Objectives

1. To establish the presence of microorganisms on multiple surfaces / items at each stage in the fabrication of dentures as well as prior to and after disinfection procedures in the clinic.
2. To study the presence of microorganisms in a variety of sites and equipment in the dental laboratory.
3. To test the current disinfecting solution used in the clinic.

2.3. Null Hypotheses

1. There is no cross contamination between the clinic and the laboratory.
2. The disinfectant currently in use is not effective

CHAPTER 3: MATERIALS AND METHOD

3.1. Study population

Ethical approval was obtained from the Human Research Ethics Committee of the University of the Witwatersrand and the clearance certificate number is M170604 (Appendix A).

Patients who attended the Prosthodontics clinics at Wits Oral Health Centre were asked to provide informed consent for swabbing of their existing dentures or saliva (if they had no previous dentures) and of the stages of the fabrication of their new dentures. Patients who had existing oral pathologies were not included in the study. Although 16 patients participated in this study only 5 patients were followed through from the commencement of fabrication to the end stage of denture construction and it was not possible to follow11 patients through all the stages. There were multiple reasons for this including timetable changes (whereby students did not inform me of the change), patients that required extensive work up before the prosthesis could be constructed, and patients who absconded treatment.

3.1.1 Sample size

For the comparison of levels of contamination between the laboratory sites the detection of a medium effect size (w=0.3), should it exist, with 80% power, at the 5% significance level, would require a minimum sample size of 273 (25 per site). The actual sample size of 110 allows the detection of only large effect sizes (w=0.47), should they exist.

Reducing the measure of contamination to the presence/absence of contamination requires a minimum sample size of 181. The actual sample size of 110 allows the detection of only moderate to large effect sizes (w=0.38), should they exist.

For the diffusion measurements, the detection of a small, medium, or large effect size (f = 0.1, 0.25 or 0.4 respectively) with 80% power at the 5% significance level, would require sample sizes of 1422, 231, and 93, respectively. The actual sample size of 80 is thus adequate only for the detection of large effect sizes, should they exist.

Sample size calculations were carried out in G*Power (Faul et al, 2007).

3.2. Collection of samples for Microbiology

Samples of denture debris, impressions, trial bases, primary and master casts and articulators were collected with sterile cotton tipped swabs after completion of each procedure prior to disinfection (if carried out) and then after disinfection. At least 10 samples were collected for each stage of denture manufacture.

An initial swab was taken of the existing denture or of the mucosa/saliva in cases where patients did not have previous dentures to gauge the existing microflora of the patient prior to swabbing the different stages of the denture making process.

Primary impressions were swabbed after acquisition and thereafter another swab was taken if disinfection took place. At the secondary impression stage the primary casts came from the laboratory were swabbed, as was the secondary impression immediately after acquisition and after disinfection if carried out. At the jaw registration stage, the final casts and occlusion rims from the laboratory were swabbed. In addition, the final casts and occlusion rims were swabbed after the jaw registration procedure and the occlusion rims were swabbed after disinfection, if it took place.

At the try in stage, the casts, articulator and trial dentures from the laboratory were swabbed. Thereafter at the try in procedure the casts, articulator and trial dentures were swabbed again and the trial dentures were swabbed if they were disinfected before sending to the laboratory. At the delivery stage, the articulator, remount models and the finished denture from the laboratory were swabbed. The dentures were swabbed again before being taken for polishing with pumice and again if disinfected. Thereafter they were swabbed after they had been polished and again if they were disinfected before final delivery to the patient. At the recall stage, a swab was taken of the fitting surface of the dentures.

Similarly, laboratory surfaces and equipment were swabbed for 10 days. In the laboratory the following were swabbed: the impression collection area, the two pumice brushes, the pumice, the grinding wheel and the working surface of each laboratory technician.

Samples were processed in the Oral Microbiology laboratory within an hour of collection under normal laboratory procedures that ensured no contamination.

3.3. Microbiological analysis

Microbiological analysis was performed using a technique described by Matthews and Patel (2018) with modification, to isolate and identify common oral aerobic bacteria and *Candida species*. Swabs were cultured onto Blood agar (BA) for total bacterial count, Mitis Salivarius agar (MSA) for total Streptococci, Rogosa agar (RA) for Lactobacilli, Baird Parker media (BP) for Staphylococci, MacConkey agar for aerobic gram negative bacteria (AGNB) and Sabouraud agar (SAB) for *Candida species* . MSA and RA were incubated at 37^{o}C for 48 hours under CO_2, whereas BA, BP, MacConkey and SAB were incubated at 37^{o}C for 48 hours aerobically. On BA all the colonies were graded as + (1-10 colonies), ++ (11-100 colonies) or

+++ (101 and above) (Figure 3.5). Similarly blue colonies on MSA (Figure 3.6), black colonies with halo on BP (Figure 3.4), white colonies on RA (Figure 3.1), pink colonies on MacConkey (Figure 3.3) and creamy colonies on SAB (Figure 3.2) were read and recorded.

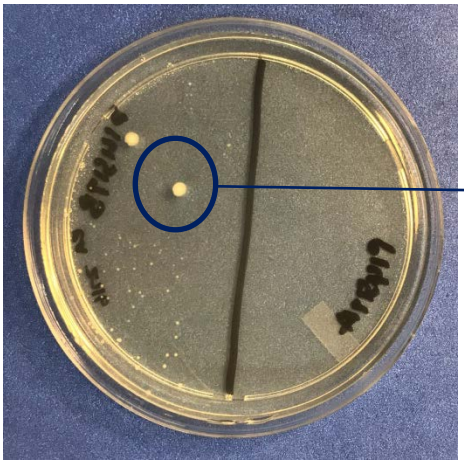

Figure 1 Rogosa agar plate with white colonies of Lactobacilli.

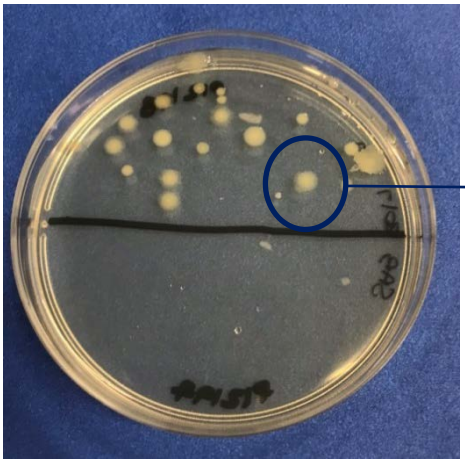

Figure 2 Sabouraud agar plate with white creamy colonies of Candida species.

Figure 3 MacConkey agar plate with pink colonies of aerobic gram negative bacteria.

Figure 4 Baird Parker agar plate with black colonies with halos of *S. aureus*.

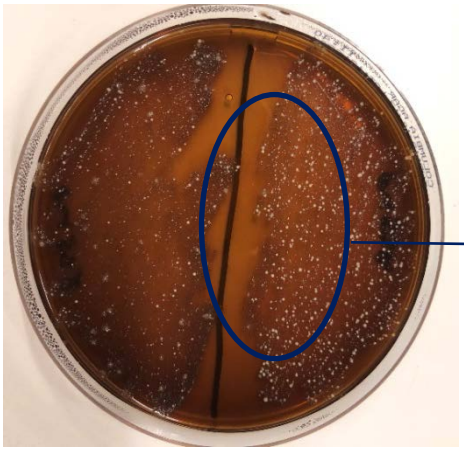

Figure 5 Blood agar plate with mix colonies of various bacteria.

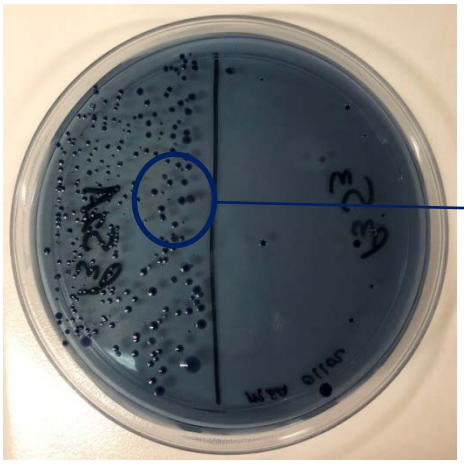

Figure 6 Mitis Salivarius agar plate with blue colonies of Streptococci species.

3.4. Efficacy of disinfectants

The antimicrobial activity of Germicide 3 and chlorhexidine were determined using the disc diffusion test (Baygar et al, 2017). The test microorganisms were *Candida* ATCC 90027, *S. aureus* ATCC25923, and clinical isolates of *S. mutans* and Lactobacilli.

For each experiment, fresh subcultures were used. Throughout the experiments, for *Candida* Sabouraud agar was used, for Lactobacilli, Rogosa agar and for *S. mutans* and *S. aureus,* blood agar was used.

For the disc diffusion test, cultures were suspended in phosphate buffered saline to obtain an optical density of 0.2, and spread onto appropriate agar plates using a swab. Paper discs (3 mm) impregnated with test disinfectant were placed onto seeded agar plates with even spacing. Plates with *S. aureus* were incubated aerobically at 37^{o}C for 24 hours. Plates with *S. mutans* and Lactobacilli were incubated under CO_2 at 37^{o}C for 48 hours. Plates with *Candida* were incubated aerobically at 37^{o}C for 48 hours. Water as a negative was also included. These experiments were repeated 10 times. A zone of inhibition was measured to determine the efficacy of the disinfectant.

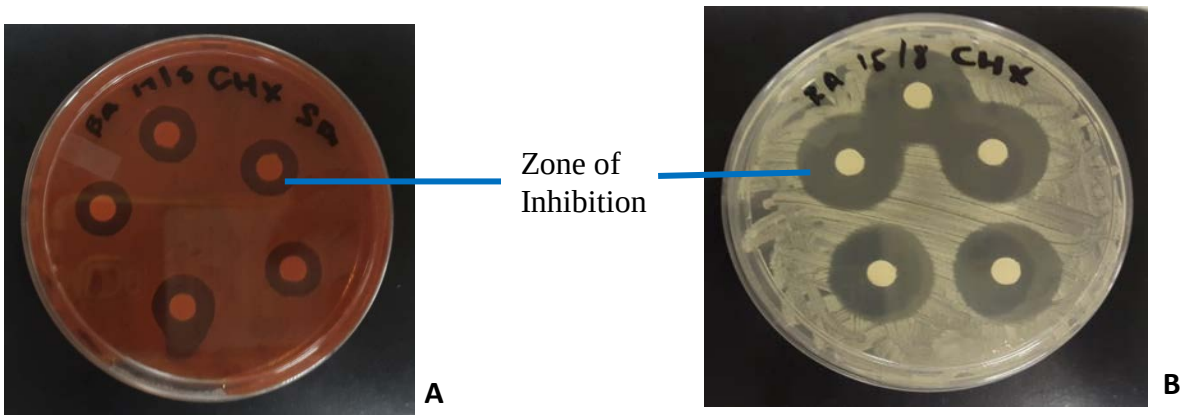

Figure 7 Agar plates with culture and zone of inhibition. A: Blood agar, B: Rogosa agar plate.

3.5. Statistical Analysis

3.5.1 Clinical Study

The microbiological results obtained before and after disinfecting during the different stages and the results obtained from samples going to and coming from the lab were descriptively

analysed, using both quantitative and qualitative analyses. The quantitative results helped establish the level of contamination and the qualitative study helped establish the presence / absence of contamination. The presence and absence of contamination was determined by a Chi-squared test.

3.5.2 Laboratory Study

Microbiological results were descriptively analysed, using both quantitative and qualitative analyses. The quantitative results helped establish the level of contamination and the qualitative study helped establish the presence / absence of contamination. The presence and absence of contamination was determined by a Chi-squared test. Fisher's exact test was used for 2 x 2 tables or where the requirements for the Chi-squared test could not be met. The strength of the associations was measured by Cramer's V and the phi coefficient respectively. The following scale of interpretation was used:

0.50 and above	high/strong association
0.30 to 0.49	moderate association
0.10 to 0.29	weak association
below 0.10	little if any association

3.5.3 Disinfectant efficacy

Microbiological result were analysed using a quantitative analysis. The determination of the effect of bacteria and disinfectant on diffusion measurement required a two-way Analysis of Variance (ANOVA) with the factors being bacteria and disinfectant, and their interaction. Data analysis was carried out using SAS (version 9.4 for Windows). The 5% significance level was used.

CHAPTER 4: RESULTS

4.1 Clinical Study

4.1.1 Level of contamination in the clinic

There were 239 samples available for analysis, and whilst not all sites were equally represented, it was decided to view them collectively (Figure. 4.1). Not surprisingly, 87% of the samples showed mixed flora. Streptococci had the highest prevalence (71.5%), with a cell count of >100 cfu/ml (32.6%) and the least prevalent microorganism was Lactobacilli (11.7%). In addition all test microorganisms sometimes exceeded 100 cfu/ml, except for *S. aureus*.

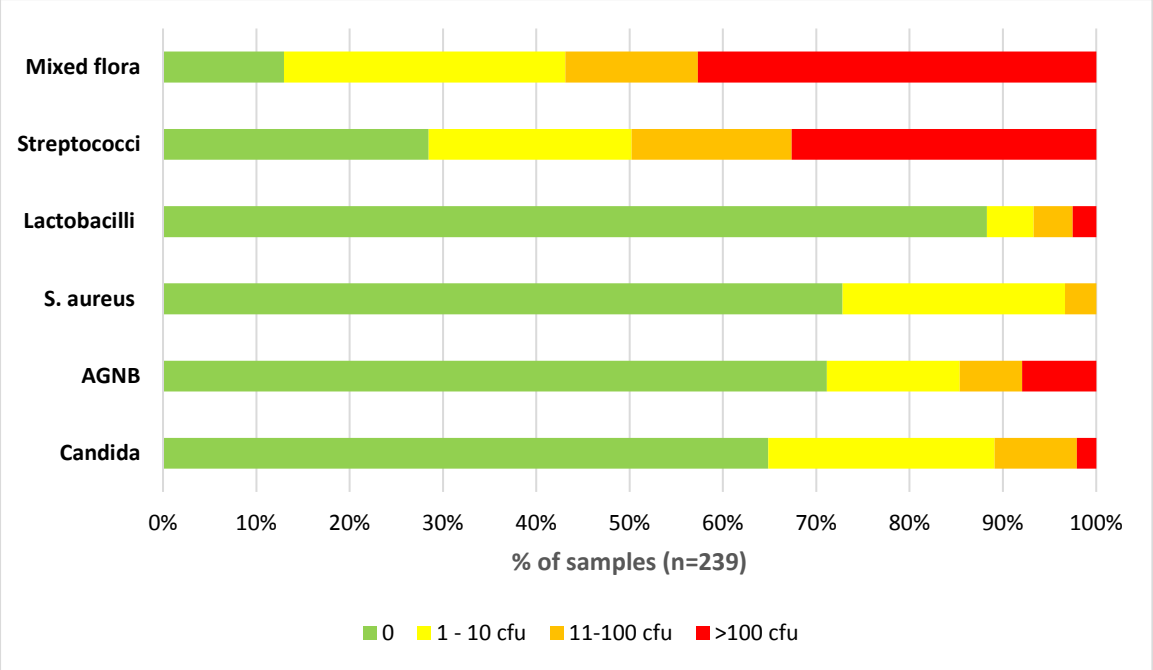

Figure 8 A comparison of the total counts of the test microorganisms at all stages of denture construction

4.1.2 Effect of disinfection at each clinical stage

The results of the microorganisms at the primary impression stage are shown in Figure 4.2. Before disinfection there was a presence of all test microorganisms Streptococci were the most prevalent (100%) and *Candida* the least prevalent (30%). Not all impressions were disinfected, but in the 7 that were, there was a decrease in all test microorganisms, with Lactobacilli and *S. aureus* absent after disinfection.

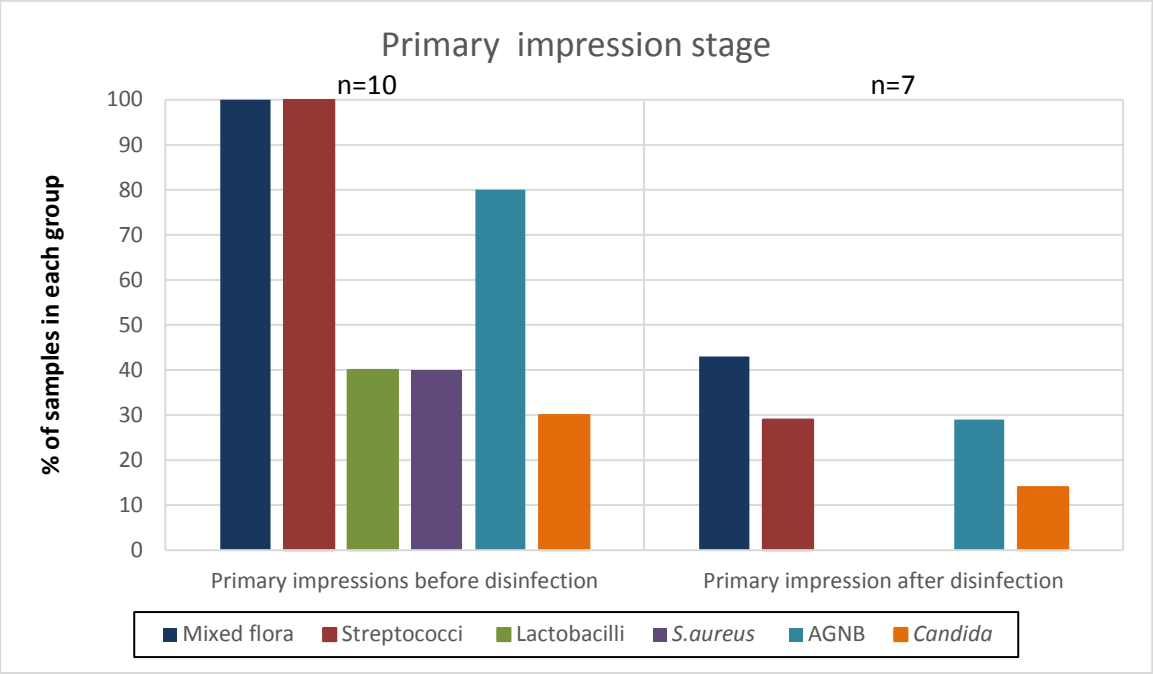

Figure 9 A comparison of the presence of microorganisms at the primary impression stage before and after disinfection.

The results of the microorganisms at the secondary impression stage are shown in Figure 4.3. The microbiological results of the primary casts from the laboratory show a presence of all test microorganisms except Lactobacilli. Streptococci, AGNB and *Candida* are equally prevalent (70%). The final impression before disinfection had a presence of all test microorganisms with mixed flora and Streptococci being most prevalent (90%) and AGNB being least prevalent (10%). After disinfection there was a decrease of most microorganisms, but an increase in AGNB (17%)

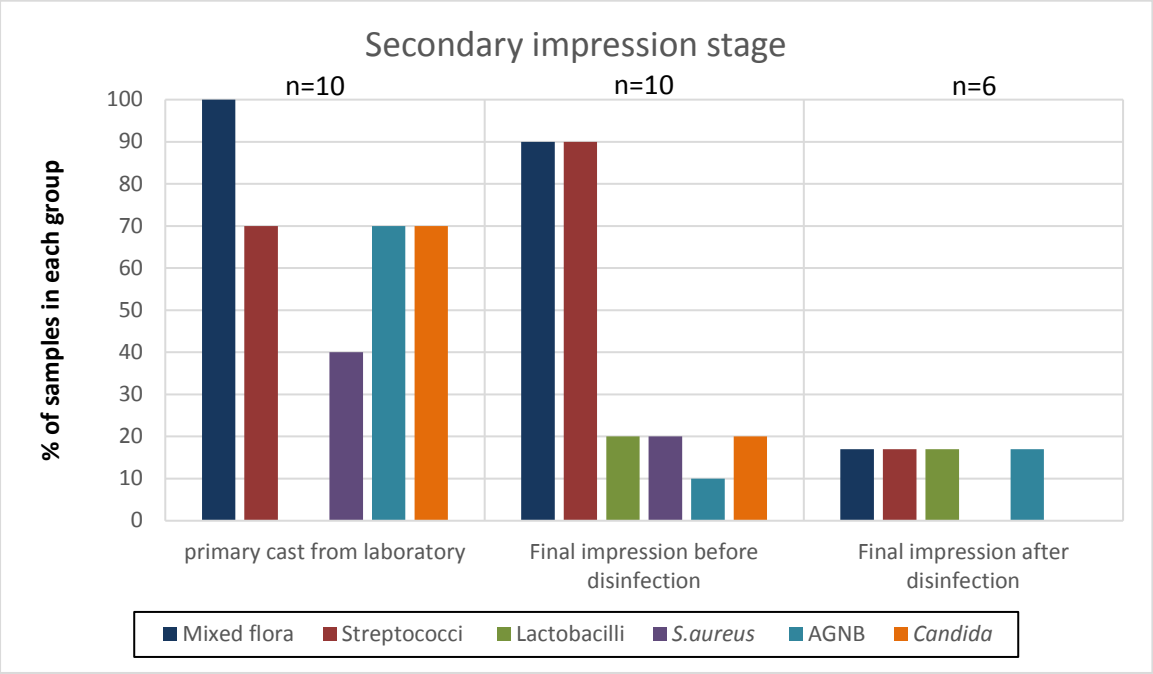

Figure 10 The presence of contamination at the secondary impression stage including the laboratory work and the secondary impressions before and after disinfection.

The results of the microorganisms at the jaw relation registration stage are shown in Figure 4.4. It was found that all the final casts from the laboratory were contaminated with mixed flora. Streptococci were more prevalent (70%) and Lactobacilli were absent. Half of the trial bases were contaminated with mixed flora. Streptococci were most prevalent (40%) and Lactobacilli and *S. aureus* were absent.

After carrying out the jaw relations procedure, all the trial bases contained mixed flora with Streptococci most prevalent (60%) and Lactobacilli least prevalent (30%). The bases after disinfection showed a decrease of all microorganisms. As the trial bass are often placed on the final casts during jaw registration, all of them then had mixed flora with Streptococci most prevalent (90%) and Lactobacilli least prevalent (10%)

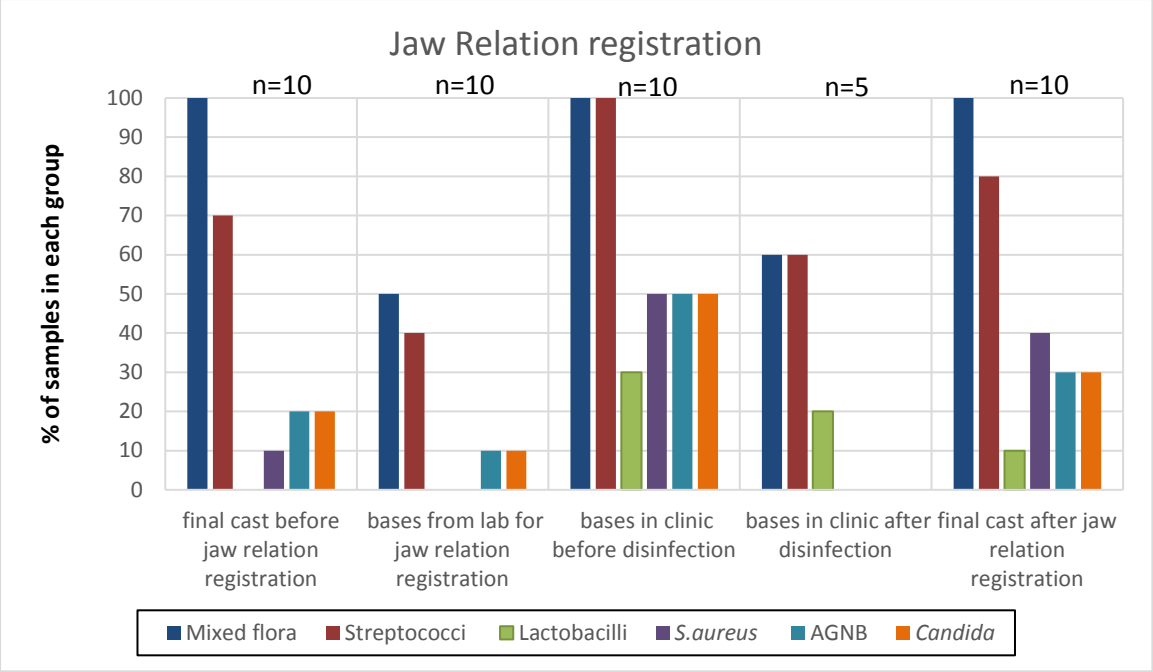

Figure 11 The presence of contamination at the jaw relation registration stage including the laboratory work and trial bases before and after disinfection.

The results of the microorganisms at the try in stage are shown in Figure 4.5. At the try in stage, 90% of the work coming from the laboratory (articulator, bases and casts) had mixed flora but Lactobacilli were absent. After the try in procedure the trial bases had all microorganisms present the most prevalent being mixed flora and Streptococci (100%), and the least prevalent being Lactobacilli and AGNB (20%). After disinfection the only microorganisms which remained were mixed flora (67%) and Streptococci (33%). All the final casts after try in, contained mixed flora followed by Streptococci (70%) and Lactobacilli was absent. *Candida* had the same presence on the final cast as well as on the trial bases before disinfection (50%). The articulator after use in the clinic had 70% of the samples containing mixed flora, most prevalent Streptococci (60%) and an absence of Lactobacilli.

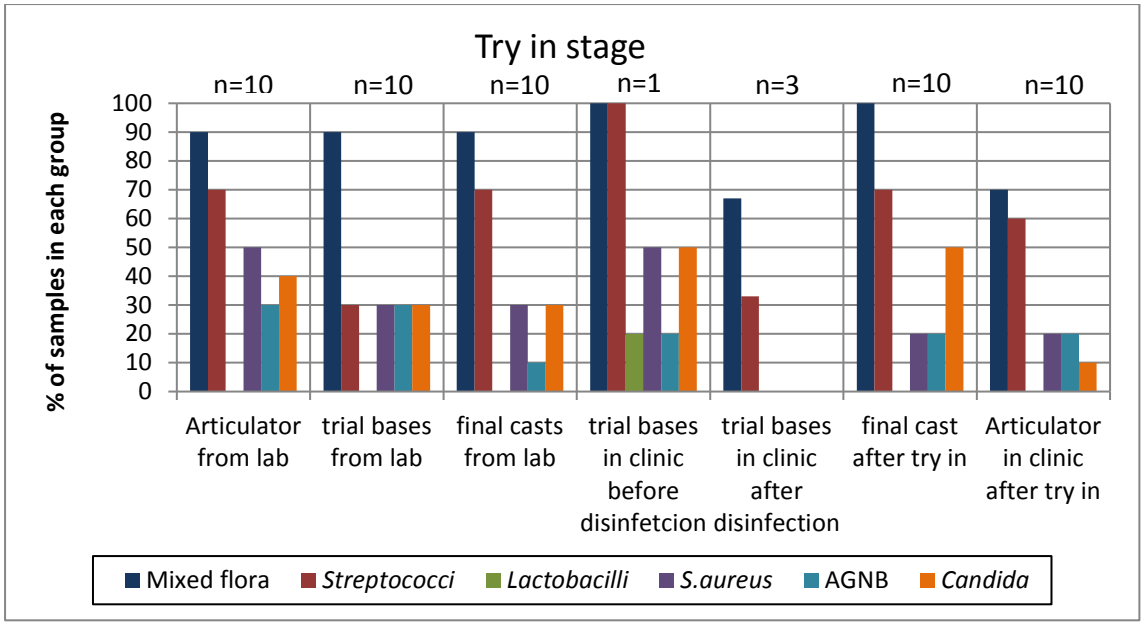

Figure 12 The presence of contamination at the try in stage

The results of the microorganisms at the delivery stage are shown in Figure 4.6. Lactobacilli were absent from the work coming from the laboratory. Of the final dentures 90% contained mixed flora and all the remount casts had mixed flora with Streptococci most prevalent (40%). The final denture before polishing had all test microorganisms with 100% mixed flora and Streptococci, 60% of *Candida* and 30% the remaining microorganisms. After disinfection the microorganisms were decreased and Lactobacilli, *S. aureus* and *Candida* were absent. The remount cast in the clinic had 90% mixed flora, 80% Streptococci, 20% *Candida* , 10% *S. aureus* and AGNB and an absence of Lactobacilli. The final denture after polishing in the laboratory before disinfection had 100% presence of mixed flora and Streptococci, 80% *Candida*, 30% of *S. aureus* and AGNB and an absence of Lactobacilli. After disinfection there was a decrease in the microorganisms.

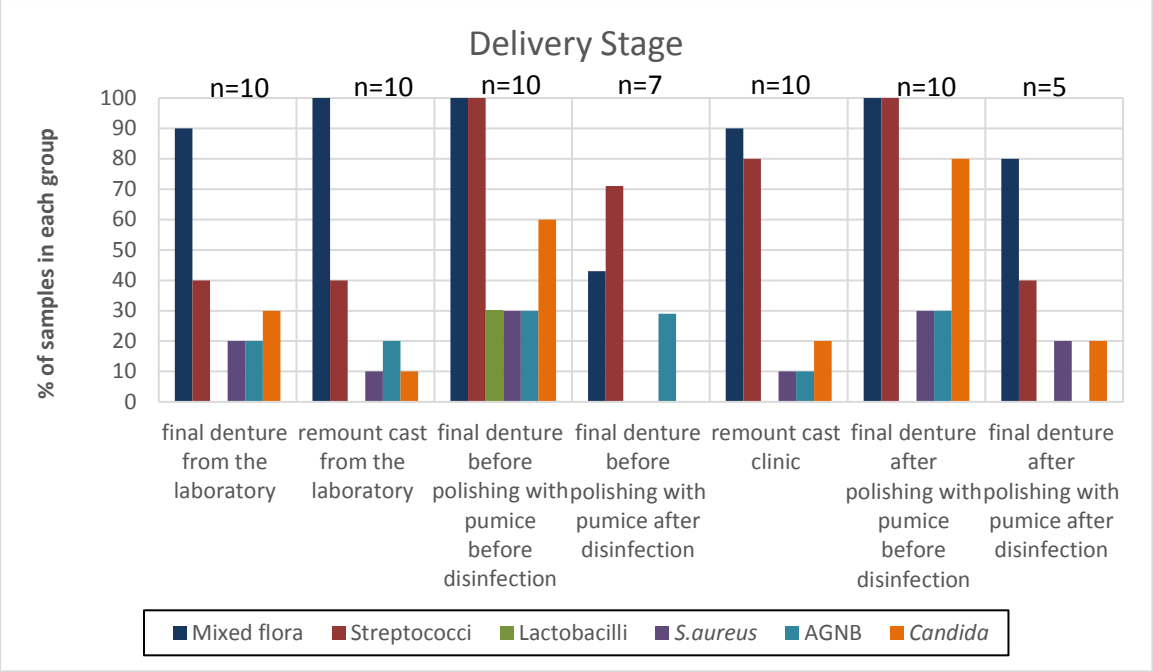

Figure 13 The presence of contamination at the delivery stage

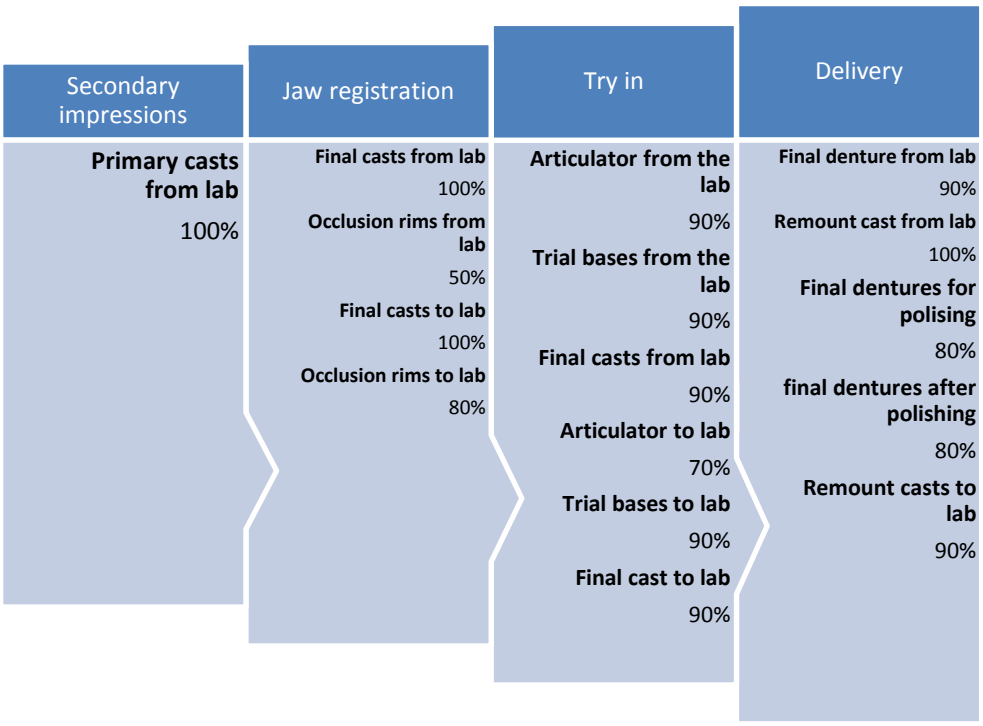

Figure 14 A summary of the overall presence of microorganisms (mixed flora) at each stage of denture construction coming and going to the laboratory

4.1.3 Efficacy of disinfection in the clinic

In order to compare the presence or absence of microorganisms before and after disinfection, all 6 sites of testing were considered together, because of the low sample sizes. The results are shown in Figure 4.8 and Table 4.1. The effect sizes were strong for mixed flora and Streptococci; these had the highest prevalence of organisms before disinfection, and showed the greatest decrease. There were significant reductions in the number of samples containing all the organisms with p values ranging from 0.045 to <0.01 (Table 4.1). Overall, no organisms were eradicated completely, although in some cases with some organisms this did occur as detailed in the previous section.

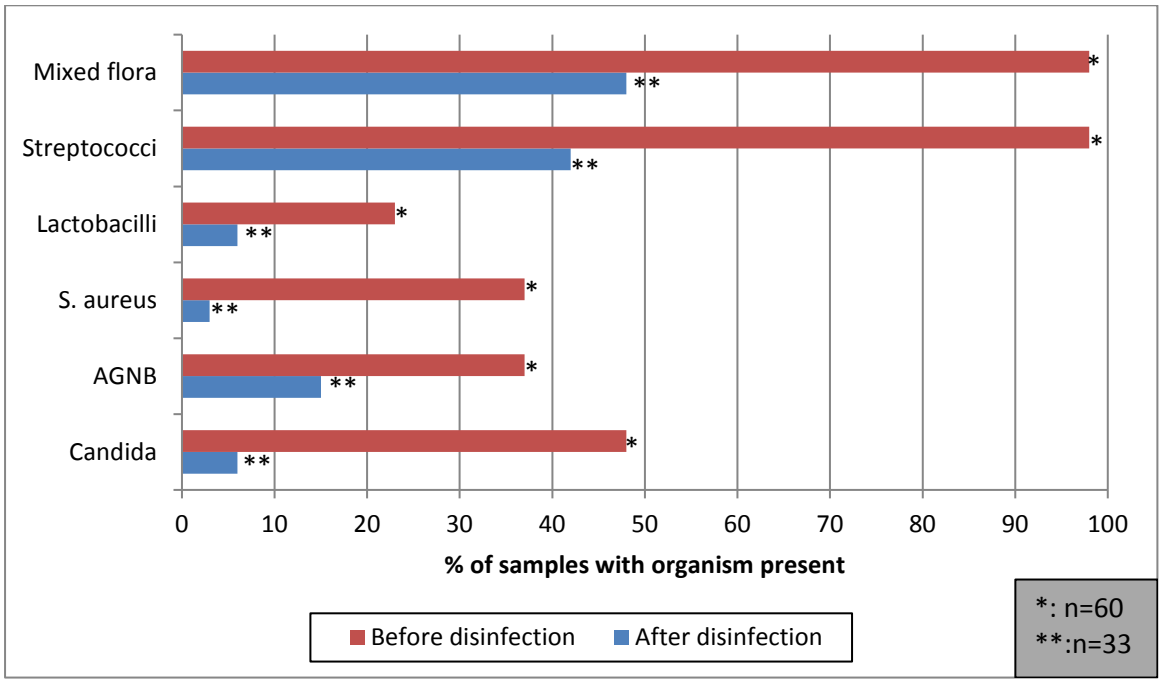

Figure 15 The presence of microorganisms before and after disinfection of all stages of denture construction

Table 4.1 The p values of the microorganism before and after disinfection

Microorganism	Comparison		P value
	Before disinfection (% of samples)	After disinfection (% of samples)	
Mixed flora	98	48	<0.01
Streptococci	98	42	<0.01
Lactobacilli	23	6	0.045
S. aureus	37	3	0.0003
AGNB	37	15	0.033
Candida	48	6	0.0001

The results for each test microorganism before and after disinfection are shown in Figures 4.9 - 4.14.

For the mixed flora, the reduction due to disinfection in samples containing >100 cfu/ml was from 67% to 18%. Similarly for 11- 100 cfu/ml the reduction was 20% to 9% (Figure 4.9). The reduction in number of samples with high counts was significant (p<0.0001).

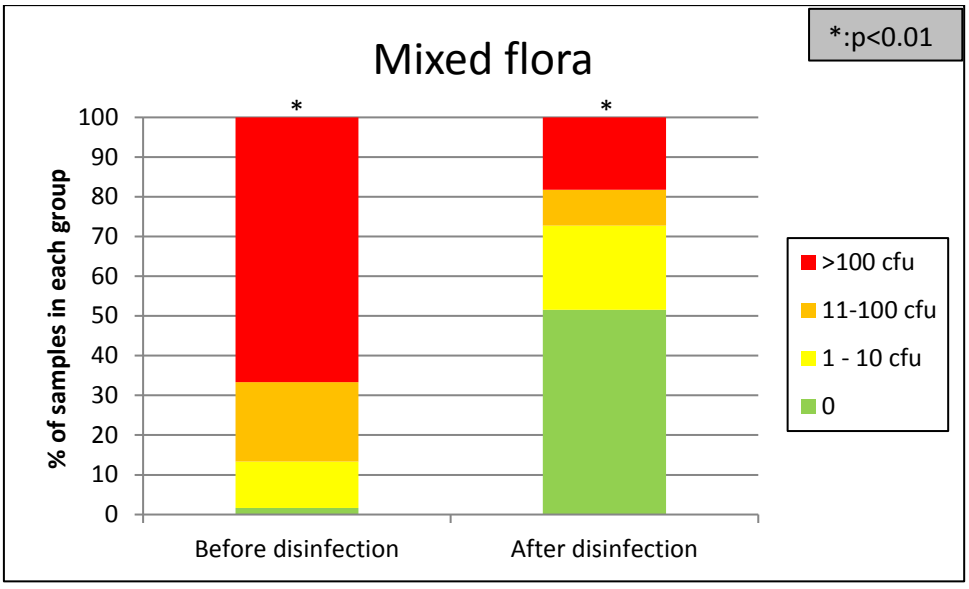

Figure 16 A comparison of the total cell count of mixed flora before and after disinfection.

For the Streptococci, the reduction due to disinfection in samples with >100 cfu/ml was from 60% to 6%. Similarly for 11 - 100 cfu/ml the reduction was 25% to 9%. These reductions were significant with a p value of <0.01 (Figure 4.10).

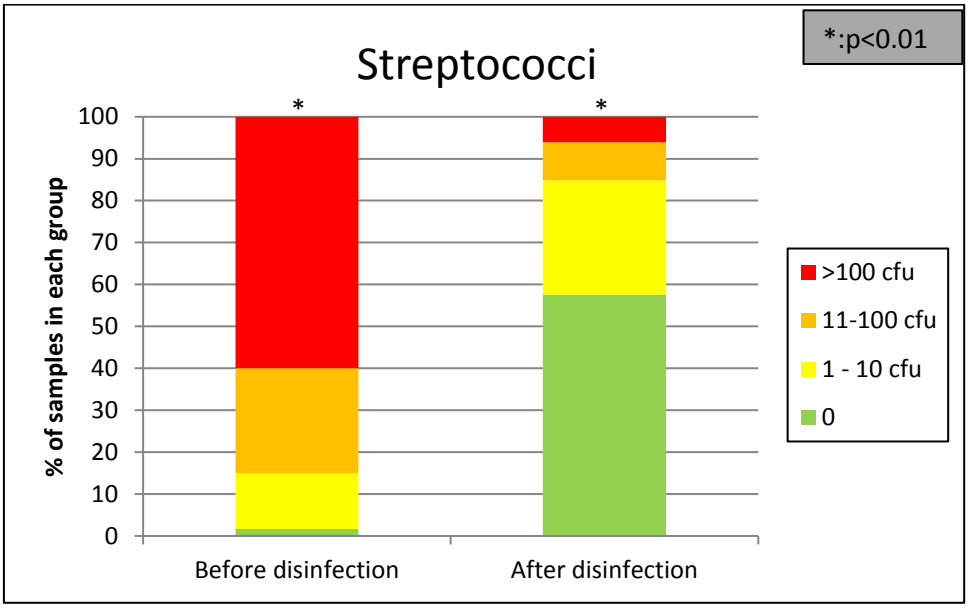

Figure 17 A comparison of the total cell count of Streptococci before and after disinfection.

Candida counts were generally lower than the other organisms. Before disinfection, 12% of samples had *Candida* of 11-100 cfu/ml which decreased to 0% after disinfection (Figure 4.11). Similarly samples with counts of 1-10 cfu/ml also decreased from 35% of samples to 6%. The reduction in microorganisms due to disinfection was significant (P=0.01).

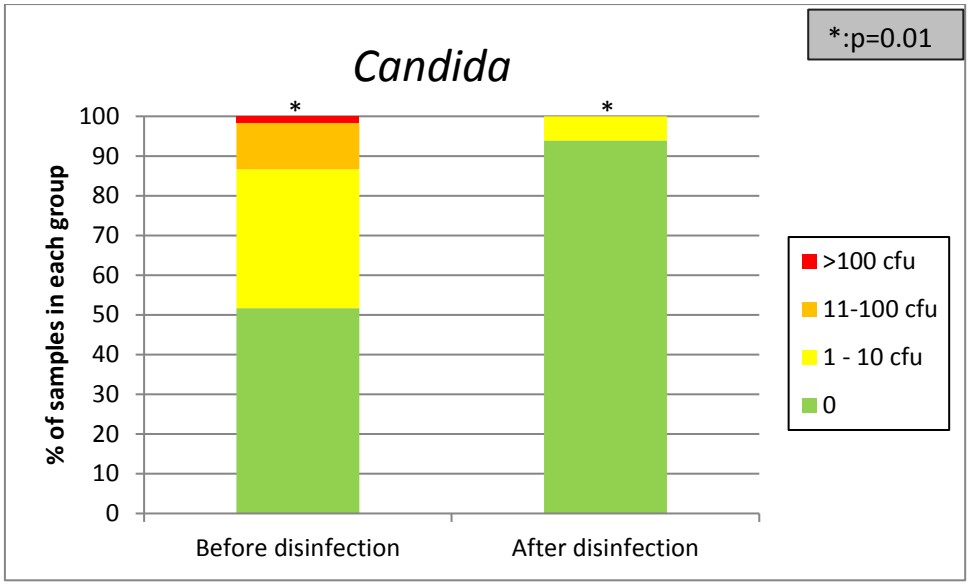

Figure 18 A comparison of the total cell count of *Candida* before and after disinfection.

Before and after disinfection, *S. aureus* counts did not exceed 100 cfu/ml (Figure 4.12). The percentage of samples with counts of 1-10 cfu/ml was reduced from 33% to 3%.

Figure 19 A comparison of the total cell count of *S. aureus* before and after disinfection.

Disinfection reduced the number of samples containing 11-100 cfu/ml of Lactobacilli from 10% to 0% and 1-10 cfu/ml from 10% to 6% which was not significant (Figure 4.13).

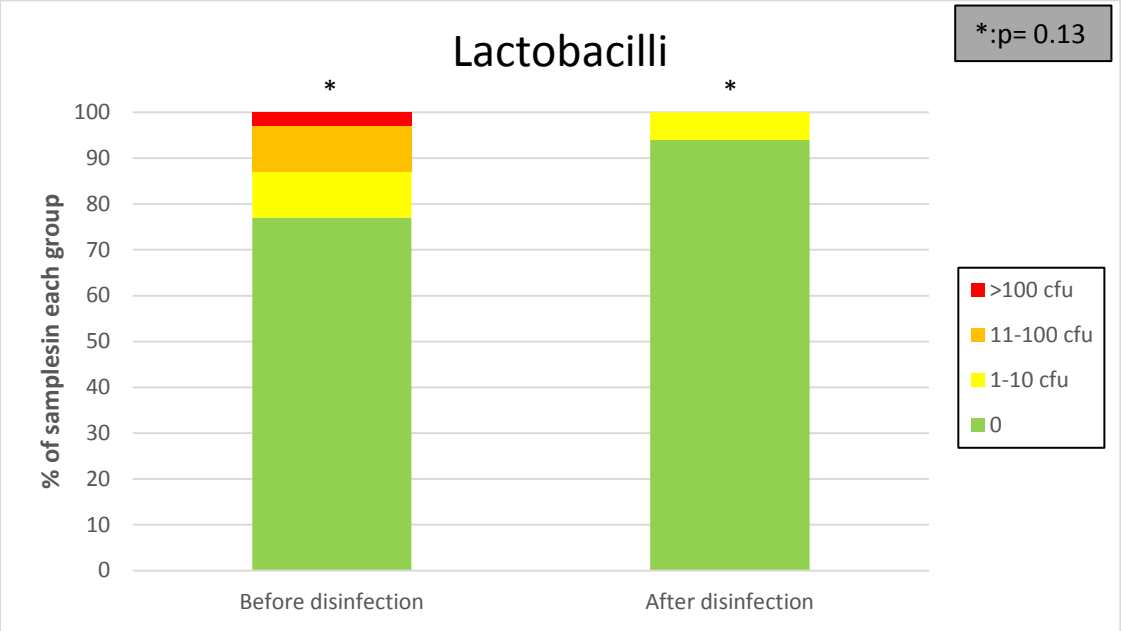

Figure 20 A comparison of the total cell count of Lactobacilli before and after disinfection.

Before disinfection when AGNB were present in 23% of samples containing 11 - >100cfu/ml, disinfection reduced it to 6% (Figure 4.14).

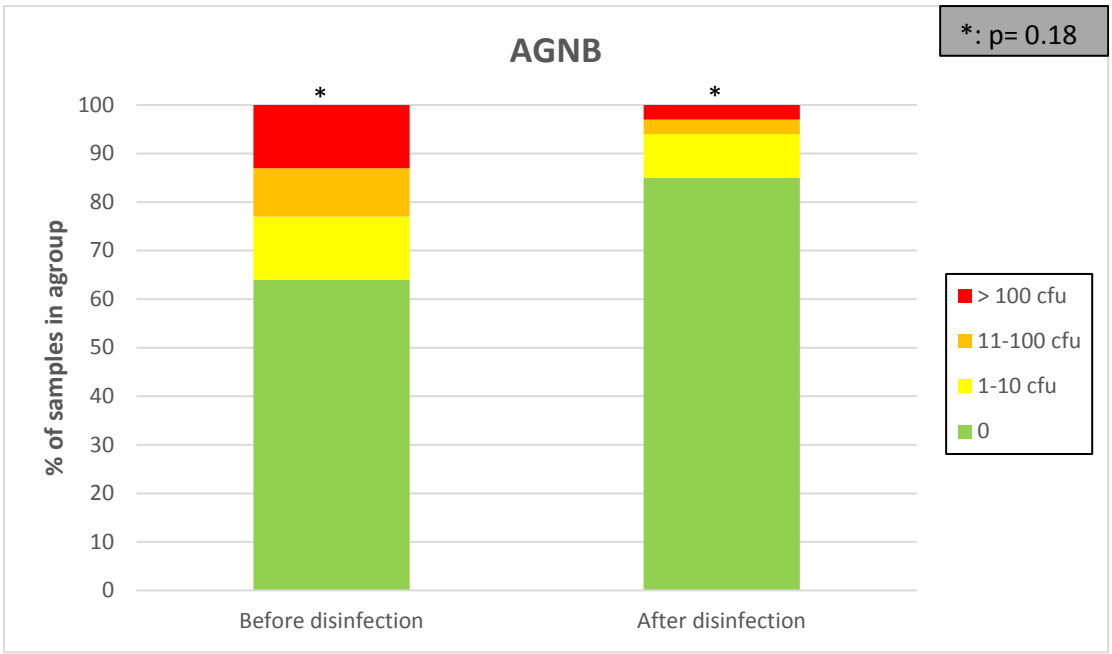

Figure 21 A comparison of the total cell count of AGNB before and after disinfection.

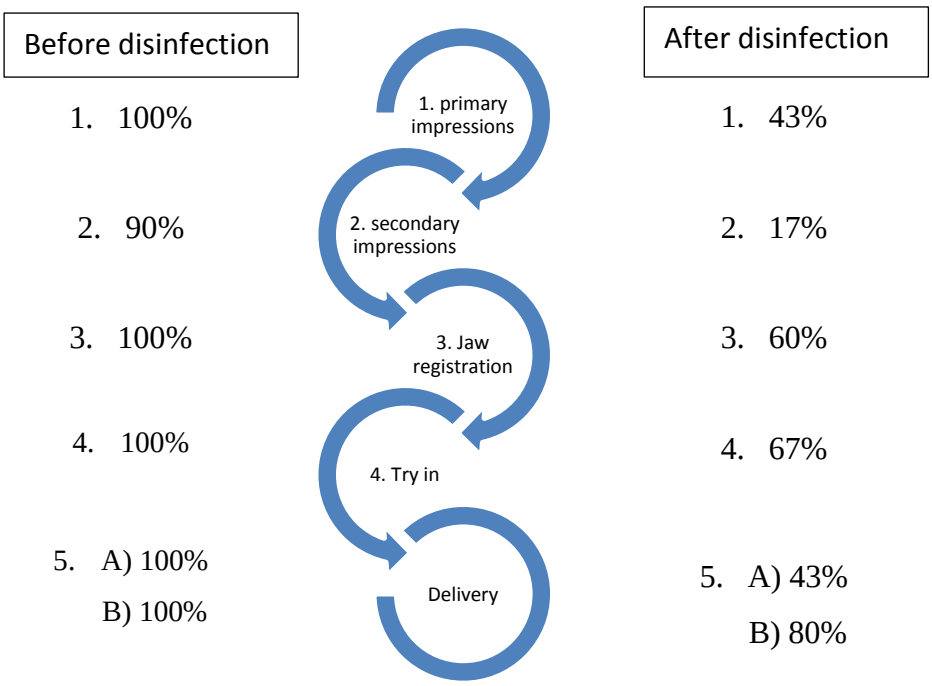

Figure 22 A summary of the reduction in the overall organisms (mixed flora) during disinfection at each stage of disinfection A) before polishing before disinfection, B) after polishing before disinfection

4.1.4 Occurrence of disinfection at each stage in the clinic

Disinfection practices varied with the stages of denture construction. Generally disinfection occurred between 30% and 70% of the time. Overall it can be observed that disinfection occurred most often at the primary impression stage (70%) and decreased as denture construction continued up to the try in stage (30%) where the least disinfection took place (Figure 4.16). At the delivery stage there were two points where disinfection could occur; before the dentures leave the clinic to go to the laboratory to be polished (70% of the time); and after the denture is polished in the laboratory and before delivery to the patient (50% of the time).

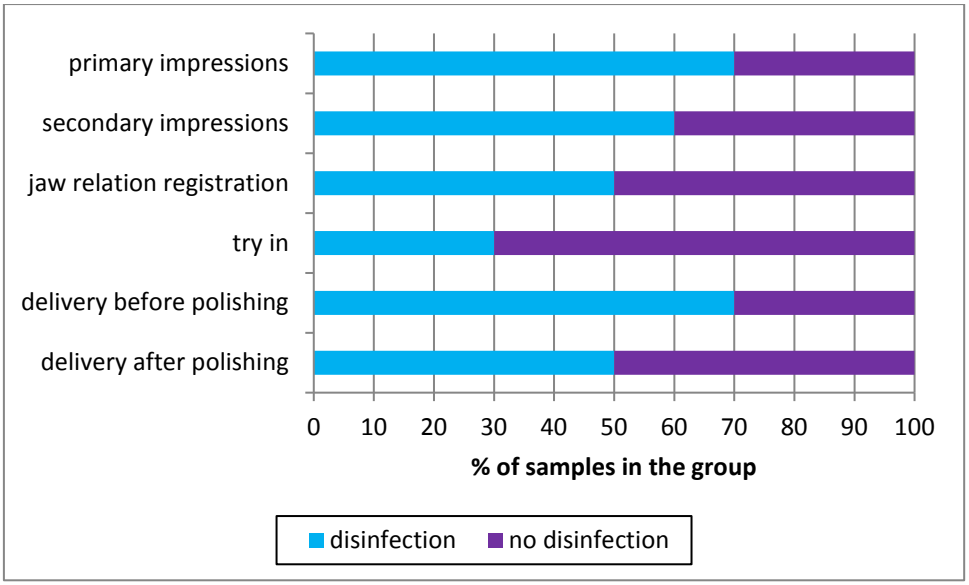

Figure 23 The occurrence of disinfection at each stage of denture construction.

4.1.5 Contamination to and from the laboratory

Mixed flora were the most prevalent in work received from the laboratory (88%) but Lactobacilli were absent (Figure. 4.17). With regards to the samples going to the laboratory, all the microorganisms were present with mixed flora being most prevalent (79%) and Lactobacilli least prevalent (10%). There was only a significant association between presence/absence of Streptococci and Lactobacilli as seen in Table 4.2

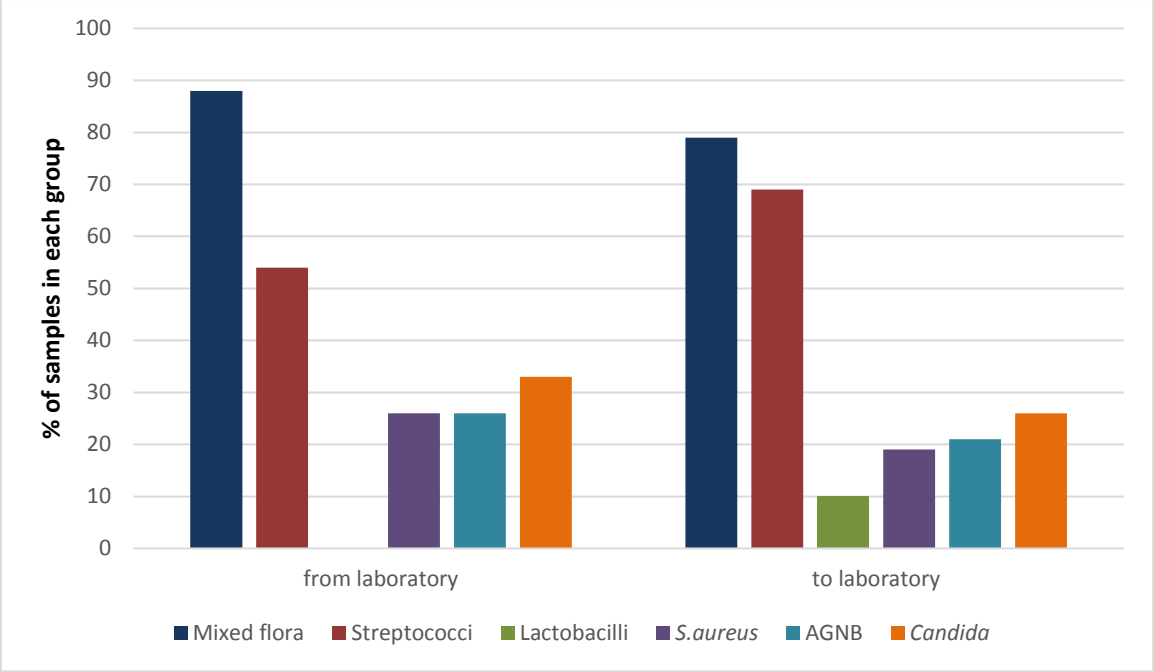

Figure 24 The overall amount of contamination that came from and went to the laboratory during the stages of denture construction.

Table 4.2 The p values of the microorganisms present coming from the laboratory and going to the laboratory.

Microorganism	**Comparison**		**P - value**
	Presence from laboratory (%)	Presence to laboratory (%)	
Mixed flora	88	79	0.17
Streptococci	54	69	0.045
Lactobacilli	0	10	0.025
S. aureus	26	19	0.28
AGNB	26	21	0.48
Candida	33	26	0.41

4.2 Laboratory Study

Ten samples had been collected and analysed from all 11 of the laboratory sites. Taking all 110 samples together, as to be expected, the most prevalent microorganisms were mixed flora. The least prevalent were Lactobacilli (Figure 4.18). When mixed flora were present, 73.3% of sites contained 11 to > 100 cfu/ml and when Streptococci were present, 45.5% of sites contained 11 to >100 cfu/ml. When AGNB were present, 19.1% of the sites contained 11- 100 cfu/ml. However for *Candida*, *S. aureus* and Lactobacilli when they were present, the counts did not exceed 100 cfu/ml.

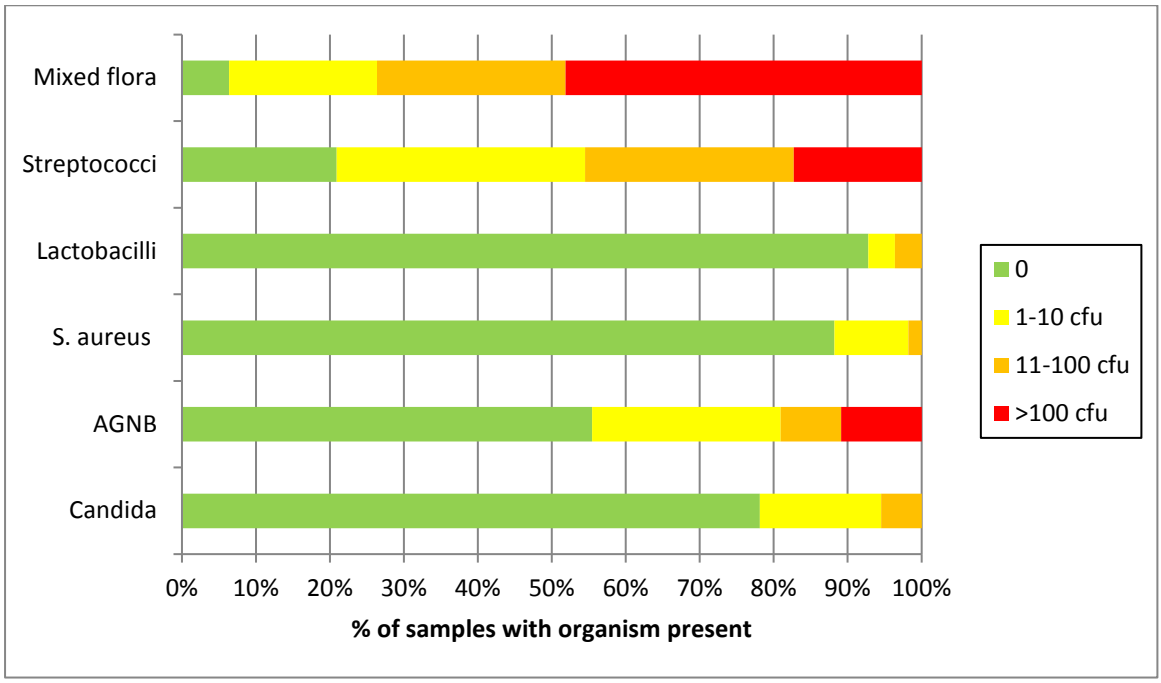

Figure 25 The total cell count of each microorganism present in all the areas of the laboratory.

Associations between bacterial count category and site could not be tested as the data in each category and the large number of sites meant that the data did not met the requirements of the appropriate tests (Chi-squared and Fisher's exact). But by collapsing the bacterial count categories into presence or absence of each organism, Fisher's exact test could be used. Then

significant associations were found at each site. The percentage of samples with organisms present are shown in Figure. 4.19 and the associations in Table 4.3.

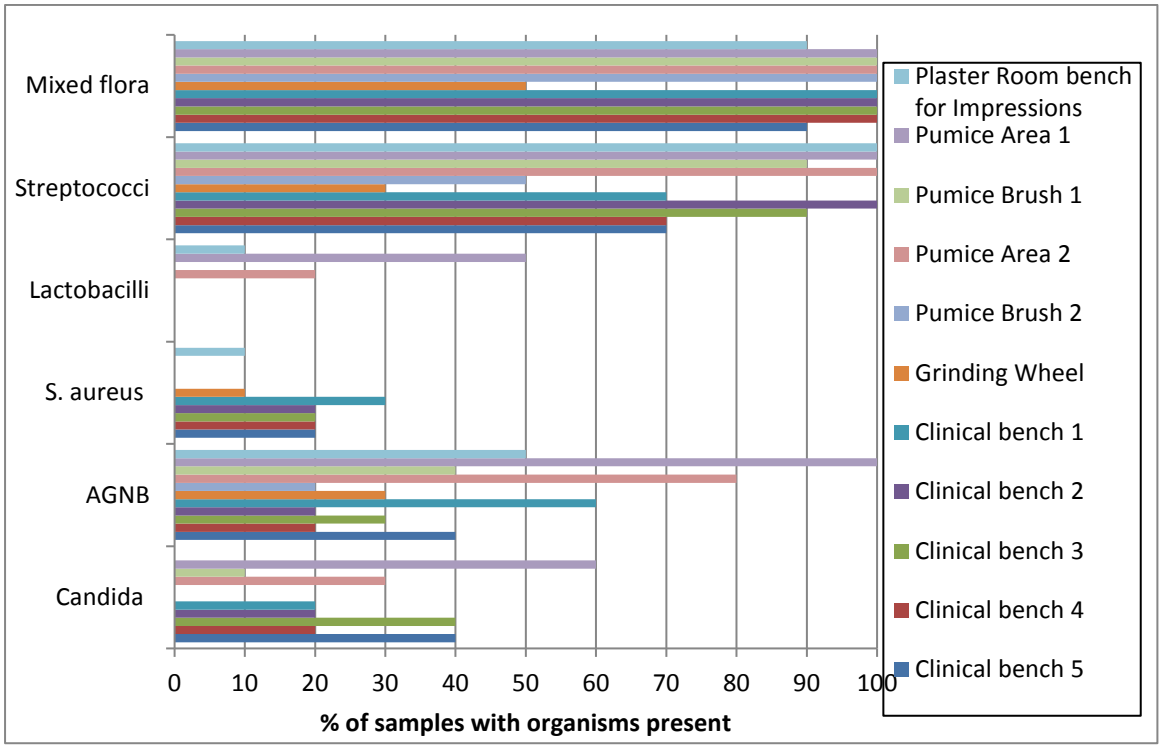

Figure 26 The percentage of microorganisms present at each site.

Table 4.3 Associations for the overall presence and absence of each microorganism in the laboratory.

Microorganism	Comparison		P-value	Effect size (phi)	Effect
	Presence (%)	Absence (%)			
Mixed flora	93.6	6.4	<0.0001	0.59	Strong
Streptococci	79.1	20.9	<0.0001	0.55	Strong
Lactobacilli	7.3	92.7	<0.0001	0.57	Strong
S. aureus	11.8	88.2	<0.0001	0.32	Moderate
AGNB	44.5	55.5	=0.0010	0.50	Strong
Candida	21.8	78.2	=0.0095	0.45	Moderate

The following significant differences were identified when comparing this prevalence with that of the other sites:

- For Mixed flora and Streptococci, the grinding wheel had a lower prevalence;
- For the AGNB, pumice Areas 1 and 2 had a higher prevalence;
- For *Candida* and Lactobacilli, pumice Area 1 had a higher prevalence;
- For *S. aureus*, the Clinical Bench 1 had a higher prevalence.

The results of the microorganisms at each site are shown in Figure 4.20. At the impression area all microorganisms were present except *Candida* with Streptococci being the most prevalent microorganism (100%) at the site. At the pumice area all microorganisms were present except *S. aureus* with mixed flora, Streptococci and AGNB being equally prevalent (100%) and followed by *Candida* (60%). At pumice brush 1 only four microorganisms were present with mixed flora being the most prevalent (100%) followed by Streptococci (90%). At pumice area 2 all microorganisms except *S. aureus* were present, with mixed flora and Streptococci being the most prevalent (100%). At pumice brush 2 only three microorganisms were present with mixed flora being the most prevalent followed by Streptococci (50%). The grinding wheel had four microorganisms present with mixed flora being the most prevalent (50%) followed by Streptococci and AGNB. At all clinical benches all microorganisms were present except Lactobacilli.). At clinical bench 1 mixed flora was the most prevalent followed by Streptococci (70%). At clinical bench 2 mixed flora and Streptococci were the most prevalent (100%) and the rest of the microorganisms were present having equally low prevalence (20%). At clinical bench 3 after mixed flora, Streptococci were the most prevalent (90%) and then *Candida* (40%). At clinical bench 4 after mixed flora, Streptococci were the most prevalent (70%) with AGNB, *Candida* and *S. aureus* having an equally low prevalence (20%). At clinical bench 5 mixed flora were the most prevalent followed by Streptococci (70%) and AGNB and *Candida* (40%).

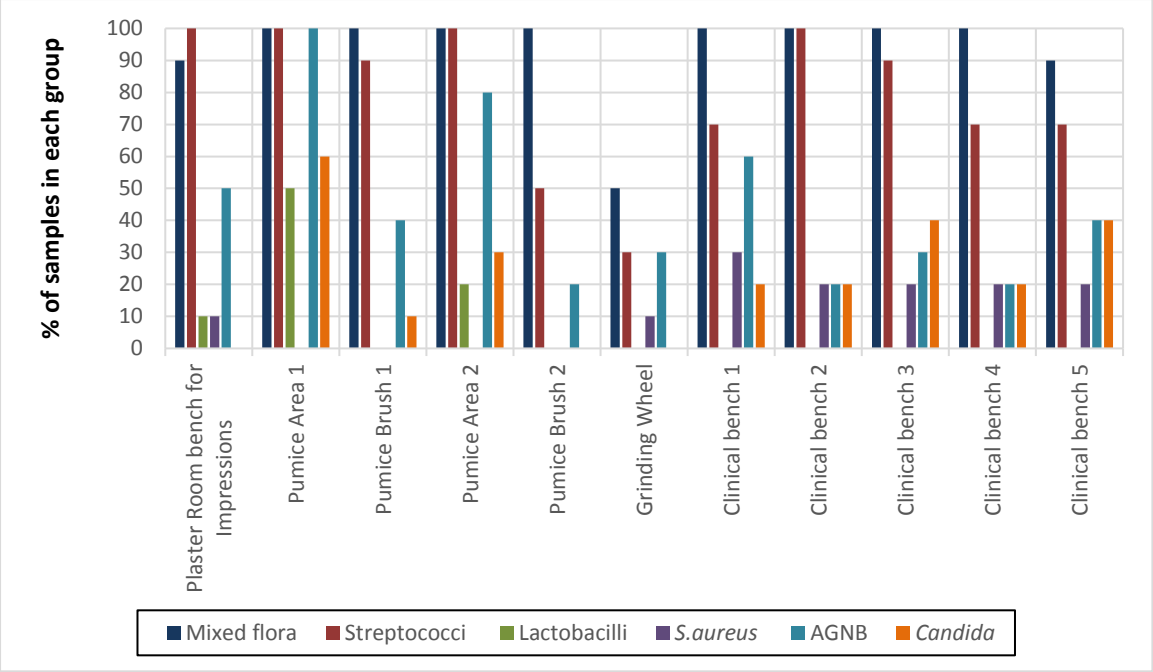

Figure 27 The various test microorganisms at each laboratory site.

When the counts of mixed flora at each site were compared, the samples in all sites ranged from 20 to 100% contamination at >100 cfu/ml (Figure 4.21).

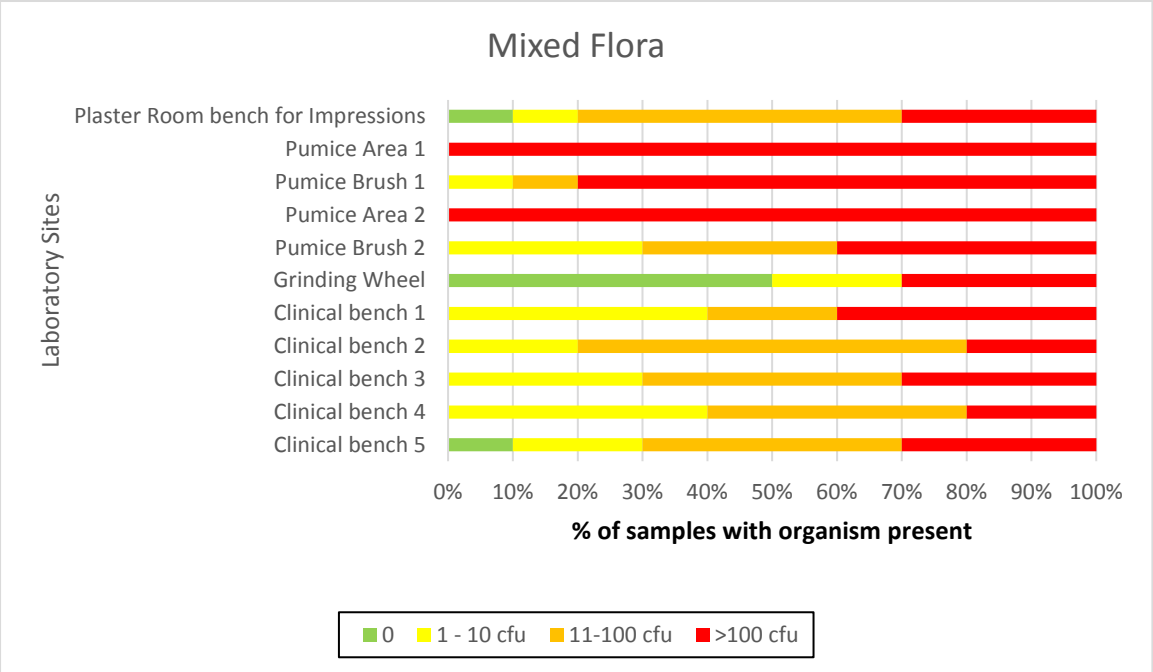

Figure 28 The total cell count of mixed flora at different sites in the laboratory.

Streptococci were present at all of the laboratory sites (Figure 4.22). At pumice area 1 there was a very high total cell count never being below 10 cfu/ml, however at the grinding wheel which is least prevalent (30%) it did not exceed 10 cfu/ml. In addition at pumice area 1& 2 the total cell count exceeded 100cfu/ml 50% of the time.

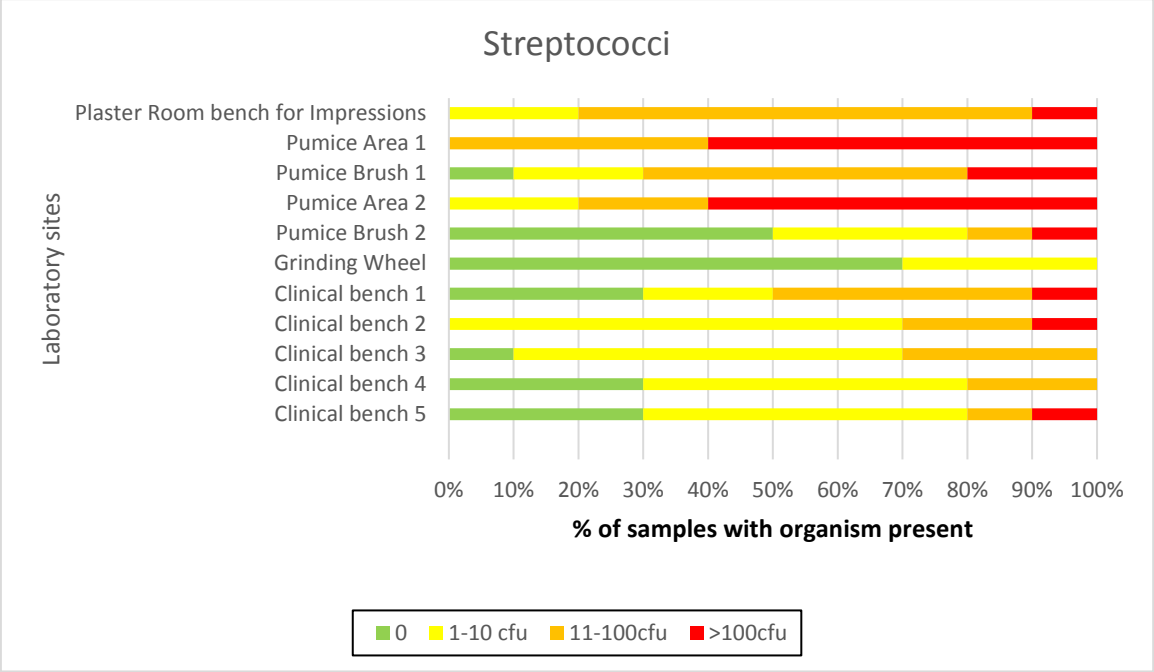

Figure 29 The total cell count of Streptococci at various sites in the laboratory.

Lactobacilli were largely absent and when it was present did not exceed 100 cfu/ml. At pumice area 1 it was most prevalent (50%). (Figure 4.23)

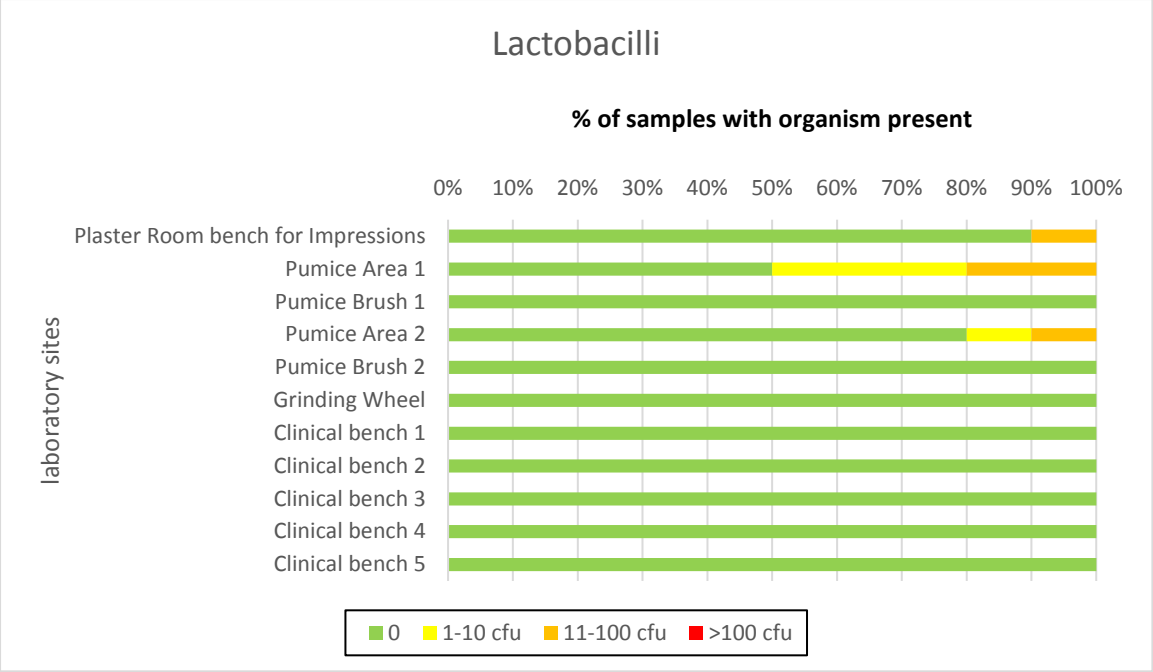

Figure 30 The total cell count of Lactobacilli at various sites in the laboratory

S. aureus were also largely absent, when it was present it did not exceed 10 cfu/ml except at clinical bench 1 and 3 where only 10% of the samples ranged from 11-100 cfu/ml (Figure 4.24).

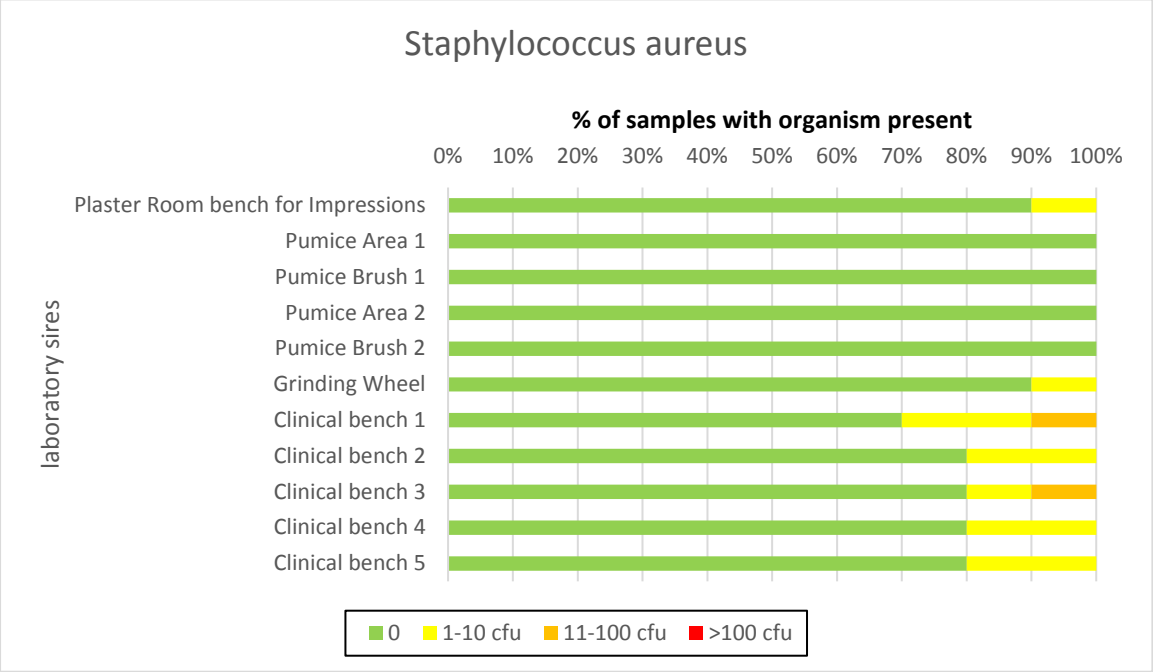

Figure 31 The total cell count of *S. aureus* at various sites in the laboratory.

AGNB were present at all the sites (Figure 4.25). At three sites the bacterial count exceeded 100cfu/ml and majority of the samples did not exceed 100cfu/ml.

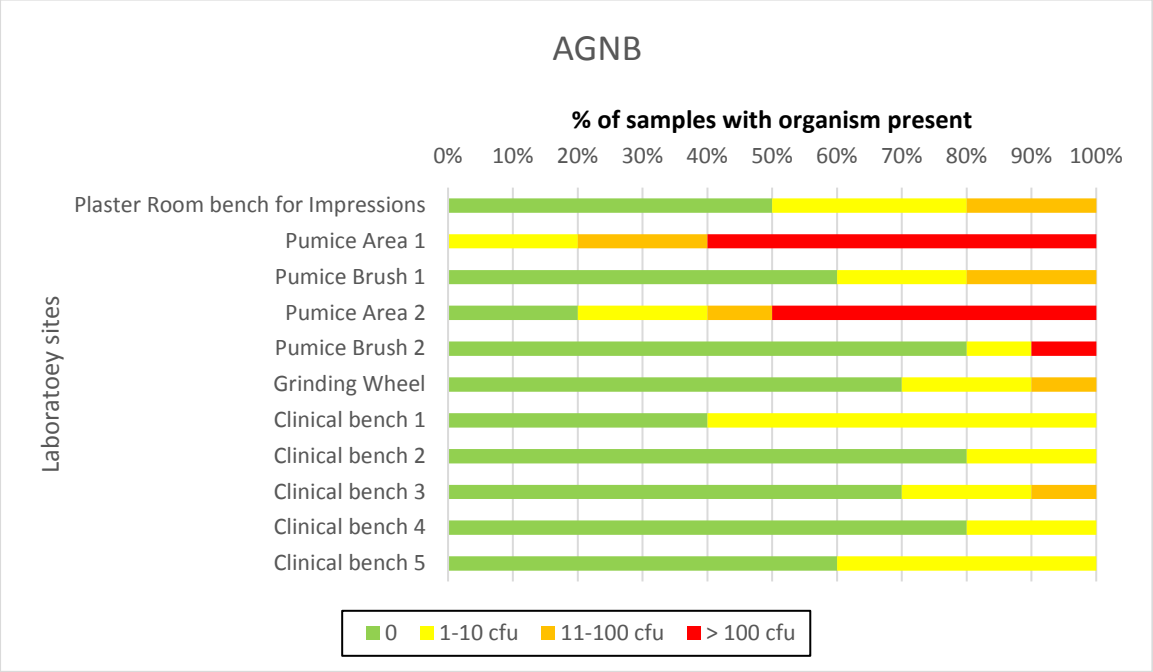

Figure 32 The total cell count of aerobic gram negative bacteria at various sites in the laboratory.

Candida was present at 8 sites with pumice area 1 being most prevalent and when it was present did not exceed 100cfu/ml (Figure 4.26).

Overall there was no significant difference between the cfu frequencies of the various microorganisms.

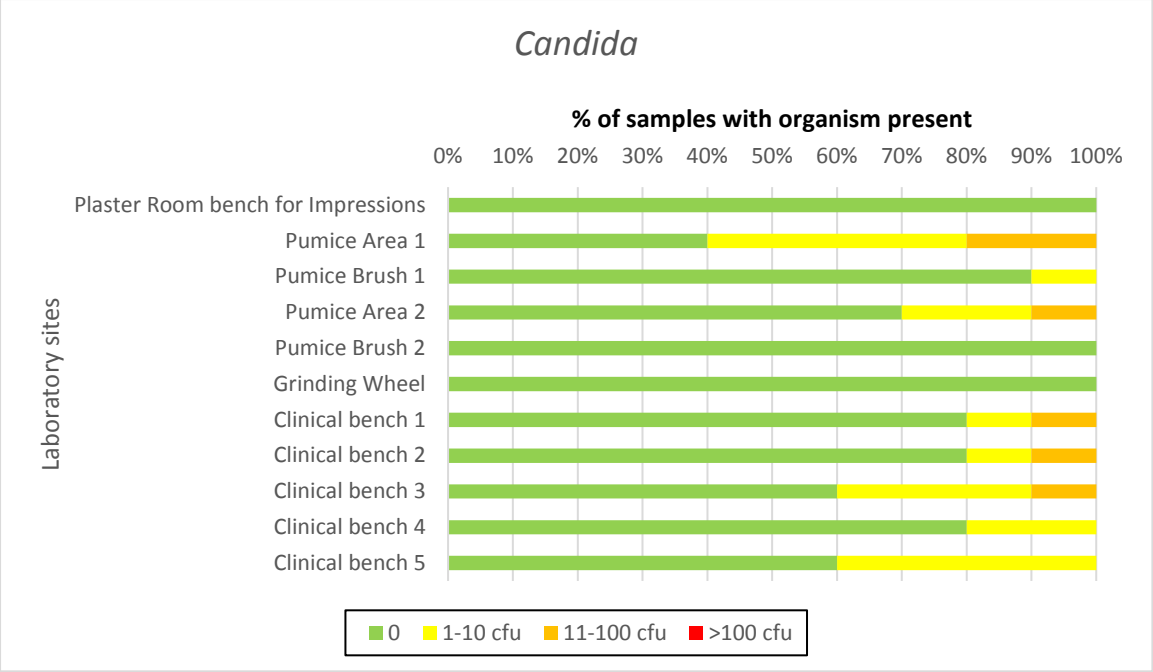

Figure 33 The bacterial cell count of *Candida* at various sites in the laboratory.

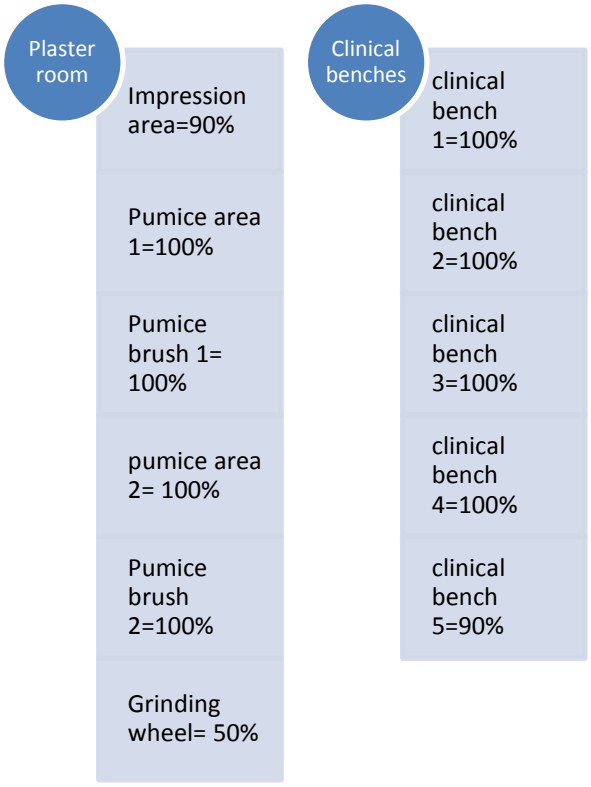

Figure 34 A summary of the presence of the mixed flora at each site in the laboratory

4.3 Efficacy of disinfectants

In Table 4.4 it can be seen that the mean zone of inhibition of Germicide was greater with *S. aureus* and *S. mutans*. The zone of inhibition was comparable for both disinfectants with *Candida.* However, the zone of inhibition with Lactobacilli was greater with the 0.2% Chlorhexidine gluconate than the Germicide.

Table 4. 4 Antimicrobial activity of chlorhexidine (CHX) and germicide against S. *mutans*, S. *aureus*, Lactobacilli and *Candida*.

No.	**Zone of inhibition (mm)** Diameter of disc: 3 mm							
	S. mutans		***S. aureus***		***Lactobacilli***		***Candida***	
	CHX	**Germicide**	**CHX**	**Germicide**	**CHX**	**Germicide**	**CHX**	**Germicide**
1	16.0	18.4	110	15.3	21,3	18.5	7.1	7.5
2	15.7	17.6	11.3	14.1	20,4	15.3	7.0	9.8
3	16.0	17.3	10.0	17.4	20,2	15.5	7.6	6.0
4	15.2	19.0	12.3	19.3	19.5	21.8	6.3	6.3
5	16.2	17.3	11.6	17.4	20.1	18.9	7.8	6.0
6	16.1	18.2	11.9	17.9	19.5	25.1	6.9	7.7
7	17.8	18.5	10.4	16.0	21.2	14.5	7.3	9.4
8	16.0	18.1	11.2	14.4	20.1	15.7	7.5	7.6
9	15.2	18.1	11.3	15.2	20.7	15.4	7.0	5.9
10	15.5	19.6	10.6	16.7	19.4	18.0	7.2	8.0
Mean	**16.0**	**18.2**	**11.2**	**16.4**	**20.2**	**16.5**	**7.2**	**7.4**
± SD	**0.7**	**0.7**	**0.7**	**1.7**	**0.7**	**3.4**	**0.4**	**1.4**

The two-way ANOVA showed that the two-factor interaction of bacteria vs disinfectant was significant ($p < 0.001$) and the least squares mean diffusion is shown in Figure. 4.28. For *Candida*, there was no significant difference between the disinfectants ($p > 0.99$) and for Lactobacilli, the zone of inhibition was significantly lower for Germicide compared with Chlorhexidine ($p < 0.001$). This was reversed for *S. aureus* and *S. mutans* (both at $p < 0.001$)

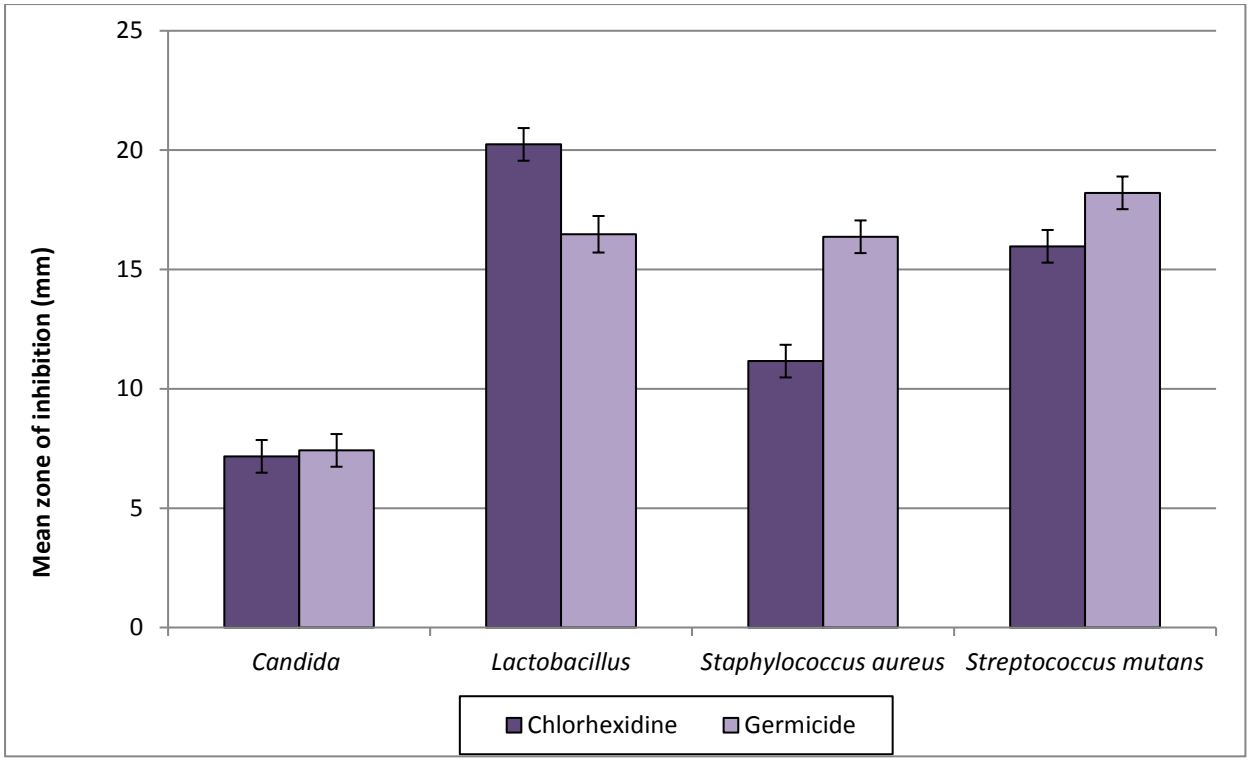

Figure 35 Comparison of antimicrobial activity of chlorhexidine (CHX) and germicide (n=10). Bars denote the 95% confidence interval.

Because each disinfectant behaves quite differently for the organisms (with the exception of Candida) it is moot whether it would be valid to consider all four organisms together, but if this is done, the mean disc diffusion of Chlorhexidine (13.6±0.3 mm) was significantly lower than that of Germicide (14.6±0.4 mm) ($p=0.002$).

CHAPTER 5: DISCUSSION

5.1 Clinical Study

During denture fabrication there are multiple stages that occur before the denture is delivered to a patient. During each stage of denture construction there are points at which contamination can occur. If disinfection does not take place contamination can occur. Contamination can come from the laboratory or it can go from the clinic to the laboratory via multiple items such as occlusion rims, articulators and casts. This study was conducted to assess the level of contamination and the types of contaminants in the clinic and in the laboratory. In addition the *in vivo* and *in vitro* efficacy of the disinfectant was also assessed.

5.1.1 Microorganisms

Blood agar supports the growth of many different types of bacteria thus mixed flora represents all of the possible microorganisms that were assessed (Streptococci, Lactobacilli, AGNB, *Candida* and *S. aureus*) as well as other organisms that were not isolated. This thus enables an assessment of the level of overall contamination.

Streptococci have been found at all sites in the mouth and constitute a large portion of the resident oral microflora (Marsh et al., 2016). In healthy individuals there is 100% carriage of Streptococci (Mathews and Patel, 2018). Therefore in this study it would be expected to find these microorganisms at most sites in high quantities. *S. mutans* and *S. mitis* can act as opportunistic pathogens and found in cases of infective endocarditis. *S. pneumoniae* is an opportunistic pathogen found in the nasopharynx, which can transfer antibiotic resistant genes amongst others in the mitis group (Marsh et al., 2016). *S. mutans* has a role in the aetiology of carious lesions (Assery et al., 1992).

Lactobacilli on the other hand constitute 1% of the total oral microflora in a healthy individual (Marsh et al., 2016). Thus in the study it would be expected to find this organism as least prevalent or absent at most sites. Lactobacilli are usually found in association with *Candida* hyphae and are often considered commensals. Lactobacilli have been implicated in dental caries, rheumatic vascular disease, septicaemia and infective endocarditis (Harty et al., 1994). Age related changes include significantly higher frequencies of Lactobacilli and *S. aureus* (Marsh et al., 2016).

Staphylococci are also not commonly isolated in the oral cavity. These bacteria are considered transient (Marsh et al., 2016). Sattar et al., (2001) reported using *S. aureus* as a test organism as it is considered a common resident in transient skin microflora, is often associated with a plethora of infections in humans, and can survive well on contaminated hands, able to withstand drying and so is a prominent nosocomial pathogen. Healthy individuals carry 36% of *S. aureus* (Mathews and Patel, 2018). It is a Gram-positive coccus, and has been found to be associated with angular cheilitis, parotitis and mucositis (Smith et al., 2003). It has also found to be the most prevalent microorganism associated with nosocomial pneumonia (Imsand et al., 2002).

Healthy individuals carry approximately 17% of aerobic gram-negative bacteria (AGNB) in the oral cavity; these bacteria are usually common in the intestine. When it is found in the oral cavity it can indicate that personal hygiene is poor or the patient is immunocompromised or has an underlying disease (Mathews and Patel, 2018). Nothing has been reported of this bacteria's presence in denture wearers. In this study it would be expected to be found in low concentrations.

Candida are fungi which are frequently encountered in the mouths of healthy individuals and are commensals. The prevalence of Candida carriage in healthy individuals is 35-55% (Marsh et al., 2016; Mathews and Patel, 2018). *Candida* can become pathogenic and can cause infection especially in immunocompromised patients (Traoré et al., 2002, Marsh et al., 2016). The incidence of candidiasis is more common in the elderly; this has been attributed to denture wearing as well as physiological changes in the oral mucosa, and malnutrition (Marsh et al., 2016). *Candida* is associated with denture stomatitis, an inflammatory process involving the oral mucosa of the denture-bearing area. According to Rodriguez-Archilla et al., (1996) persistent or recurrent denture stomatitis is associated with increased systemic levels of Interleukin 2 which can be harmful. *Candida* species has been reported to increase the risk of systemic candida infection in neutropenic patients. Endogenous infection can occur by transmission via hands and other vehicles such as porous (e.g. cloth) and non-porous (e.g. articulators) items (Traoré et al., 2002).

Overall Marsh et al., (1992) found that regardless of age, patients who wear dentures showed a significantly higher carriage in Lactobacilli, *Candida* and *S. mutans* in the oral cavity than their non-denture wearing patients. In addition Staphylococci in saliva and plaque were higher in those who wore dentures. However this study only assessed partial denture wearers.

5.1.2 Level of contamination in the clinic

During the stages of denture construction Streptococci were the most prevalent microorganism and Lactobacilli the least prevalent. This is similar to the results found in the overall laboratory samples, and was to be expected.

At the primary stage of denture construction only two points of contamination were assessed (Figure 4.2). This involved the primary impression before and after disinfection (all impressions were alginate). There was no assessment of the stock trays as they do not come from the laboratory but from the sterilisation area and were assumed to be sterile. As expected all 6 microorganisms were present before disinfection, and after disinfection there was a decrease in presence of microorganisms but not a complete eradication: *Candida* for example, was present in 14% of the samples. However, in the plaster room on the bench for impressions there was an absence of *Candida* (Figure 4.20) which may perhaps have been confined to the impression.

The primary casts received from the laboratory were assessed and the most prevalent microorganisms were mixed flora whilst Lactobacilli was absent (Figure 4.3). When compared with the primary impressions after disinfection (Figure 4.2), the primary casts had a higher presence of microorganisms with the exception of Lactobacilli. Egusa et al., (2008a) in an analysis of the persistent presence of opportunistic pathogens on impressions and gypsum casts, found that microorganisms on the impressions were viable and transferred onto the stone casts. Although a similar pattern was found here, the small number of samples meant that it was not possible to trace precisely how contamination occurred, but it was clear that disinfection of the primary impressions was insufficient and that the resultant casts were contaminated. These patterns were also found when assessing the secondary impressions and final casts.

At the jaw relation registration stage the bases from the laboratory had fewer microorganisms present than the final casts with *Lactobacilli* and *S. aureus* being absent. As was to be expected, following this procedure the bases and final casts showed an increase in

microorganisms, and once again after disinfection there was a decrease but not an absence of all microorganisms. Debattista et al., (2010) assessed occlusion rims (bases) after use in the clinic and transported to the laboratory and found *S. aureus* being absent unlike in the current study which showed that occlusion rims from the laboratory had an absence of *S. aureus,* but after use in the clinic 50% of the samples were contaminated with *S. aureus.*

At the try in stage (Figure 4.5) all work coming from the clinic to the laboratory and the work coming from the laboratory to the clinic was absent of Lactobacilli. In the clinic the bases and casts showed a similar pattern to the previous procedure. In addition, the articulator after try in showed a decrease in all microorganisms which suggests the clinicians may be disinfecting the articulators before sending it to the laboratory. Williams et al., (2011) assessed microbial contamination of trial bases which came from their laboratory and found *Bacillus spp* (57%), *pseudomonas* (22%), Staphylococci *(*13%) and *Candida* (38%); in addition they found that the method of storage contributed to the amount of contamination. In light of this, it was suggested that it is the responsibility of the clinician to disinfect work coming from the laboratory as well as before sending it to the laboratory, as part of their duty to reduce the potential for transmission of infectious agents. Debatissa et al., (2010) also assessed trial dentures and found *S. aureus* absent unlike the current study where there was a presence of *S. aureus* and only an absence after disinfection. However because disinfection is not taking place all the time there was a presence of *S. aureus* going to the laboratory.

At the delivery stage (Figure 4.6), the remount cast and the final dentures from the laboratory had Lactobacilli absent. The final dentures before being polished and before disinfection showed a presence of all microorganisms. After disinfection there was once again a decrease but not an absence of all the microorganisms. There was an increase of microorganism and

Lactobacilli were absent after the dentures had been polished in the laboratory, before disinfection. Debattista et al., (2010) found the new dentures from the laboratory after polishing with pumice had *S. aureus* absent. In this study, *Candida* had a considerably higher presence compared with any of the other times *Candida* was present in the clinic. This is correlated to the high presence of *Candida* found in the pumice areas in the laboratory (Figure 4.20).

5.1.3 Efficacy of disinfection in the clinic

The effectiveness of the current disinfectant in the clinic (Figure 4.8 and Table 4.1) shows that after disinfection there was a significant decrease but not an absence of all microorganisms. In addition to a decrease in presence of the microorganisms overall during denture construction, there was also a significant reduction in the total cell count for mixed flora, Streptococci, *Candida* and *S. aureus* with the exception of AGNB and Lactobacilli (Figure 4.9-4.14). This could suggest that Germicide 3 is not as effective against Lactobacilli and AGNB. This was further confirmed in the laboratory study as Chlorhexidine was more effective than Germicide 3 for Lactobacilli and 2% Glutaraldehyde was also effective against Lactobacilli. (Mähönen et al, 1998)

At the different stages of denture construction it shows that disinfection is not happening all the time in the clinics (Figure 4.16) and the clinicians were more inattentive with disinfection as they continued through the stages of denture construction. At the delivery stage, however, the clinicians were more mindful of disinfection before taking the denture to the laboratory for polishing but when coming back from polishing tended to be less mindful of disinfection. Connor (1991) reported that cross contamination of oral flora from a contaminated denture to a sterile denture by polishing wheel and pumice was possible.

From the results it can be observed that the disinfectant currently in use is effective in decreasing the total cell load, however there is no uniform method for the use of the disinfectant in the clinic and the clinicians are not disinfecting at all opportunities.

In addition the disinfectant was only tested to see its effectiveness as a surface disinfection and as Latib et al., (2018) found, *Candida* does penetrate the denture surface and remains viable despite the use of disinfectants. It would be useful to see if Germicide 3 is able to penetrate the denture surface. Basavanna et al., (2016) found that certain disinfectants changed the dimensional stability of heat cured resins and thus dentures exhibited dimensional change during the disinfecting procedures. This study did not test Germicide3 or any disinfectant with the same disinfectant properties. Moreover, the study was conducted on small acrylic discs and not tested under clinical conditions. Connor (1991) suggested that careful consideration should be taken to account when choosing a disinfectant especially for impressions as disinfectants can cause changes in dimensional stability and surface details. Owen and Goolam (1991) reported that a disinfectant for impressions may also potentially alter surface detail reproduction, surface roughness and dimensional stability. These effects were not assessed for Germicide 3 in this study.

No uniform method amongst clinicians was used in disinfecting during the various stages of denture construction and clinicians were not uniform with their own methods of infection control during the different stages. Clinicians also did not have a uniform disinfectant contact time, which was not assessed in this study. For a disinfectant to be fully effective it requires a certain amount of contact time to effectively decontaminate the surfaces. According to the manufacturer, Germicide 3 requires 3 min contact time to be effective. Kolstad and Lambert

(1986) found soaking a denture in a benzoic acid germicide would only achieve decontamination if the dentures were soaked for 6 hours overnight but this could be decreased to 10 minutes if soaking was combined with ultrasonic enhancement.

Most of the research papers on disinfectants in prosthodontic practice, mainly assess the effectiveness of the disinfectant on the impression stage and different types of impression material (Owen & Goolam, 1993; Egusa et al., 2008; Rweyendela et al., 2009), as well as acrylic (Da silva et al., 2008; Pavarina et al., 2003), but do not focus on the disinfectants' efficacy on other items that may become infected during denture construction.

Gluteraldhyde is a disinfectant and a sterilant at low temperatures. Formaldehyde is also a disinfectant and sterilant at low temperatures but requires longer contact time then gluteraldhyde and needs to be used in combination with steam. Chlorhexidene is used as an antiseptic and alcohol based products are used for hard surface disinfection and skin antisepsis but not recommended for sterilization. Chlorine and iodine based products are used for both antiseptic and disinfecting purposes. Hydrogen peroxide is widely used for disinfection, sterilization and antisepsis. However this study did not look at the disinfectants in a dental setting (McDonnell & Russel,1999). Chlorine dioxide has been shown to be a sterilant with a sort immersion time, for alginate impression material (Rweyendela et al., 2009).

5.1.4 Contamination between the laboratory and the clinic

There is contamination between the clinic and the laboratory with more microorganisms coming from the laboratory than going to the laboratory (Figure 4.17). Lactobacilli were not present coming from the laboratory but were present going to the laboratory (often exceeding

>100 cfu). It cannot be ignored that even though there was no significance between the other microorganisms (Table 4.2) that there was still a considerable amount of contamination occurring with the other microorganisms both coming from and going to the laboratory..

Conor (1991) assessed cross contamination between clinic and laboratory and found that contamination was through impressions, bases for jaw relation registration, trial bases, and final prostheses. However, the study did not acknowledge any other point of contamination such as casts and articulators nor possible contamination from the laboratory to the clinic. Egusa et al., (2008a) reported that the evidence was limited when assessing the transmission of pathogenic microorganisms into the dental laboratory. Da Silva., (2008) et al reported that the possibility of cross contamination between the dental office and laboratory is high and suggested that disinfection of prostheses before sending to the laboratory and when receiving the item from the laboratory is paramount. According to the current study not only do the prostheses need to be disinfected, but all items coming and going to the laboratory need to be disinfected as well.

5.2 Laboratory Study

Overall the most prevalent microorganisms in the laboratory were mixed flora, followed by Streptococci with the least prevalent being Lactobacilli (Figure 4.18). The most contaminated area was the pumice area 1 and the least contaminated area was the grinding wheel (Figure 4.20). At the pumice area it is mostly moist and the pumice slurry is not replaced often. The grinding wheel is a dry area where a lot of friction is created. Furthermore, no area had all six microorganisms present at one time. A contaminated pumice area is a particular concern as it is known to produce contaminated aerosols and splatter particles (Connor, 1991) and thus protective eye wear and gloves should be worn (currently not the case in this laboratory). The

report further suggested that pumice may be mixed with a non-irritant disinfectant or preferably dispensed when needed and then removed, as it should never be left to incubate. In addition lathes, rag wheels, and stones should all be disinfected as well. Debattista et al., (2010) also reported the pumice to be highly contaminated with 60% of the samples containing *S. aureus.* Sattar et al., (2001) found the transfer of *S. aureus* from moist fabrics was higher than dry fabrics; additionally friction increased the level of transfer, even with a short contact time the number of transfer of organisms was as high as 700 cfu/ml. This logic can be applied to the pumice wheel as this is a wet fabric which has contamination which can be transferred to denture bases as well as to the technicians / clinicians hands during handling. This is a risk if gloves are not worn as skin infections can occur due to invisible breaks and cuts in the hands (Sattar et al., 2001). The survival of *C. albicans* on fabrics was better than on non-porous and inanimate objects and was detectable after 14 days. This could be due to the fungi being retained inside the fibres of the fabric and the moist environment. *S. aureus* was found to be transferred to fingertips and can survive up to 24 hours as well as for the same amount of time on laminated surfaces, with higher contamination on cloth surfaces (Scott & Bloomfield, 1990). Gontijo Filho et al., (1985) reported that gram negative bacteria are more sensitive to drying than gram positive bacteria. In addition, the study discovered that the microbial population of the tested organisms (gram positive and gram negative bacteria) on the hands decreased by 99% after 5 mins of drying, with *S. aureus*, *K. pneumoniae* showing a greater survival rate, *P. aeruginosa* showed intermediate survival rates with *E.coli* being the most sensitive to drying. Although drying reduces bacterial strains it does not eliminate them. (Gontijo Filho et al., 1985, Scott and Bloomfield, 1990). Staphylococci successfully survive even in adverse conditions and can alter their physiology and cellular activities; this adaptation enables them to persist and affects the nature of their pathogenesis (Onyango & Alreshidi, 2018)

These results show that there is contamination in the laboratory and highlights the need to introduce infection control procedures in the laboratory.

5.3 Efficacy of disinfectant

There is very little or no recent research done which shows the efficacy of Germicide 3 and denture disinfection, as its main use is as a surface disinfectant. Chlorhexidine is considered a gold standard antiplaque agent and has been found to be effective in the decontamination of dentures. Surprisingly from the results of the disc diffusion test, Germicide 3 was more effective than 0.2% Chlorhexidine gluconate against *Candida, S. mutans and S. aureus* which supports its claim to be bactericidal and fungicidal. However, it is not as effective against Lactobacilli. In addition the disc diffusion test does not take into account the method or time in which the disinfectant needs to be used in order to achieve decontamination of a denture base as Lambert and Kolstad (1986) found that when combined with a detergent it was more effective in safely decontaminating dentures. To achieve decontamination it was reported that it needed to be soaked for six hours and at the time of the research, the active ingredient was benzoic acid. In addition the ingredients have changed and are available as a spray solution. Ultra-sonic enhancement decreased the decontamination time to ten minutes. Assery et al., (1992) found that 10 minutes was sufficient to thoroughly disinfect a denture, although disinfection is dependent on the material's ability to infiltrate denture porosities and penetrate the plaque biofilm. In the clinical situation Germicide 3 is only available as a spray and the option of soaking as suggested by the literature is not an available option. The active ingredients in Germicide 3 are alkyl dimethyl ethylbenzyl ammonium chloride, benzalkonium chloride and isopropyl alcohol. In the study done by Lambert and Kolstad (1986) the active ingredient was benzoic acid thus the Germicide that was used in this study

would be expected to behave differently Latib et al., (2018) have shown the viability of *Candida* which had penetrated the denture after disinfection but did not test either Germicide 3 or 0.2% Chlorhexidine gluconate. Egusa et al., (2018) pointed out that the effects of disinfectants *in vitro* may differ from those *in vivo* due to the presence of salivary and serum proteins or individual differences in oral flora composition. Traoré et al., (2002) conducted a study testing chemical germicides against *Candida* species and found that ethanol and ethanol based products effectively inactivated *Candida* species.

5.4 Clinical implications

Overall there is cross contamination occurring between the clinic and the laboratory, which puts patients, clinicians and laboratory staff at risk. Not only are the test organisms part of the normal flora but are also possible pathogens that can lead to opportunistic infections (Egusa et al., 2008). Onyango and Alreshidi., (2018) reported that Staphylococci are a serious public health threat as they are resistant to many antibiotics and *S. aureus* was the culprit in many difficult to treat infections. AGNB and *Candida* also cause some serious infections. This study highlights the importance of an infection control regimen during denture fabrication.

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

6.1. Summary and Conclusions

This study showed that at each stage of denture fabrication, there is a presence of Streptococci, Lactobacilli, *Candida,* AGNB and *S. aureus*. The level and combination of these microorganisms can be different. During denture fabrication if disinfectant is used, it reduces the level of microorganisms but does not eliminate them. Various surfaces in the dental laboratory were also contaminated with these test organisms with the pumice area being most contaminated and the grinding wheel being least contaminated. No significant difference in the contamination coming from and going to the laboratory samples were found. Germicide3 used in the clinics showed good antimicrobial efficacy against the test organisms. Therefore, it is important to follow infection control protocols during denture fabrication in the clinic and the laboratory to prevent cross contamination and to protect healthcare workers and patients.

6.2 Limitations

Ideally an increased sample size could have been used, and more cases could have been followed from beginning to end. In addition, more microorganisms could have been tested both *in vivo* and *in vitro* as well as more disinfectants. None of this could not be done due to time constraints as well as limited resources.

6.3. Recommendations

- A new infection control protocol is required.
- Stricter observation of infection control protocols in the clinics is necessary.
- An infection control protocol must be introduced into the laboratory such as wearing gloves, even gloves of a different colour to discriminate between clinical gloves being used in the laboratory.
- Communication between both internal and external laboratories and the clinics of whether infection control has been undertaken or not.
- More research needs to be done on the effect of different disinfectants in decontaminated all aspects of prosthodontic procedures.
- Different disinfectants need to be assessed as to their effects on the material properties of impression and denture base materials.
- The knowledge, attitude and awareness of clinicians and laboratory staff toward infection control should be tested on a regular basis.

REFERENCES

Assery M, Sugrue PC, Graser GN, Eisenberg AD. Control of microbial contaminations with commercially available cleaning solutions. J Prosthet Dent, 1992; 67(2):275-277.

ADA 2015. ADA's Guidelines for infection control. Australian Dental Association. 3rd Edition 2015

Basavanna JM, Jujare RH, Varghese RK, Singh VD, Gaurav A. Effects of laboratory disinfection agents on dimensional stability of three commercially available heat cured denture acrylic resins in India: An in vitro study. J Clin Diagn Res, 2016; 10(3):37-31

Baygar T, Ugur A, Sarac N, Balci U, Ergun G. Functional denture soft liner with antimicrobial properties and antibiofilm properties. J Dent Sci, 2018; 13(3):213-219

CDC (2003). Centers for Disease Control and Prevention. Guidelines for Infection Control in Dental Health-Care Settings. 2003. MMWR 2003;52 (No. RR-17): 2-5, 33, 62

CDC (2016). Summary of Infection Prevention Practices in Dental Settings: Basic Expectations for Safe Care. Atlanta, GA: US Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Division of Oral Health; March 2016.

Connor C. Cross contamination control in prosthodontic practice, Int J Prosthodont 1991; 4:337-344

Da Silva FC, Kimpara ET, Mancini MNG, Balducci I, Jorge AOC, Kago-Ito CY. Effectiveness of Six Different Disinfectants on Removing Five Microbial Species and Effects on the Topographic Characteristics of Acrylic Resin. J Prosthodont. 2008; 17(8):627–633

Debattista N. Zarb M, Portelli J. Bacterial cross contamination between the dental clinic and laboratory during prosthetic treatment. Malta Med J, 2010; 22:17-19

Egusa H, Watamoto T, Matsumoto T, Abe K, Kobayashi M, Akashi Y et al. Clinical evaluation of the efficacy of removing microorganisms to disinfect patient derived dental impressions. Int J Prosthodont 2008; 21:531-538

Egusa H, Watamoto T, Abe K, Kobayashi M, Kaneda Y et al. An analysis of the persistent presence of opportunistic pathogens on patient derived dental impressions and gypsum casts. Int J Prosthodont 2008a; 21:62-68

Faul F, Erdfelder E, Lang A.-G, Buchner A. G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behavior Research Methods, 2007;39:175-191.

Glass RT. Bullard JW, Conrad RS. Blewett E. Evaluation of the sanitisation effectiveness of a denture-cleaning product on dentures contaminated with known microbial flora. An in vitro study. Quintessence Int 2004;35:194-9.

Glass RT, Bullard JW, Hadley CS, Mix EW, Conrad RS. Partial spectrum of microorganisms found in dentures and possible disease implications. J Am Osteopath Assoc. 2001; 101:92-94

Glass RT, Conrad RS, Bullard JW, Goodson LB, Mehta N, Stanley JL et al. Evaluation of microbial flora found in previously worn prostheses from the Northeast and Southwest regions of the United States. J Prosthet Dent, 2010; 103:384-389.

Glass RT, Conrad RS, Bullard JW, Goodson LB, Mehta N, Lech SJ et al. Evaluation of cleansing methods on previously worn prostheses. Compend Contin Educ Dent 2011; 33:68-73

Gontijo Filho PP, Stumpf M, Cardoso CL. Survival of gram negative and gram positive bacteria artificially applied on hands. J Clin Microbiol, 1985; 21(4):652-653

Harrison Z, Johnson A, Douglas CWI. An in vitro study into the effect of a limited range of dental cleaners on surface roughness and removal of Candida from conventional heat cured acrylic resin denture base material. J Oral Rehabil, 2001; 31:460-467.

Harty DWS, Oakey HJ, Patrikakis M, Hume EBH and Knox KW. Pathogenic potential of lactobacilli. Int J Food Microbiol, 1994; 24:179-189

Imsand M, Janssens JP, Auckenthaler R, Mojon P, Budtz-Jorgensen E. Bronchopneumonia and oral health in hospitalized older patients. A pilot study. Gerodontology. 2002; 19(2):66-72.

Lambert JP, Kolstad R. Effect of a benzoic acid detergent germicide on denture bore Candida. J Prosthet Dent, 1986; 6:699-700

Latib YO, Owen CP, Patel M. Viability of *Candida* in denture base resin after disinfection: A preliminary study. Int J Prosthodont 2018; 31:436-439

Li X, Kolltiveit KM, Tronstad L, Olsen I. Systemic diseases caused by oral infection. Clin Microbiol Rev, 2000; 13(4):547-558.

Mähönen K, Virtanen K, Larmas M. The effect of prosthesis disinfection on salivary microbial levels. J Oral Rehabil, 1998;25:304-310

Marsh PD, Lewis AOL, Rogers H, Williams DW, Wilson. Marsh & Martin's Oral Microbiology. 6th ed. London: Elsevier, 2016

Marsh PD, Percival PS, Challacome SJ. The influence of denture-wearing and age on the oral microflora. J Dent Res, 1992; 71(7):1374-1381.

Mathews J, Patel M. Bacterial endotoxins and microorganisms in the oral cavities of patients on cancer therapy. Microb Pathog, 2018;123:190-195.

McDonnell G, Russell AD. Antiseptics and disinfectants: activity, action, and resistance. Clin Microbiol Rev. 1999; 12(1):147-79.

Onyango LA, Alreshidi MM. Adaptive metabolism in Staphylococci: survival and persistence in environmental and clinical settings. J Pathog, 2018, 2018: article 1092632

Owen CP, Goolam R. Disinfection of impression materials to prevent viral cross contamination: A review and a protocol. Int J Prosthodont, 1993; 6:480-494

Patel M, Ebonwu J, Cutler E. Comparison of chlorine dioxide and dichloroisocyanurate disinfectants for use in the dental setting. SADJ 2012; 67(7):364-368

Pavarina AC, Pizzolitto AC, Machado AL, Vergani CE, Giampaolo ET. An infection control protocol: Effectiveness of immersion solutions to reduce the microbial growth on dental prostheses. J Oral Rehabil, 2003; 30:532-536

Rodriguez-Archilla A, Urquia M, Cutando A, Ascensio R. Denture Stomatitis: Quantification of Interlukin-2 production by mononuclear blood cells cultured with Candida. J Prosthet Dent, 1996; 75:426-431.

Rweyendela IH, Patel M, Owen CP. Disinfection of irreversible hydrocolloid impression material with chlorinated compounds. SADJ 2009; 64(5):208-212

Sattar SA, Springthrope S, Mani S, Gallant M, Nair RC, Scott E, et al. The transfer of bacteria from fabrics to hands and other fabrics: development and application of a quantitative method using Staphylococcus aureus as a model. J Appl Microbiol, 2001; 90(6):962-970

Scott E, Bloomfield SF. The survival and transfer of microbial contamination via cloths, hands and utensils. J Appl Bacteriol, 1990; 68(3):271-278.

Smith AJ, Robertson D, Tang MK, Jackson MS, MacKenzie D, Bagg J. Staphylococcus aureus in the oral cavity: a three year retrospective analysis of clinical laboratory data. Br Dent J, 2003; 195:701-703

Traoré O, Springthorpe V.S, Sattar SA. A quantitative study of the survival of two species of Candida on porous and non-porous environmental surfaces and hands. J Appl Microbiol, 2002; 92:549-555.

Valentini-Mioso F, Maske TT, Cenci MS, Boscato N, Pereira-Cenci T. Chemical hygiene protocols for complete dentures: A cross over randomized clinical trial. J Prosthet Dent. 2019; 121(1):83-89.

Watamoto T, Egusa H, Sawase T, Yatani H. Clinical Evaluation of Chlorine dioxide for disinfection of dental instruments. Int J Prosthodont 2013; 26:541-544

Williams DW, Chamary N, Lewis MAO, Milward PJ, McAndrew R. Microbial contamination of removable prosthodontic appliances from laboratories and impact of clinical storage. Br Dent J 2011; 211:163-166

APPENDIX A: ETHICS CERTIFICATE

R14/49 Dr Krystle L'oreal Moodley

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M170604

NAME: (Principal Investigator)	Dr Krystle L'oreal Moodley
DEPARTMENT:	Oral Rehabilitation Wits Oral Health Centre and Department of Oral Biological Sciences
PROJECT TITLE:	Cross Contamination between the Clinic and Laboratory during Denture Fabrication
DATE CONSIDERED:	30/06/2017
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof CP Owen and Prof M Patel
APPROVED BY:	Professor P Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	26/07/2017

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 301, Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in June and will therefore be due in the month of June each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

Date 26/07/17

APPENDIX B: FACULTY PROTOCOL APPROVAL

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

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Reference: Mrs Sandra Benn
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10 January 2018
Person No: 359387
PAG

Dr KL Moodley
P O Box 1661
Mondeor
2110
South Africa

Dear Dr Moodley

Master of Science in Dentistry: Approval of Title

We have pleasure in advising that your proposal entitled *Cross contamination between the clinic and laboratory during denture fabrication* has been approved. Please note that any amendments to this title have to be endorsed by the Faculty's higher degrees committee and formally approved.

Yours sincerely

Mrs Sandra Benn
Faculty Registrar
Faculty of Health Sciences

APPENDIX C: MEDIA AND REAGENTS

Blood Agar

The base was created using Columbia agar and distilled water. 500ml of water was boiled, to which 44g of Columbia agar was dissolved. Thereafter 500ml was added and heated again to boiling point. This solution was poured into Boston bottles, 500ml in each bottle.

The Columbia base was then autoclaved at 121°C for 15 minutes. The medium was allowed to cool to 45°C and 10ml of blood was added to the media, swirled to mix well and petri dishes were filled with the solution under the laminar wood.

Mitis Salivarius Agar

Prepared as per instructions on the packaging.

90g MSA agar was mixed with 1000ml distilled water and boiled till it dissolved. This solution was dispensed to 500ml Boston bottles and autoclaved at 121°C for 15 minutes. The medium was allowed to cool to 45°C and 0.50ml potassium tellurite was added to the media, swirled to mix well and petri dishes were filled with the solution under the laminar wood.

Rogosa Agar

Prepared as per instructions on the packaging

75g Rogosa Agar was mixed with 1000ml distilled water this was heated until dissolved. The solution was allowed to cool and then 1.32ml of acetic acid was added and heated again to 90°C for two minutes. The medium was allowed to cool to 45°C and petri dishes are filled with the solution under the laminar wood. This media was not autoclaved.

Saboraud Agar

60g of Saboraud Agar was weighed on an electronic weighing balance. This powder was dispensed into an Ellern Meyer Flask with a magnetic stirrer. This was placed on a magnetic hot plate and 1000ml of distilled water is added 500ml at a time. Foil was placed over the flask opening to prevent spillage and the mix was stirred with heat until the powder was completely dissolved. Once it was fully dissolved, the solution was dispensed into 500ml Boston bottles. It was then autoclaved at 121°C for 15 minutes, thereafter allowed to cool to 45°C and petri dishes were filled with the solution under the laminar wood.

MacConkey Agar

Heat 500mL water to boiling point, dissolve 50g medium powder in water add 500 mL water and heat to boiling point.

Dispense into 500mL Boston bottles and Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Baird Parker Agar

Suspend desired quantity (as per manufacturer's instruction) of the medium in 950 ml purified water. Heat with frequent agitation and boil for one minute to completely dissolve the medium. Autoclave at 121°C for 15 minutes. After cooling to 45- 50°C, add 50 mL of Egg Yolk Tellurite Supplement and 3 ml sterile 3.5% Potassium Tellurite solution or 50 ml Egg Yolk Tellurite Emulsion and mix thoroughly before dispensing.

APPENDIX D: TURNITIN REPORT

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